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EFFECT OF DIET ON VASCULAR HEALTH IN TYPE 2 DIABETES AND
COMMUNITY-DWELLING ADULTS

A Dissertation in
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by
Katherine A. Sauder

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The dissertation of Katherine A. Sauder was reviewed and approved* by the following:

Sheila G. West  
Associate Professor of Biobehavioral Health and Nutritional Sciences  
Dissertation Advisor  
Chair of Committee

Penny M. Kris-Etherton  
Distinguished Professor of Nutritional Sciences

David N. Proctor  
Professor of Kinesiology and Physiology

Jan S. Ulbrecht  
Professor of Biobehavioral Health

Collins O. Airhihenbuwa  
Professor of Biobehavioral Health  
Head of the Department of Biobehavioral Health

*Signatures are on file in the Graduate School.
ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death in the United States. Lifestyle modification, including dietary changes, continues to be the first line of therapy for risk management. While notable progress has been made in identifying foods and nutrients that optimize health, it remains unclear as to what eating patterns are most effective at reducing risk in vulnerable populations. The purpose of this dissertation is to examine the relationship between diet and vascular risk factors in two high-risk populations: individuals with type 2 diabetes and older adults. Additionally, it will examine the reliability of a novel method of measuring vascular health that has the potential to improve risk assessment in the medical clinic.

The first study in this dissertation evaluated the effects of nut consumption on blood pressure, systemic hemodynamics, heart rate variability, and endothelial function in adults with type 2 diabetes. Cardiovascular disease is the leading cause of death in this population, and interventions to reduce risk are urgently needed. We enrolled 30 adults with type 2 diabetes in a randomized, crossover, controlled-feeding study. After a 2-week baseline period, participants consumed a low-fat control diet (27% fat) and a moderate-fat pistachio diet (33% fat) containing pistachios (20% of daily energy) for 4 weeks each. Following each diet period, we assessed systemic hemodynamics and heart rate variability at rest and during acute mental stress, endothelial function, and, in a subset of participants (n=21), 24-hour ambulatory blood pressure. There was no difference between treatments in blood pressure at rest and during mental stress; however, total peripheral resistance was significantly reduced by approximately 4% following the pistachio diet and did not change following the control diet. Furthermore, 24-hour ambulatory systolic blood pressure, which is a better predictor of target organ damage than resting blood pressure, was significantly reduced by 3.5 mmHg following the pistachio diet. Several measures of heart rate variability were improved following the pistachio diet, and there was no difference between the treatments on endothelial function. This study demonstrates that, for individuals with type 2 diabetes, consuming a moderate-fat diet that includes pistachios improves multiple cardiovascular risk factors.

The second study in this dissertation examined whether adherence to the 2010 Dietary Guidelines for Americans is independently associated with endothelial dysfunction and arterial stiffness in a cross-sectional sample of adults from the Framingham Heart Study.
There is strong evidence from intervention studies that diet is related to vascular health, but the majority of these studies have tested individual foods or nutrients and relied on small samples of mostly younger adults. The effect of overall diet quality, as defined by public health recommendations, on measures of vascular health is unknown. This study included diet and vascular data previously collected in 6020 adults in the Framingham Heart study. Diet quality was quantified with the 2010 Dietary Guidelines for Americans Index (DGAI-2010), endothelial dysfunction was assessed via brachial flow-mediated dilation, and arterial stiffness was assessed via carotid-femoral pulse wave velocity. Regression analyses were used to determine whether DGAI-2010 scores were independently associated with vascular health. Baseline brachial mean flow velocity significantly decreased with increasing DGAI-2010 scores \( (P=0.002) \) and hyperemic mean flow ratio significantly increased with increasing DGAI-2010 scores \( (P=0.02) \). Augmentation index significantly decreased with increasing DGAI-2010 scores; age-stratified analyses revealed that this relationship was statistically significant only in older adults \( (>50 \text{ years}) \). Diet was not significantly related to measures of endothelial dysfunction. Our results suggest a link between diet quality and vascular health and as such may be useful for designing interventions to treat or prevent age-related vascular decline.

The third study in this dissertation assessed the test-retest reliability of a novel method of measuring endothelial dysfunction. Peripheral arterial tonometry (PAT) is a promising method for non-invasive assessment of endothelial dysfunction that is operator-independent, easily standardized between laboratories, and FDA-approved for risk assessment in medical clinics. Furthermore, given the increased risk of diabetes and CVD in adults with the metabolic syndrome, it is important to verify the reliability of PAT in the metabolic syndrome and to provide population-specific power and sample size estimates that can guide clinical trial design. This study included 20 adults with the metabolic syndrome who completed 5 PAT tests each separated by 1 week. The PAT-derived index of endothelial dysfunction (the reactive hyperemia index, RHI) showed robust repeatability (intra-class correlation \( = 0.74 \)), and was not related to daily fluctuations in glucose and insulin. We show that a parallel arm study powered at 0.90 would require 22 participants to detect an absolute change in RHI of 0.40 units (equal to ~25% change in this sample), whereas a crossover study would require only 12
participants. These results indicate that PAT can be used to assess endothelial dysfunction in adults with the metabolic syndrome as reliably as in healthy subjects.

In conclusion, dietary modification is a key element of CVD prevention. This dissertation provides evidence that a specific food (pistachios) and health dietary pattern (the Dietary Guidelines for Americans) may reduce risk in vulnerable populations. It also demonstrates the reliability of a novel, non-invasive method of measuring vascular endothelial dysfunction in a population with elevated risk. These findings can help researchers and clinicians develop interventions to reduce the burden of CVD in the United States and globally.
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CHAPTER 1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States (1). Lifestyle modification, including dietary changes, continues to be the first line of therapy for risk management. While notable progress has been made in identifying foods and nutrients that optimize health, it remains unclear as to what eating patterns are most effective at reducing risk in vulnerable populations. Furthermore, residual risk of events remains even after successful treatment of risk factors such as dyslipidemia, hyperglycemia, and hypertension (2), and additional intervention strategies are needed to reduce the burden of CVD in the modern world.

Low-fat diets have traditionally been recommended, but this dietary pattern excludes foods, such as nuts, that are rich in healthy unsaturated fats. Nut consumption has been associated with beneficial health outcomes in numerous studies and populations (3). For example, research has shown that frequent nut consumers are 35-58% less likely to experience cardiovascular events compared to those who rarely consume nuts (4, 5). This effect is often attributed to the fatty acid profile of nuts (low in saturated fat and high in unsaturated fat), which has repeatedly been shown to reduce LDL cholesterol by an average of 7.4% in 4 weeks (6). However, the effect of nuts on other cardiovascular risk factors, namely blood pressure, is less conclusive. A recent review of approximately two dozen clinical trials assessed the effect of nuts on blood pressure (see Casas-Agustench *et al.* (7) for review), and the majority reported non-significant changes in resting blood pressure over 3-24 weeks. However, some studies, including one from our research group, indicate that regular nut consumption can have a beneficial effect on blood pressure, particularly during stress (8). The first study in this dissertation (Chapter 3) evaluates the effects of regular nut consumption on blood pressure in adults with type 2 diabetes, and also examines treatment effects on the underlying hemodynamics that determine blood pressure to further understand physiological changes.

Another dietary pattern recommended for risk management is presented in the 2010 Dietary Guidelines for Americans (9). Updated every 5 years, the Dietary Guidelines for Americans are the result of a rigorous scientific review process that considers the evidence for and against consumption of particular foods and nutrients. In the 2010 version, three major goals are emphasized: balancing calories with physical activity to manage weight; increased consumption of fruits, vegetables, whole grains, low-fat dairy,
and seafood; and reduced consumption of sodium, saturated fat, trans fat, cholesterol, added sugars, and refined grains. While these guidelines are evidence-based (at least for the individual recommendations), there is limited subsequent evidence that adhering to these guidelines results in health improvements. Given the burden of CVD in the United States, it is important to evaluate if adherence to the Dietary Guidelines for Americans is associated with reduced risk. Therefore, the second study in this dissertation (Chapter 4) evaluates the association between adherence to the 2010 Dietary Guidelines for Americans and important precursors of CVD (arterial stiffness and endothelial dysfunction) in a large, cross-sectional sample of community-dwelling adults in the Framingham Heart Study. Additionally, given that age is the major risk factor for CVD, this study also examines whether adherence to the Dietary Guidelines for Americans modifies the effect of age on vascular decline.

Finally, as nutrition scientists strive to improve the cardiovascular health of Americans through dietary modification, it is vital that accurate and reliable assessments of risk factors are used in research. Arterial stiffness and endothelial dysfunction are two markers of vascular health that have been widely assessed in nutrition research and show promise as surrogates of cardiovascular disease that can potentially be modified by diet. However, the traditional methods of assessment, carotid-femoral pulse-wave velocity and flow-mediated dilation, respectively, have technical limitations that prevent widespread application to a clinical setting. Peripheral arterial tonometry (PAT) is a promising method for non-invasive assessment of endothelial dysfunction that is operator-independent, easily standardized between laboratories, and FDA-approved for risk assessment in medical clinics. However, before PAT can be widely adopted for clinical risk assessment, it is important to understand day to day variations, especially in high-risk populations such as adults with the metabolic syndrome. Therefore, the third study in this dissertation (Chapter 5) assesses the PAT test-retest reliability in adults with the metabolic syndrome and uses variability metrics to provide sample size and power estimates for a range of study designs. It also examines the correlation between PAT scores and fasting glucose and insulin to determine whether PAT variability is associated with fluctuations in fasting glucose and insulin.

Together, the three studies in this dissertation demonstrate the importance of diet in CVD risk management. These findings can help researchers and clinicians develop interventions to reduce the burden of CVD in the United States and globally.
CHAPTER 2. REVIEW OF THE LITERATURE

This chapter reviews the literature support for the studies presented in Chapters 3-5.

Cardiovascular Risk Factors

Blood Pressure

Blood pressure is one of the most prominent CVD risk factors. Numerous observational studies have documented the association between increases in blood pressure and CVD mortality. A meta-analysis of 61 studies that included over 1 million individuals concluded that for every 20 mmHg increase in systolic blood pressure (SBP) or 10 mmHg increase in diastolic blood pressure (DBP) above 115/75, the risk of CVD mortality doubles (10). Diagnostic criteria for hypertension were developed on the basis of these studies, with optimal blood pressure classified as <120/80 mmHg, prehypertension classified as 120-140/80-90 mmHg, and hypertension beginning at 140/90 mmHg. Approximately 1 in 3 Americans has hypertension, with 25% being undiagnosed (11). Lifestyle modification and pharmacotherapy are recommended to reduce blood pressure to below 140/90, and numerous clinical trials have demonstrated that successfully controlling blood pressure significantly reduces morbidity and mortality (12).

The traditional method for assessing blood pressure in the clinic involves a trained clinician using a stethoscope and sphygmomanometer to listen for Korotkoff sounds following brief occlusion of the brachial artery with a blood pressure cuff. Two readings should be taken 1 minute apart following 5 minutes of seated rest (13). A second method of assessing blood pressure includes using an automated cuff for a 24-hour period. The patient wears a blood pressure cuff on the brachial artery, which is connected to a small monitor that can be attached to a belt. Automated readings are typically taken every 20-30 minutes during the patient’s normal daily activities. Studies have shown that compared to standard clinic measurements, 24-hour ambulatory blood pressure is a better predictor of target organ damage (see Verdecchia 2000 for review (14)). An important benefit of 24-hour monitoring is that blood pressure is observed in a variety of conditions, including sleep periods. Blood pressure normally decreases (or “dips”) during sleep, and individuals who do not display this physiological change can be 66% more likely to experience a cardiovascular event (15). Finally, a third method of measuring
blood pressure involves taking repeated readings under standardized, acute mental stress tasks. This procedure typically involves a baseline resting period, one or more stress tasks (e.g. mental arithmetic, simulated public speaking, hand cold pressor), and post-task recovery periods. Blood pressure is measured throughout the procedure, and the stress reactivity, or change in blood pressure during stress compared to baseline, is calculated. The stress reactivity protocol has been used by psychophysiologists since the 1930s (16, 17), and a recent meta-analysis of 36 studies concluded that exaggerated stress reactivity is independently predictive of future cardiovascular outcomes (18).

**Systemic Hemodynamics**

Mean arterial blood pressure is determined by cardiac output (the amount of blood in liters pumped by the heart each minute) and total peripheral resistance (the resistance of the peripheral blood vessels, not including the pulmonary vasculature). Assessing the underlying systemic hemodynamics is useful in understanding the mechanisms behind changes in blood pressure, and why an intervention that reduced blood pressure was effective. Additionally, understanding the underlying systemic hemodynamics can enable clinicians to treat hypertension more effectively (19). For example, if a patient has tachycardia contributing to increased cardiac output, beta-blockers can be given to slow the heart rate. If a patient has increased stroke volume, diuretics can be given to reduce blood volume. If a patient has increased peripheral resistance, angiotension-converting enzyme inhibitors or calcium channel blockers can be given to reduce vasoconstriction.

Systemic hemodynamics can be measured non-invasively with impedance cardiography (20). Four Mylar band electrodes are placed on the body: two around the neck (>3 cm apart), and two around the chest at the xiphesternal junction (>3 cm apart). The outer two bands serve as current electrodes, and the inner two are used to measure the thoracic impedance. Three spot electrodes are used to record the electrocardiogram, and simultaneous blood pressure is obtained with an external device. Stroke volume (ml/beat) is calculated with the Kubicek equation, which includes the resistivity of blood (assumed to be 150 ohm*cm), the distance between the inner two recording bands, left ventricular ejection time, and the maximum rate of change in impedance during each heartbeat (21). Stroke volume is multiplied by heart rate to determine cardiac output (l/min), and total peripheral resistance (dyne*sec/cm$^5$) is calculated by dividing cardiac output into mean arterial pressure (mmHg). Numerous studies have shown that
impedance cardiography is a valid, non-invasive method for measuring relative changes in stroke volume (see Woltjer et al. for review) (22).

**Heart Rate Variability**

Increased sympathetic nervous system function is believed to contribute to the development of CVD, whereas increased parasympathetic activity appears to protect against sudden cardiac death (23, 24). Heart rate variability (HRV) is a noninvasive method used to index autonomic influence on cardiac function by quantifying the beat-to-beat fluctuations in R-R interval collected by electrocardiogram (25). Spectral analysis of these fluctuations allows identification of several frequency bands, each with their own physiological determinants (26). Oscillations in the high frequency (HF) band (0.15 Hz to 0.4 Hz) are widely considered a marker of parasympathetic activation (27). Oscillations occurring in the low frequency (LF) band (0.05 Hz to 0.15 Hz) are believed to be influenced by both the sympathetic and parasympathetic nervous systems. The ratio of LF to HF activity has been proposed as a marker of sympathovagal balance, with higher numbers indicating a relative dominance of the sympathetic nervous system (28). While there is controversy over what these parameters represent and which are most important for assessing CVD risk (29, 30), there is robust evidence that reduced HRV is associated with greater CVD morbidity (31-33). For example, even when controlling for age, race, gender, education, diabetes, hypertension, and beta-blocker use, individuals in the lowest quartile of HF power were 1.72 times more likely to develop incident coronary heart disease compared to individuals in the upper three quartiles (32). Additionally, several studies have reported that reductions in HRV at rest that persist during standardized laboratory stress tasks can predict increases in cardiovascular risk above and beyond traditional risk factors (34-36), indicating that evaluating autonomic tone during stress may be beneficial to risk assessment.

**Arterial Stiffness**

Arteries undergo structural and functional changes with advancing age that increase cardiovascular risk. Thickening of the arterial wall, increases in collagen, and breakdown of elastin promote arterial stiffness, particularly in the large elastic central arteries (37). These changes increase the velocity of the pulse wave as it travels through the arterial tree (38). When the pulse wave moves from the larger arteries to the smaller arterioles, a portion of the wave is reflected back towards the aorta. In the absence of arterial stiffness, the reflected wave reaches the heart during diastole, which maintains diastolic
blood pressure and enhances coronary perfusion (39). However, in the presence of arterial stiffness, the reflected wave reaches the heart during systole, and thereby increasing the central systolic blood pressure. A stiffened aorta is also less able to cushion the pulsations generated by the heart, which is how healthy arteries normally promote a continuous blood flow to the peripheral tissues between cardiac contractions via the Windkessel effect. Pulsatile perfusion of the microvasculature due to age-related arterial stiffness is associated with an increased risk of microhemorrhages, microinfarcts, and target organ damage (40).

The clinical gold standard for assessing large elastic central arterial stiffness is carotid-femoral pulse wave velocity (PWV) (41). This procedure is based on the principle that pulse waves travel through stiffer arteries faster than more compliant arteries (38). Waveforms at the carotid and femoral arteries are recorded simultaneously with mechano-transducers (42) or sequentially with applation tonometers (43) (less commonly used is an echotracking (Doppler) system, in which transit time is determined by tracking distention waves) (44). The transit distance (in meters) is measured from the suprasternal notch to the recording sites, and then divided by the transit time (in seconds) to yield PWV (m/s). This method has been widely applied in research settings, and has been shown to predict cardiovascular outcomes above and beyond traditional risk factors (45-48). A recent meta-analysis concluded that the relative risk for cardiovascular events and mortality was 1.90-2.26 for individuals in the top tertile of PWV (stiffer arteries) compared to the lowest tertile (less stiff arteries) (48).

Arterial stiffness can also be estimated by analyzing pressure waveforms. Again, as the pressure waveform travels from the heart through the vasculature, some of the forward pressure waveform is reflected back toward the heart. The difference between the first and second central systolic peaks, divided by the pulse pressure (the difference between systolic and diastolic blood pressures), is called the augmentation index (AI). Positive AI scores are indicative of increased arterial stiffness, whereas negative AI scores reflect a healthy, distensible vasculature. Pressure waveforms are typically recorded with strain gauge transducers or tonometers in carotid or radial arteries (41). The waveform at the carotid artery is more difficult to measure than the radial artery, because the radial artery is supported by bony tissue. However, when the radial artery is used, a transfer function must be applied to more accurately estimate the shape of the central waveform. Prospective studies of patients with end-stage renal disease (49) and patients
undergoing percutaneous coronary intervention (50) have provided evidence that both methods can be used to independently predict future events.

**Endothelial Function**

The endothelium is the single layer of cells that lines the interior of blood vessels. This dynamic barrier is involved in anti-thrombotic, anti-inflammatory, and vasodilatory activities that are vital to maintaining vascular health (51). Physical and chemical receptors on endothelial cells sense changes in blood flow and blood-derived signaling. These receptors stimulate the release of vasoactive molecules and growth factors that inhibit platelet aggregation, reduce expression of pro-inflammatory cytokines and adhesion molecules, and regulate vascular smooth muscle tone. Most notable of these molecules is nitric oxide, a powerful vasorelaxing substance that is synthesized from L-arginine by nitric oxide synthase (52). When nitric oxide is released by the endothelium, it causes relaxation of the underlying vascular smooth muscle cells and, hence, vessel dilation. Nitric oxide also plays a critical role in the initiation, development, and progression of atherosclerosis by inhibiting leukocyte adhesion and infiltration into the intima-media space, oxidation of low-density lipoprotein (LDL) cholesterol, and vascular smooth muscle cell migration (53, 54). Nitric oxide also reduces thrombosis by preventing platelet aggregation and clot formation (55). Reductions in nitric oxide bioavailability and function of the endothelium are early indicators of vascular decline and can be present years before clinically-evident disease (56).

The gold standard for non-invasive assessment of endothelial function is brachial flow-mediated dilation. Developed by Celermajer and colleagues in the early 1990s (56), this technique utilizes high-resolution ultrasonography to measure the diameter of a target artery before and after a shear-raising stimulus. A cuff is applied to the target conduit artery and inflated to above systolic pressure, which induces ischemia in the distal tissues supplied by that artery. After five minutes of occlusion, the cuff is released and reactive hyperemia ensues. Increased blood flow creates a shear stress stimulus through the conduit artery that induces mechanical deformation of structures on the endothelial cell membranes. This signal is transduced by the endothelial cell and stimulates production of vasodilating substances (e.g. nitric oxide, endothelial-derived hyperpolarizing factor, prostaglandin), which diffuse through the cell wall to the underlying vascular smooth muscle cells and cause vasodilation (41). The artery diameter during vasodilation is compared to the baseline artery diameter by calculating a
percent change (%FMD). Higher %FMD values are reflective of better endothelial function, and have been shown to predict cardiovascular morbidity and mortality above and beyond traditional risk factors (57-60).

A newer, alternative method of measuring endothelial function non-invasively is peripheral arterial tonometry (PAT). The EndoPAT device (Itamar, Israel) utilizes fingertip plethysmography to measure digital blood volume before and after a flow stimulus (61). Thimble-like probes are placed on one finger of each hand, and balloons inside the probes are inflated to just below diastolic pressure to prevent venous pooling. Metal tips inside the probes detect changes in blood volume. Similar to the FMD procedure, the EndoPAT procedure requires three distinct periods: baseline, distal artery cuff occlusion, and reactive hyperemia-induced vasodilation, with each period lasting 5 minutes. The ratio of digital blood volume during reactive hyperemia to baseline in the occluded arm is divided by the corresponding ratio in the control arm (to adjust for systemic effects). This provides a reactive hyperemia index (RHI), of which greater RHI is indicative of normal endothelial function, and lower PAT ratio has been reported in high risk and clinical populations (62-64).

Compared to FMD, PAT equipment is less expensive, the PAT procedure is more easily standardized between laboratories, and the data is instantly analyzed by the computer software. While both FMD and PAT have been associated with coronary artery vasodilation (64, 65) and CVD (57, 66) it is unclear whether the data derived from these techniques are equivocal. A small study comparing FMD and PAT obtained simultaneously in 89 adults complaining of chest pain reported a linear correlation of 0.55 between % FMD and PAT ratio (63). In contrast, data from 1843 participants in the Framingham Heart Study revealed no correlation between % FMD and PAT ratio obtained simultaneously (67). Differences in participant demographics, particularly in prevalence of CVD, may explain this discrepancy, but it is also possible that FMD and PAT are assessing distinct components of vascular health. Further research is needed to determine the relative importance of these two methods and their ability to identify changes in endothelial function.
Dietary Interventions for Cardiovascular Risk Factors

Lifestyle modification is the first line of therapy recommended for cardiovascular risk management, and there is substantial scientific evidence that dietary changes can improve multiple CVD risk factors.

Blood Pressure

The American College of Cardiology/American Heart Association Task Force on Practice Guidelines has recommended a dietary pattern rich in fruits, vegetables, whole grains, low-fat dairy, lean protein, and nuts and limited in sweets and red meats (68) for individuals who would benefit from lowering their blood pressure. The Dietary Approaches to Stop Hypertension (DASH) adheres to these recommendations and has been shown to reduce blood pressure. In a controlled-feeding setting where food is provided to individuals, this dietary pattern reduced blood pressure by 11.4/5.5 mmHg in hypertensive adults and by 3.5/2.1 mmHg in normotensive adults (69). In a free-living setting where individuals select their own foods, it is effective in reducing blood pressure by 6.3/3.6 mmHg in hypertensive adults and by 3.1/2.0 mmHg in normotensive adults (70). By limiting sodium intake to 1500 mg per day, blood pressure was reduced by an additional 3.0/1.6 mmHg (71). Finally, a meta-analysis of 15 trials evaluating the effect of alcohol consumption on blood pressure concluded that moderate consumption of alcohol (< 2 drinks/day for males and ≤ 1 drink/day for females) can reduce blood pressure by 3.3/2.0 mmHg (72).

Another dietary pattern that has been associated with reduced blood pressure is the Mediterranean Diet. This diet is rich in olive oil, fruits, and vegetables and low in red meat and saturated fat. In a cross-sectional analysis of over 20,000 adults in the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study, greater adherence to the Mediterranean Diet was inversely associated with blood pressure, with the strongest relationship evident for olive oil specifically (73). This study indicates that unsaturated fatty acids may be particularly important to blood pressure control. Individual foods that are rich in unsaturated fatty acids have also been shown to improve blood pressure control, with some evidence existing for nuts (8) and omega-3 fish oil (74). Other foods that may potentially improve blood pressure include chocolate/cocoa (75, 76) and dairy products (77).
Systemic Hemodynamics

Dietary patterns over short-term periods have been associated with changes in hemodynamics. West and colleagues have shown in two studies of adults with hypercholesterolemia that stroke volume, cardiac output, and total peripheral resistance at rest and during stress can be significantly altered by dietary modification. Diets containing walnuts (78), flax oil (78), or pistachios (8) significantly reduced total peripheral resistance at rest and during mental stress by approximately 4%, and the flax oil (78) and pistachio diets (8) also increased cardiac output by approximately 4-6%. Straznicky and colleagues reported that stress reactivity was significantly lower after two weeks on a low fat diet compared to a high saturated fat diet (79).

In addition to habitual diet, hemodynamics and reactivity can be affected by a single meal. Studies have demonstrated postprandial increases in resting heart rate, cardiac output, and stroke volume, and a decrease in total peripheral resistance (80-88). These postprandial shifts have minimal effect on the magnitude of stress responses when compared to fasting conditions (85), but meal content (relative amounts of fat, protein, and carbohydrates) may moderate postprandial stress reactivity. A high carbohydrate meal was reported to augment reactivity compared to a high protein meal (85), and greater reactivity was observed after a meal high in saturated fat compared to a meal with minimal saturated fat (89, 90).

Heart Rate Variability

Epidemiological studies suggest that several components of diet may be related to HRV. Three studies reported that individuals who consume relatively greater amounts of fish, particularly fatty fish rich in long-chain omega-3 fatty acids, have higher HRV (91-93). One study of almost 600 men in the Normative Aging Study reported that HRV was not related to fish intake but was positively correlated with consumption of dark green leafy vegetables (94). Finally, a study of 276 middle-aged male twins reported that greater adherence to the Mediterranean Diet was associated with elevations in HRV (95).

Intervention studies examining the relationship between diet and heart rate variability have predominantly focused on fish oil, with several studies providing evidence that EPA+DHA supplementation can improve or prevent a decline in HRV in individuals at increased risk of CVD (96-98) and those with established CVD (99-102). In overweight or obese adults without existing CVD, Ninio et al. observed an 18% increase in resting
HF-HRV after 12 weeks of EPA+DHA supplementation, reflecting enhanced parasympathetic modulation of heart rate (96). Two studies assessing EPA+DHA supplementation in post-myocardial infarction patients reported improvements in HRV due to increases in vagally-mediated HRV (measured by HF-HRV (99) and the standard deviation of all normal R-R intervals (100)). Conversely, Hamaad et al. reported no change in HRV after 12 weeks of supplementation in post-myocardial infarction patients (103). It is possible that EPA+DHA supplementation occurred at too low of a dose (0.8 g/day) to have an effect in this study, or HRV may have already stabilized post-myocardial infarction due to standard therapies that also affect autonomic activity. In general, studies including healthy populations did not find a beneficial effect of EPA+DHA supplementation on HRV (104-106), indicating that EPA+DHA supplementation may be most beneficial in individuals with cardiac impairment.

Arterial Stiffness
Epidemiological studies have provided some evidence that arterial stiffness is related to diet. Dietary patterns that are associated with arterial stiffness include diets rich in meat intake and high alcohol consumption (107, 108), whereas diets with moderate alcohol consumption (109-112), low sodium intake (113), greater fruit and vegetable consumption (114), and increasing dairy intake (115) have been associated with less arterial stiffness. Greater adherence to the Mediterranean Diet has also been associated with reduced arterial stiffness in both adults and children (116, 117).

Intervention studies provide limited evidence that arterial stiffness can be reduced by dietary modification. Sodium-restricted diets have been shown to reduce carotid-femoral pulse wave velocity in hypertensive adults (118-120). Cranberry juice (500 ml/day) reduced carotid-femoral pulse wave velocity by 4% in adults with coronary artery disease, but had no effect in healthy men (121). Other dietary patterns and foods that have been shown to have no effect on arterial stiffness include diets rich in saturated fat (122), walnuts (123), and tomatoes (124).

Endothelial Function
There is strong evidence from nutrition research that endothelial function can be altered by individual foods and overall dietary patterns. To date, only one large epidemiological study has examined the cross-sectional correlation between diet and flow-mediated dilation. The Multi-Ethnic Study of Atherosclerosis (MESA) study conducted FMDs in
over 3000 adults and found that among women (but not men), regular fish intake was positively associated with FMD (125).

In clinical intervention trials, the most commonly studied individual food are those rich in antioxidants. Cocoa and chocolate (46-100g/day) has been shown to improve FMD by 15-78% in up to 4 weeks in adults who are healthy (126, 127), hypertensive (127, 128), have type 2 diabetes (129), or smoke (130), but had no effect in overweight adults (131) or those with coronary artery disease (132). Grape juice (500-640 ml/day) (133-136), pomegranate juice (240 ml/day) (136), and red wine (250 ml/day)(135, 137) are reported to improve FMD by 12-190% in adults with coronary artery disease or hypercholesterolemia and adolescents with the metabolic syndrome, although cranberry juice (480 ml/day) had no effect in adults with coronary artery disease (138). Black tea (900-1250 ml/day) improved FMD by 60 and 41% in adults with dyslipidemia (139) and coronary artery disease (140), respectively. Walnuts (18% daily energy)(141) and hazelnuts (18-20% of daily energy)(142) improved FMD by 56-73% in adults with dyslipidemia, while pistachios (10-20% of daily energy) improved FMD by 43% in young healthy males but had no effect in adults with dyslipidemia (8).

Nutrition studies using EndoPAT to assess changes in endothelial function have been less promising. One study of flavanol-rich cocoa supplementation in healthy adults demonstrated an improvement in RHI after just 5 days (143), but a second study by the same group in a similar sample showed no effect (144). Studies that examined mixed nuts (30 g/day) in adults with the metabolic syndrome (145) and cranberry juice in adults with coronary artery disease (480 ml/day)(138) also reported no vascular effects. Given that these foods (except for cranberry juice) have demonstrated vascular benefits measured by FMD, these null findings indicate that FMD and EndoPAT could be measuring distinct components of vascular health.

Among the studies of dietary patterns, the most common pattern is the Mediterranean diet and similar diets described as rich in olive oil-based monounsaturated fatty acids. Controlled-feeding studies have examined the vascular effects of these diets in adults with metabolic syndrome (146), hypercholesterolemic adults (147), young healthy adults (148-151), and older adults (152). Most reported relative improvements of vascular health when compared to the control condition (Step 1 diet (147), high saturated fat diets (146, 148-150, 152)). Only Ambring and colleagues reported no benefit of
Mediterranean diet compared to a high saturated fat diet on vascular health (measured by venous occlusion plethysmography) in young healthy adults (151). In free-living trials, advice to follow the Mediterranean diet or a diet rich in monounsaturated fatty acids has been shown to improve vascular health for up to 24 months relative to control groups. These studies have been conducted in samples with type 2 diabetes (control: diet high in linoleic acid) (153), ischemic heart disease (control: no dietary advice) (154), the metabolic syndrome (control: prudent diet) (155), erectile dysfunction (control: prudent diet) (156), and healthy older adults (control: no dietary advice) (157). In contrast, no relative effect of a Mediterranean-style or high-monounsaturated fat dietary advice was reported in overweight/obese females (control: Atkins diet) (158) or healthy adults (control: low fat diet) (159) after 1-2 months. While there is great variation in the study populations, the control diet used, and the degree of improvement observed, these studies collectively present strong evidence that the Mediterranean diet (or high intake of monounsaturated fatty acids) improves vascular health.

A few studies have evaluated the vascular effects of the National Cholesterol Education Program’s Step 1 diet (now known as the Therapeutic Lifestyle Changes diet), which contains <7% saturated fat and <200 mg of cholesterol (160). In two samples of CAD adults, this diet was demonstrated to improve FMD by 19-32% after 12-14 weeks; however, neither study included a control condition (161, 162). This diet also had no effect on FMD adults with high cholesterol compared to a diet rich in saturated fat (147, 163). A low-fat dietary pattern has been shown to improve FMD by 34% in obese adults (relative to a low carbohydrate diet) after 6 weeks, whereas the low carbohydrate diet was shown to reduce FMD by 14% (164). Finally, West et al. reported a 34% improvement in FMD in hypercholesterolemic adults following 6-weeks of a diet high in alpha-linoleic acid (a polyunsaturated fatty acid found in walnuts) compared to an average Western diet relatively higher in saturated fat (78). Collectively, these studies provide strong evidence that diets rich in monounsaturated fat (such as the Mediterranean diet) or polyunsaturated fat can improve vascular health in a variety of populations. The evidence supporting the vascular benefits of low saturated fat diets (such as the Step 1 diet) or diets low in total fat is less conclusive and requires further research.
CHAPTER 3. PISTACHIO CONSUMPTION MODIFIES SYSTEMIC HEMODYNAMICS, INCREASES HEART RATE VARIABILITY, AND REDUCES AMBULATORY BLOOD PRESSURE IN TYPE 2 DIABETES

Abstract

Background – Managing cardiovascular risk factors is important for reducing vascular complications in type 2 diabetes, even in individuals who have achieved glycemic control. Regular nut consumption is associated with reduced cardiovascular risk in observational and controlled clinical studies, an effect often attributed to lipid-lowering effects. There is mixed evidence, however, about the role of nuts in modulating blood pressure, and limited research on the underlying hemodynamics that mediate blood pressure. This study assessed the effect of pistachio consumption on blood pressure, systemic hemodynamics, heart rate variability, and endothelial function in adults with well-controlled type 2 diabetes.

Methods – We enrolled 30 adults (40-74 years) with type 2 diabetes in a randomized, crossover, controlled feeding study. After a 2-week run-in period, participants consumed a low-fat control diet (27% fat) and a moderate-fat diet (33% fat) containing pistachios (20% of total energy) for 4 weeks each, separated by a 2-week washout. Following each diet period, we assessed blood pressure, systemic hemodynamics, and heart rate variability at rest and during acute mental stress. Endothelial function was assessed via brachial flow-mediated dilation and peripheral arterial tonometry, and a subset of participants (n=21) completed 24-hour ambulatory blood pressure monitoring.

Results – Blood pressure at rest and during mental stress did not differ between treatments. The pistachio diet significantly reduced total peripheral resistance (-3.7%±2.9%, P=0.004) and increased cardiac output (3.1%±2.3%, P=0.002). Heart rate variability was significantly improved following the pistachio diet (P<0.05). Systolic ambulatory blood pressure was significantly reduced by 3.5±2.2 mmHg (P=0.046) following the pistachio diet, with the greatest reduction observed during sleep (-5.7±2.6 mmHg, P=0.052). Measures of endothelial function were unchanged.
**Conclusions** – Daily pistachio consumption reduces total peripheral resistance, improves heart rate variability, and lowers systolic ambulatory blood pressure in adults with type 2 diabetes.
Introduction

With the global burden of diabetes continuing to grow, the resulting cardiovascular morbidity in this population is threatening to reverse the downward trend in cardiovascular mortality observed in recent years (165, 166). Despite successful treatment of modifiable risk factors, individuals with type 2 diabetes retain residual risk of vascular complications. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (167), the Action in Diabetes and Vascular Disease (ADVANCE) trial (168), and the Veterans’ Administration Diabetes Trial (VADT) (169) all reported that improved glucose control does not significantly lower risk of major cardiovascular events. Additionally, the Action for Health in Diabetes (Look AHEAD) trial recently reported that the risk of CVD remains elevated even in individuals with type 2 diabetes that lose weight and see an improvement in metabolic risk factors (170). Dietary modification has long been the first line of therapy recommended for risk management (171), and much recent attention has focused on the residual risk that remains after successful pharmacological treatment of metabolic risk factors (2). In a 2013 Position Statement (172), the American Diabetes Association recommends five different eating patterns for the management of diabetes: Mediterranean style, vegetarian and vegan, low fat, low carbohydrate, and Dietary Approaches to Stop Hypertension. All of these eating patterns except low fat include nuts, but there is a notable absence of rigorous randomized controlled trials testing the effects of nut consumption in type 2 diabetes, particularly in regards to blood pressure.

Several epidemiological studies have shown that regular nut consumption lowers blood pressure and the risk of hypertension (173-175). Approximately two dozen clinical trials assessed the effect of nuts on blood pressure (see Casas-Agustench et al. (7) for review), and the majority reported non-significant changes in resting blood pressure over 3-24 weeks. We have previously shown that, in adults with hypercholesterolemia, consumption of 10% of daily energy from pistachios (approximately 1.5 ounces/day for most adults) reduced systolic blood pressure at rest and during acute mental stress by an average of 4.8 mmHg (8). We also observed significant shifts in systemic hemodynamics, including a dose-dependent reduction in total peripheral resistance.

Among individuals with type 2 diabetes, management of blood pressure is key to reducing vascular complications (176). Thus, the purpose of the present study was to
evaluate the effects of pistachio consumption on blood pressure and systemic hemodynamics in adults with well-controlled type 2 diabetes. We selected this population as proof-of-concept that dietary changes can provide additional health benefits, even in adults who are early in the progression of metabolic disease. We hypothesized that daily pistachio consumption (20% of total energy) for 4 weeks would reduce total peripheral resistance at rest and during acute stress. Secondary analyses assessed the effect of pistachio consumption on heart rate variability, endothelial function, and 24-hour ambulatory blood pressure.

Methods

Participants
Recruitment began in July 2009 and continued through November 2012. Adults with well-controlled type 2 diabetes (defined below) were recruited through campus and community advertisements (Figure 3.1). Interested individuals completed a phone interview during which trained research assistants assessed eligibility. Participants were required to be between the ages of 30-75 (women had to be post-menopausal) with a body mass index (BMI, kg/m$^2$) between 18.5-45.0 and managing their blood glucose by: a) diet and exercise alone; or b) any diabetes medication(s) except insulin. This study was specifically designed to include individuals who were early in the progression of diabetes and had achieved moderate glycemic control (defined as glycated hemoglobin < 7.4%). Initial exclusion criteria included self-reported history of chronic disease other than type 2 diabetes (including cardiovascular disease and diabetes complications such as retinopathy and neuropathy), history of bariatric surgery, major surgery in the prior six months, nut or latex allergies, use of tobacco, use of daily aspirin, anti-inflammatory medications, oral steroids, hormone replacement therapy, or medication for hypertension. Individuals meeting the phone interview criteria completed a clinic screening visit that included blood pressure measurement, an electrocardiogram (ECG), and assessment of lipids, glucose, and inflammation. Individuals with blood pressure $\geq$ 160/100 mmHg, an abnormal ECG, fasting triglycerides $\geq$ 5.65 mmol/L, or glycated hemoglobin $\geq$ 7.4% were excluded. Participants were instructed to discontinue all dietary supplements 2 weeks prior to study enrollment, except for omega-3 supplements which were discontinued 8 weeks prior.
Figure 3.1. CONSORT diagram of recruitment and study completion.

Responded to advertisement (n=970)

- Ineligible after phone interview (n=893)

Completed clinic screening visit (n=77)

- Ineligible (n=36) due to:
  - Glycated hemoglobin ≥ 7.4% (57 mmol/mol) (n=11)
  - Blood pressure ≥ 160/100 mmHg (n=4)
  - Body mass index ≥ 45 (n=1)
  - C-reactive protein ≥ 10 mg/l (n=2)
  - Abnormal ECG (n=4)
  - Medication change (n=3)
  - No longer interested (n=5)
  - Other (n=6)

Began run-in period (n=41)

- Withdrew during run-in period (n=7) due to:
  - Elevated blood pressure (n=2)
  - Medication change (n=1)
  - No longer interested (n=4)

Randomized (n=34)

- Withdrew during diet periods (n=4) due to:
  - Developed food intolerance (n=3)*
  - Pre-existing medical condition revealed (n=1)

Completed full protocol (n=30)

*Of these, 2 developed intolerances to tomatoes and 1 experienced an allergic reaction to pistachios. It was confirmed that this participant reported no history of nut allergies prior to study enrollment, but during questioning that followed the allergic reaction, this participant stated he had not previously eaten pistachios.
In January 2010, due to low enrollment, the criteria were revised to allow individuals taking a single drug for hypertension to participate when a) their primary care physician gave written permission for them to discontinue drug monotherapy during the study, and b) seated, resting blood pressure remained below 160/100 mmHg for the duration of the study. In November 2010, after discovery of extreme inflammation in a participant (who, in the interest of safety, was withdrawn from the study and referred for follow-up care; Figure 3.1), the criteria were revised to exclude individuals with C-reactive protein (CRP) \( \geq 10.0 \text{ mg/l} \).

Thirty participants (50% female) completed the full study, and their baseline characteristics are displayed in Table 3.1. Written informed consent was obtained from all participants, and approval for the study was granted by the Institutional Review Board of The Pennsylvania State University. All data was collected at the Clinical Research Center at The Pennsylvania State University between July 2009 and March 2013. This study was registered at ClinicalTrials.gov (NCT00956735).

**Intervention**

The study employed a 2-period, randomized, crossover, controlled-feeding design. A 2-week run-in period preceded the first test diet to establish baseline values for a typical

<table>
<thead>
<tr>
<th>% Female</th>
<th>50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>56.1 ± 1.4</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>31.2 ± 1.1</td>
</tr>
<tr>
<td>Resting systolic blood pressure mmHg</td>
<td>116.2 ± 2.5</td>
</tr>
<tr>
<td>Resting diastolic blood pressure mmHg</td>
<td>71.0 ± 0.9</td>
</tr>
<tr>
<td>Fasting glucose mmol/L</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>Glycated hemoglobin %</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol mmol/L</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>LDL cholesterol mmol/L</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>HDL cholesterol mmol/L</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides mmol/L</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>% Taking diabetes medication(s)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>16.7</td>
</tr>
<tr>
<td>One*</td>
<td>66.7</td>
</tr>
<tr>
<td>Two or more</td>
<td>16.7</td>
</tr>
<tr>
<td>% Taking statin therapy</td>
<td>43.3</td>
</tr>
</tbody>
</table>

*Of these, 90% were on metformin only.
Western diet. Test diets were presented in a counterbalanced order for 4 weeks each, with order determined by simple randomization (www.randomization.com) and equal allocation to each treatment order assignment. The study coordinator (KAS) generated the random allocation sequence and assigned participants to the interventions. Due to the nature of the dietary intervention, metabolic kitchen staff and participants were aware of treatment assignments; however, technicians who measured outcome variables were blinded to the diet assignments. At the end of each diet period (including the run-in period), participants underwent testing to assess blood pressure, cardiovascular reactivity to acute mental stress, heart rate variability, and 24-hour ambulatory blood pressure. Short compliance breaks (average of 2 weeks) separated the diet periods.

The nutrient composition of the study diets is presented in Table 3.2. Participants were assigned to calorie levels (range: 1800-3900 kcal/day) according to the Harris-Benedict equation (177), and adjustments were made as needed for weight stability. The run-in diet was a nutritionally-adequate diet with total fat based on a typical Western diet (36% total fat, 11.5% saturated fat, and 278 mg/day of cholesterol). The control diet was designed in accordance with the National Cholesterol Education Program’s Therapeutic Lifestyle Changes diet (178), with reduced total fat (26.9% of energy), saturated fat (6.7% of energy) and cholesterol (186 mg/day). The pistachio diet was similar to the control diet with regard to saturated fat (6.8% of energy) and cholesterol (172 mg/day), but included pistachios equivalent to 20% of daily energy (range: 59-128 grams). The pistachios replaced low-fat or no-fat carbohydrate snacks (i.e. pretzels, string cheese,

<table>
<thead>
<tr>
<th>Table 3.2. Nutrient composition of the study diets*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run-In</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Energy kcal</td>
</tr>
<tr>
<td>Total fat g (%)</td>
</tr>
<tr>
<td>Saturated fat g (%)</td>
</tr>
<tr>
<td>Monounsaturated fat g (%)</td>
</tr>
<tr>
<td>Polyunsaturated fat g (%)</td>
</tr>
<tr>
<td>Protein g (%)</td>
</tr>
<tr>
<td>Carbohydrate g (%)</td>
</tr>
<tr>
<td>Fiber g</td>
</tr>
<tr>
<td>Sodium mg</td>
</tr>
<tr>
<td>Potassium mg</td>
</tr>
<tr>
<td>Cholesterol mg</td>
</tr>
</tbody>
</table>

*Values calculated using Nutrient Data System for Research (NDSR), 2009.
etc.) in the control diet, and resulted in a total fat content of the pistachio diet of 33.2% of total energy. All meals and snacks were prepared in the Metabolic Kitchen at the Pennsylvania State University Clinical Research Center. For 3-5 days each week (depending on travel distance), participants ate one meal per day in the Metabolic Kitchen and had the other meals prepared and packed for off-site consumption. Daily compliance questionnaires indicated that adherence to the diets was very good.

Assessments

**Systemic hemodynamics.** At the end of each diet period, systemic hemodynamics were assessed in a seated position during a 20-minute rest period, two stress tasks, and two 10-minute recovery periods. Participants listened to classical music during the rest and recovery periods. The first stress task was the Paced Auditory Serial Addition Task (PASAT), during which participants were instructed to add together the last two numbers played from an audio recording and state the sum aloud (179). This task lasted for 5 minutes, and the speed at which the numbers were played increased at each testing session to account for habituation effects. The second stress task was the hand cold pressor, during which participants placed their right hand into 4°C water for 2.5 minutes. Each task was followed by a 10-minute recovery period. Throughout these five tasks (baseline rest, math, recovery, cold pressor, recovery), systolic and diastolic blood pressures (SBP, DBP) were obtained at 1-2-min intervals (depending on task) with an automatic oscillometric blood pressure monitor attached to the left arm (Dinamap, Critikon Pro 100, GE Medical Systems). Impedance cardiography utilizing a tetrapolar band configuration with spot ECG electrodes was used to estimate heart rate and stroke volume (Hutcheson Impedance Cardiograph and the Cardiac Output Program, Bio-Impedance Technology, Inc., Chapel Hill, NC) at the same time as the blood pressure assessments. Cardiac output and total peripheral resistance were calculated according to standard formulae (20). To control for the acute effect of eating on systemic hemodynamics (180), participants consumed a standard snack (10% of daily calories) that matched the macronutrient content of the corresponding treatment diet 2 hours prior to the cardiovascular reactivity testing. During the pistachio treatment period, this snack included 6.4 – 9.6 grams of pistachios (depending on calorie assignment).

**Heart rate variability.** Heart rate variability was assessed in the resting state and during two acute stress tasks (mental arithmetic and hand cold pressor, described above) at the end of each diet period. Three electrocardiogram electrodes were placed according to
guidelines for impedance cardiography (20). The electrocardiogram was used to obtain raw interbeat intervals (R-R) recorded at a frequency of 1,000 Hz. The R-R interval sequences were visually inspected, and the data considered artifactual was manually replaced by interpolated or extrapolated data. The square root of the mean squared differences of successive R-R intervals (RMSSD, ms) was calculated using commercial software (Nevrokard, Medistar Inc.). Frequency-domain measures of HRV (using autoregressive spectra) were calculated using standard methods (181). Oscillations in the high frequency band (0.15 Hz to 0.4 Hz) are thought to reflect vagal modulation of heart rate, while oscillations occurring in the low frequency band (0.05 Hz to 0.15 Hz) may reflect a complex interplay between sympathetic and parasympathetic modulation of heart rate as well as baroreceptor activity (27). The HRV variables examined in the frequency domain include high frequency (ms$^2$), low frequency (ms$^2$), the ratio of LF-HRV to HF-HRV (LF:HF), and total power (ms$^2$).

*Flow-mediated dilation.* The brachial artery above the elbow was scanned in a longitudinal section and continuous, cross-sectional images were recorded during baseline, occlusion, and post-deflation. Changes in arterial diameter were measured by external B-mode ultrasound imaging (Acuson Aspen equipped with a 10-mHz linear array transducer; Acuson, Mountain View, CA) by a single, well-trained sonographer (P Wagner). The images were gated by using R-wave detection so that the scans were assessed at end diastole. Automated edge detection software (Brachial Analyzer; MIA, Iowa City, IA) was used to quantify artery diameter continuously throughout the test. Resting diameters were the average of all images collected over 1-minute of the baseline recording. Peak artery diameter was determined as the largest diameter recorded in the first 2 minutes of the post-deflation period. Percent change in brachial diameter at peak dilation compared to baseline (%FMD) was calculated by 2 independent scorers, and average values are presented. If %FMD values differed by >2%, a third technician reviewed the scan.

Average flow velocity (m/s) across the cardiac cycle, maximal flow velocity, and velocity time integral across the cardiac cycle (m) were measured by using duplex pulsed Doppler during the resting baseline and immediately after cuff release. Flow (ml/min) was calculated using the following equation: velocity time integral × cross-sectional area of the vessel × heart rate.
Peripheral arterial tonometry. Relative changes in digital pulse wave amplitude before and after occlusion was assessed with the EndoPAT 2000 (Itamar Medical Ltd, Caesarea, Israel). Two flexible probes were placed on the index fingers of the right (occluded) and left (control) hands. The reactive hyperemia index (RHI) was calculated as the ratio of the average pulse wave amplitude during hyperemia (60 to 120 seconds of the post-deflation period) to the average pulse wave amplitude during baseline in the occluded hand divided by the same values provided by the control hand, and then multiplied by a baseline correction factor. The Framingham RHI (F-RHI) is an alternative calculation that uses the period of 90 to 120 seconds of the post-deflation period, does not incorporate a baseline correction factor, and has a natural log transformation applied to the resulting ratio. Both RHI and F-RHI have been shown to correlate with cardiovascular risk factors (62). Vascular stiffness was indexed by augmentation index (AI), and calculated from the shape of the pulse wave recorded by the probe during baseline. This measure is typically standardized to a heart rate of 75 beats/min (AI-75) to correct for the independent effect of heart rate. Both unadjusted and adjusted indices are reported.

Ambulatory blood pressure monitoring. At study enrollment, participants were invited to participate in an optional sub-study of ambulatory blood pressure. Twenty-one participants agreed, and there was no significant difference in resting blood pressure between individuals who accepted and declined participation in this sub-study. Near the end of each diet period, but while still on the relevant diet, participants were fitted with an ambulatory blood pressure monitor (model 90207, Spacelabs Healthcare, Snoqualmie, WA) and brachial cuff that was worn for an average of 21.3±0.16 hours. Automated readings were taken every 20 minutes during the day (6 am – 10 pm) and every 30 minutes overnight (10 pm – 6 am). To test differences between daytime and nighttime blood pressure, wake and sleep times were conservatively estimated to be 10 am – 9 pm (wake) and 1 am – 5:30 am (sleep) (182). The average number of successful readings obtained in a 24-hour period was 50±2, with 26±1 wake readings and 8±1 sleep readings.

Statistical Analyses
This study was designed with 80% power and alpha set at 0.05 to detect significant between-treatment differences in total peripheral resistance of 4%, based on our previous study (8). Treatment effects were tested per protocol using the mixed models
procedure in SAS (v9.3, Cary, NC). Differences in baseline characteristics according to treatment order assignment were examined for all variables and uniformly non-significant (data not shown). For the outcome analyses, diet, diet period (first or second), and (for relevant analyses) task (rest, math, recovery 1, cold pressor, recovery 2), and their interactions were entered as fixed effects; subject was a random effect. Diet by task and diet by period interactions were uniformly non-significant (no evidence of carryover). All analyses were adjusted for age, sex, BMI, and the baseline (run-in) value. Alpha < 0.05 was considered statistically significant, and Tukey tests were used to adjust for multiple comparisons. Tables and figures reflect means ± standard errors.

Results

Blood pressure and systemic hemodynamics at rest and during acute mental stress are presented in Table 3.3. Regardless of treatment, systolic and diastolic blood pressure, heart rate, cardiac output, and total peripheral resistance increased during the stress tasks (all $P<0.0001$). There were no differences between the treatments in resting measurements for any variables. However, when data from the rest period, stress tasks, and recovery periods were averaged and analyzed using a repeated-measures analysis, stroke volume and cardiac output were significantly greater following the pistachio diet (+3.1±2.5%, $P=0.011$ and +3.1±2.3%, $P=0.002$) than the control diet. In contrast, total peripheral resistance was significantly lower following the pistachio diet than the control diet (-3.7±2.9%, $P=0.004$).

Mean heart rate variability at rest and during the acute stress tasks are shown in Table 3.4. Both RMSSD and high frequency power, which reflect parasympathetic activity, were significantly higher following the pistachio diet (+13.7±4.8%, $P=0.028$ and +24.4±10.6%, $P=0.007$). Low frequency power, which reflects both sympathetic and parasympathetic activity, was also greater following the pistachio diet (+19.8±10.6%, $P=0.041$). There was no difference between the treatments in total power or the ratio of low to high frequency power.

Measures of endothelial function are displayed in Table 3.5. There was no significant difference between the treatments in any variables. There was a trend for a significant difference ($p=0.076$) in brachial baseline diameter, with a larger diameter observed after the pistachio diet than the control diet (4.56 mm vs 4.51 mm).
Table 3.3. Systemic hemodynamics at rest and during acute mental stress

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Baseline</th>
<th>Control</th>
<th>Pistachio</th>
<th>Condition $P^*$</th>
<th>Treatment $P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic BP mmHg</strong></td>
<td>Rest</td>
<td>116.2 ± 2.5</td>
<td>112.1 ± 2.2</td>
<td>112.3 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Math</td>
<td>135.0 ± 4.1</td>
<td>127.8 ± 3.9</td>
<td>128.0 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>118.5 ± 2.6</td>
<td>114.6 ± 2.4</td>
<td>114.7 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>146.7 ± 4.2</td>
<td>139.6 ± 4.1</td>
<td>139.9 ± 4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>119.8 ± 2.8</td>
<td>117.6 ± 2.5</td>
<td>115.7 ± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>127.0 ± 1.8</td>
<td>122.1 ± 1.6</td>
<td>121.9 ± 1.5</td>
<td>0.0001</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Diastolic BP mmHg</strong></td>
<td>Rest</td>
<td>71.0 ± 0.9</td>
<td>69.2 ± 0.9</td>
<td>69.2 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Math</td>
<td>78.9 ± 1.5</td>
<td>76.2 ± 1.4</td>
<td>75.9 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>70.3 ± 1.0</td>
<td>70.1 ± 0.9</td>
<td>69.2 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>83.6 ± 1.6</td>
<td>82.0 ± 1.5</td>
<td>81.4 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>71.7 ± 1.3</td>
<td>70.0 ± 1.0</td>
<td>69.8 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>75.0 ± 0.7</td>
<td>73.4 ± 0.7</td>
<td>73.0 ± 0.6</td>
<td>0.0001</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Heart rate bpm</strong></td>
<td>Rest</td>
<td>63.8 ± 1.1</td>
<td>64.7 ± 1.4</td>
<td>64.9 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Math</td>
<td>72.0 ± 1.4</td>
<td>71.9 ± 1.5</td>
<td>71.5 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>64.4 ± 1.2</td>
<td>65.5 ± 1.4</td>
<td>65.7 ± 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>71.1 ± 1.3</td>
<td>70.1 ± 1.6</td>
<td>71.0 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>64.7 ± 1.2</td>
<td>64.3 ± 1.3</td>
<td>64.9 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>67.2 ± 0.6</td>
<td>67.3 ± 0.7</td>
<td>67.6 ± 0.7</td>
<td>0.0001</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Stroke volume ml/beat</strong></td>
<td>Rest</td>
<td>68.3 ± 3.1</td>
<td>66.1 ± 3.5</td>
<td>67.9 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Math</td>
<td>69.3 ± 3.9</td>
<td>65.5 ± 4.3</td>
<td>68.5 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>67.3 ± 3.1</td>
<td>67.4 ± 3.8</td>
<td>68.4 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>68.0 ± 3.8</td>
<td>64.6 ± 3.6</td>
<td>67.9 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>66.2 ± 3.6</td>
<td>68.2 ± 3.8</td>
<td>69.4 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>67.8 ± 1.6</td>
<td>66.4 ± 1.7</td>
<td>68.4 ± 1.6</td>
<td>0.17</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>Math</td>
<td>Recovery</td>
<td>Cold</td>
<td>Recovery</td>
<td>Average</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Cardiac output l/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>4.36 ± 0.20</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Math</td>
<td>5.01 ± 0.30</td>
<td>4.7 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>4.32 ± 0.20</td>
<td>4.4 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>4.82 ± 0.27</td>
<td>4.5 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>4.28 ± 0.23</td>
<td>4.3 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>4.55 ± 0.11</td>
<td>4.4 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total peripheral resistance dyne-sec/cm²</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>1695 ± 105</td>
<td>1695 ± 107</td>
<td>1641 ± 97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Math</td>
<td>1694 ± 113</td>
<td>1738 ± 125</td>
<td>1691 ± 111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>1702 ± 102</td>
<td>1665 ± 98</td>
<td>1618 ± 94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>1918 ± 157</td>
<td>1965 ± 148</td>
<td>1853 ± 158</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>1789 ± 127</td>
<td>1680 ± 91</td>
<td>1622 ± 91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>1758 ± 54</td>
<td>1746 ± 51</td>
<td>1682 ± 50</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± standard error, n=30. BP, blood pressure.

*Statistical significance assessed by PROC MIXED in SAS, after adjustment for age, sex, BMI, and baseline (run-in) values. The condition effect tested differences in hemodynamics between resting and stress conditions. The treatment effect tested differences in hemodynamics between the Control and Pistachio diets.
Table 3.4. Heart rate variability

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Control</th>
<th>Pistachio</th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSSD ms</td>
<td>24.9 ± 1.1</td>
<td>23.9 ± 1.1</td>
<td>27.2 ± 1.3</td>
<td>0.028</td>
</tr>
<tr>
<td>High Frequency ms²</td>
<td>273.5 ± 24.4</td>
<td>253.3 ± 25.1</td>
<td>315.1 ± 28.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Low Frequency ms²</td>
<td>494.9 ± 43.1</td>
<td>480.0 ± 43.8</td>
<td>575.0 ± 58.4</td>
<td>0.041</td>
</tr>
<tr>
<td>Low:High Frequency ms²</td>
<td>2.7 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>0.59</td>
</tr>
<tr>
<td>Total Power ms²</td>
<td>1256.0 ± 87.3</td>
<td>1236.3 ± 116.8</td>
<td>1401.0 ± 115.4</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Data are mean ± standard error from the rest, stress tasks, and recovery periods, n=30. RMSSD, square root of the mean squared differences of successive R-R intervals.

*Statistical significance for comparison between Control and Pistachio diets by PROC MIXED in SAS, after adjustment for age, sex, BMI, and baseline (run-in) values.
Table 3.5. Endothelial function

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Control</th>
<th>Pistachio</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated dilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter <strong>mm</strong></td>
<td>4.49 ± 0.15</td>
<td>4.50 ± 0.15</td>
<td>4.48 ± 0.17</td>
<td>0.076</td>
</tr>
<tr>
<td>Peak diameter <strong>mm</strong></td>
<td>4.72 ± 0.15</td>
<td>4.74 ± 0.16</td>
<td>4.69 ± 0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>Flow-mediated dilation %</td>
<td>5.28 ± 0.41</td>
<td>5.46 ± 0.50</td>
<td>4.95 ± 0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>Baseline flow velocity <strong>ml/min</strong></td>
<td>147.5 ± 9.1</td>
<td>158.8 ± 14.3</td>
<td>153.4 ± 12.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Peak flow velocity <strong>ml/min</strong></td>
<td>898.8 ± 40.9</td>
<td>926.9 ± 48.3</td>
<td>875.5 ± 49.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Peripheral arterial tonometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive hyperemia index</td>
<td>2.27 ± 0.12</td>
<td>2.31 ± 0.12</td>
<td>2.27 ± 0.09</td>
<td>0.66</td>
</tr>
<tr>
<td>Framingham reactive hyperemia index</td>
<td>0.64 ± 0.08</td>
<td>0.60 ± 0.07</td>
<td>0.61 ± 0.07</td>
<td>0.94</td>
</tr>
<tr>
<td>Augmentation index</td>
<td>17.21 ± 3.60</td>
<td>13.32 ± 3.17</td>
<td>12.83 ± 3.24</td>
<td>0.76</td>
</tr>
<tr>
<td>Augmentation index at 75 bpm</td>
<td>9.84 ± 3.51</td>
<td>5.59 ± 3.00</td>
<td>5.07 ± 3.17</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Data are mean ± standard error. Flow-mediated dilation is calculated as: (peak − baseline)/baseline x 100. Reactive hyperemia is an indicator of endothelial function (higher numbers indicate better function), and augmentation index is an indicator of arterial stiffness (higher numbers indicate greater stiffness).

*Statistical significance for comparison between Control and Pistachio diets by PROC MIXED in SAS, after adjustment for age, sex, BMI, and baseline (run-in) values.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Period*</th>
<th>Baseline</th>
<th>Control</th>
<th>Pistachio</th>
<th>( P^t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP <em>mmHg</em></td>
<td>24-hour</td>
<td>115.8 ± 2.0</td>
<td>117.3 ± 2.3</td>
<td>113.8 ± 2.0</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Wake</td>
<td>119.1 ± 2.3</td>
<td>122.4 ± 2.8</td>
<td>119.4 ± 2.3</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Sleep</td>
<td>107.0 ± 2.8</td>
<td>106.7 ± 2.9</td>
<td>101.1 ± 2.4</td>
<td>0.052</td>
</tr>
<tr>
<td>Diastolic BP <em>mmHg</em></td>
<td>24-hour</td>
<td>70.0 ± 1.2</td>
<td>71.2 ± 1.2</td>
<td>69.2 ± 1.2</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Wake</td>
<td>73.0 ± 1.5</td>
<td>74.4 ± 1.9</td>
<td>73.5 ± 1.7</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Sleep</td>
<td>62.3 ± 1.7</td>
<td>63.0 ± 1.6</td>
<td>58.8 ± 1.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Data are mean ± standard error, \( n=21 \). BP, blood pressure.

*24-hour includes all readings; wake includes readings from 10 am – 9 pm; sleep includes readings from 1 am – 5:30 am.

\(^t\)Statistical significance for comparison between Control and Pistachio diets by PROC MIXED in SAS, after adjustment for age, sex, BMI, and baseline (run-in) values.
Twenty-four-hour systolic blood pressure was significantly lower following the pistachio diet compared to the control diet (-3.0±1.8%, $P=0.046$; Table 3.6), with the largest difference occurring during sleep (-5.3±2.5%, $P=0.052$). Diastolic blood pressure did not differ between treatments, and there were no differences in dipping status (defined as ≥ 10% reduction in systolic blood pressure during sleep) between the treatments (data not shown).

**Discussion**

In this randomized, crossover, controlled-feeding study, we have shown that consuming 20% of total energy from pistachios for 4 weeks modifies systemic hemodynamics, increases heart rate variability, and reduces 24-hour systolic ambulatory blood pressure in adults with well-controlled type 2 diabetes. Our goal was to demonstrate that diet can improve cardiovascular risk factors, even in adults early in disease progression with well-controlled type 2 diabetes. This is also the primary limitation of this study: we acknowledge that our results may not be relevant to individuals with more advanced disease. However, we offer evidence of an effective, preventative, low-risk strategy in a group who remains at risk of vascular events in spite of their glycemic control.

Previous studies of nuts and blood pressure have relied on the traditional clinic method of measuring blood pressure under resting conditions. However, blood pressure during acute mental stress is an independent predictor of cardiovascular risk (18), and the shifts we observed in underlying hemodynamics (reduced total peripheral resistance and increased cardiac output) could have important clinical implications even in the absence of blood pressure reductions. In the majority of patients with essential hypertension, total peripheral resistance is elevated while cardiac output is normal (183). Elevated peripheral resistance is an important contributor to left ventricular hypertrophy, which is an independent predictor of cardiovascular outcomes. Furthermore, a reduction in left ventricular hypertrophy lowers cardiovascular risk independent of changes in blood pressure (184). Based on a meta-analysis of 50 studies, the medications that most effectively reverse left ventricular hypertrophy are those that reduce peripheral resistance (angiotension-converting enzyme inhibitors and calcium channel blockers) (185). Thus, it is plausible that the reduction in total peripheral resistance that we observed following pistachio consumption could contribute to a reversal of left ventricular hypertrophy. Unfortunately, we did not measure left ventricular mass in the present
study, and it is unlikely that a clinically significant change in left ventricular mass could occur during a 4-week dietary intervention. Future research about the mechanisms underlying the hemodynamic shifts that we observed should include longer diet periods and assessment of left ventricular mass and remodeling.

To our knowledge, this is the first study on nuts to include ambulatory blood pressure monitoring, which is a better predictor of target organ damage than traditional clinic blood pressure measurements (186). We observed reductions of both 24-hour and sleep systolic blood pressure following the pistachio diet. Blood pressure normally decreases (or “dips”) during sleep, and individuals who do not display this physiological change can be 66% more likely to experience a cardiovascular event (15). Therefore, a significant reduction in 24-hour blood pressure that is driven primarily by a reduction in sleep blood pressure could be particularly beneficial to cardiovascular health. Unfortunately, ambulatory assessment of cardiac output and peripheral resistance is difficult to perform, so it is unknown how the underlying hemodynamics shifted during the ambulatory monitoring to reduce blood pressure. The observed reduction in ambulatory systolic blood pressure was small, but such a change on a population level is estimated to reduce coronary heart disease and stroke mortality by 5-8% (187). Importantly, it indicates that among adults with type 2 diabetes and normal blood pressure at baseline (Table 3.1), pistachio consumption can further reduce blood pressure and potentially lower residual vascular risk. These findings must be replicated in a larger sample, including individuals with more advanced diabetes.

Heart rate variability is also a recognized cardiovascular risk factor (31-33), and we observed improvements in three indices of heart rate variability following pistachio consumption: RMSSD, high frequency, and low frequency. Both RMSSD and high frequency reflect vagal tone, and low frequency is believed to reflect both sympathetic and parasympathetic function. In the Atherosclerosis Risk In Communities studies, individuals with type 2 diabetes who were in the lowest quartile of either high or low frequency heart rate variability were 50-80% more likely to develop coronary heart disease during an 8-year follow-up (188). The effect of pistachios on heart rate variability that we observed in the present study may be a yet unexplored mechanism through which nuts benefit cardiovascular health.
We observed no effect of pistachio consumption on measures of endothelial function. Previous studies have demonstrated that daily consumption of pistachios (189), walnuts (141, 190), or hazelnuts (142) improve brachial FMD by 16-73% in healthy adults or those with cardiovascular risk factors. To our knowledge, this is the first study to examine the effect of nut consumption on endothelial function in type 2 diabetes, and it is possible that this population will not exhibit improvements in vascular markers in response to dietary interventions that healthier populations do. However, it is interesting that we observed a trend towards significance for an increase in baseline brachial diameter following the pistachio treatment. Considering the significant decrease in total peripheral resistance that followed the pistachio diet, this trend suggests that pistachios may reduce basal vascular constriction. More research is needed to determine if this is a true effect and, if so, to explore the potential mechanisms driving it.

Given that this study utilized a controlled-feeding protocol with whole pistachios, we can only speculate which component(s) of pistachios may be responsible for the observed changes. Like all nuts, pistachios have a heart-healthy fatty acid profile (low in saturated fat, high in unsaturated fat) and are rich in plant protein, fiber, vitamins, and minerals. Pistachios are a source of potassium, vitamin A, and phytosterols. Notably, in the present study the ratio of sodium to potassium was substantially lower for the pistachio diet (0.63) than the control diet (0.81). This difference was due to the high potassium content of pistachios (approximately 600 mg for 69 g/d for the 2100 calorie level) and the sodium content of the control diet snacks (i.e. pretzels, string cheese, etc.) that were replaced by the pistachios (approximately 500 mg per day for the 2100 calorie diet). While it is possible that a single bioactive component of pistachios is responsible for the observed changes in cardiovascular risk factors, it is more likely that the macro- and micronutrient profiles combined contribute to their health benefits.

The present study has a few limitations. First, the sample included only adults with well-controlled (average HbA1c <6.5% at baseline) and relatively “early” diabetes (17% taking no diabetes medication, 66% on a single diabetes medication, and no insulin users). We are unable to draw conclusions about pistachio consumption in amounts other than the 20% of energy daily that was evaluated here. We compared the pistachio diet to a low-fat control diet that was designed in accordance with recommendations for type 2 diabetes, but did not test it against other recommended dietary patterns such as the Mediterranean style or vegetarian diet (172). The controlled-feeding protocol enabled
us to assess the efficacy of pistachio consumption in 4 weeks, but does not provide information on the effectiveness of a pistachio intervention over a longer period of time. Finally, the total sample was relatively small, and only a portion of the subjects completed the ambulatory blood pressure monitoring. Future studies should enroll larger samples, include ambulatory blood pressure as a primary outcome, and test the effectiveness of pistachio consumption on cardiovascular risk factors in a free-living setting.

In conclusion, this carefully controlled randomized clinical trial indicates that consuming pistachios equal to 20% of energy daily modifies systemic hemodynamics, increases heart rate variability, and reduces ambulatory blood pressure in adults with well-controlled type 2 diabetes. This study provides further evidence that daily nut consumption benefits multiple cardiovascular risk factors and may be an effective strategy for reducing residual vascular risk.
CHAPTER 4. ENDOTHELIAL DYSFUNCTION, ARTERIAL STIFFNESS, AND ADHERENCE TO THE 2010 DIETARY GUIDELINES FOR AMERICANS: A CROSS-SECTIONAL ANALYSIS OF THE FRAMINGHAM HEART STUDY

Abstract

Background – Endothelial dysfunction and arterial stiffness are early predictors of cardiovascular disease. There is strong evidence from intervention studies that diet is related to vascular health, but the majority of these studies have tested individual foods or nutrients and relied on small samples of mostly younger adults. The purpose of this study was to determine if adherence to the 2010 Dietary Guidelines for Americans is independently associated with vascular health in a large, cross-sectional study.

Methods – This study included diet and vascular data previously collected in 6020 adults in the Framingham Heart study. Diet quality was quantified with the 2010 Dietary Guidelines for Americans Index (DGAI-2010). Endothelial dysfunction was assessed via brachial flow-mediated dilation and arterial stiffness was assessed via carotid-femoral pulse wave velocity. Regression analyses were used to determine whether DGAI-2010 scores were independently associated with vascular health, and whether these relationships were moderated by age.

Results – Baseline brachial mean flow velocity significantly decreased with increasing DGAI-2010 scores ($P=0.002$) and hyperemic mean flow ratio significantly increased with increasing DGAI-2010 scores ($P=0.02$). Augmentation index significantly decreased with increasing DGAI-2010 scores; age-stratified analyses revealed that this relationship was statistically significant only in older adults ($\geq 50$ years). Diet was not significantly related to measures of endothelial dysfunction.

Conclusions – We have shown that adherence to the 2010 Dietary Guidelines for Americans is significantly associated with blood flow velocity and arterial stiffness but not endothelial dysfunction in the Framingham Heart Study. Importantly, we have demonstrated that diet may particularly important for arterial stiffness in older adults. Our results suggest a link between diet quality and vascular health and as such may be useful for designing interventions to treat or prevent age-related vascular decline.
Introduction

Endothelial dysfunction and arterial stiffness are early independent predictors of atherosclerosis, hypertension, and cardiovascular disease (CVD) (48, 59). There is strong evidence that diet is related to endothelial dysfunction, and, to a lesser degree, arterial stiffness. Clinical trials have documented improvements of 10-73% in flow-mediated dilation (FMD) of the brachial artery following consumption of foods (e.g. nuts (141), cocoa (108)), beverages (e.g. tea (140), wine (135), fruit juice (133)), and dietary patterns (low saturated fat diets (78), a Mediterranean diet (147)). Observational studies indicate that diets rich in meat intake and high alcohol consumption increase arterial stiffness (107, 108), whereas diets with moderate alcohol consumption (109-112), low sodium intake (113), greater fruit and vegetable consumption (114), and increasing dairy intake (115) have been associated with reduced arterial stiffness. However, the weight of existing literature is limited by methodology, as most studies have relied on small (n<200) homogenous samples of younger adults (<65 years) with short treatment periods (4 – 12 weeks). There has also been significant variety in the methods used to quantify diet in previous observational studies, with the majority examining just a small component of habitual diet rather than the overall pattern. Given that consumption of specific foods and nutrients occur within the context of the larger habitual diet, it is important to understand the effect of an individual's overall diet on cardiovascular risk.

Diet quality, as defined by public health recommendations, is a comprehensive approach that can provide valuable insight into the relationship between diet and vascular health.

The Dietary Guidelines for Americans are evidence-based recommendations that provide guidance for choosing an eating pattern that promotes health and prevents disease. The 2010 Guidelines emphasize greater intake of fruits, vegetables, low-fat dairy products, whole grains, and a variety of lean meats while maintaining appropriate weight through caloric balance and physical activity (9). The Dietary Guidelines Adherence Index (DGAI) is a tool that quantifies the degree to which habitual diet meets these recommendations. Developed in regard to the 2005 Guidelines (191) and updated for the 2010 Guidelines (P.F. Jacques, Personal communication, 2012), the DGAI provides an objective index of diet quality that is useful for standardizing dietary assessments across studies. Previous research utilizing the DGAI-2005 indicates that increasing diet quality is associated with increasing age, female sex, lower body mass index, and non-smoking status (191). In addition, metabolic syndrome and insulin...
resistance were significantly more prevalent in the lowest quintile of DGAI scores compared to the highest quintile (192, 193). However, no studies have evaluated whether diet quality is associated with vascular health, particularly in a large, community-based sample.

Additionally, vascular health declines with age despite control of traditional risk factors. It is unclear whether age-related vascular decline is part of a normal physiological aging process or a consequence of repeated exposure to lifestyle risk factors. Physiological changes with age likely interact with lifestyle risk factors to exacerbate arterial stiffness and endothelial dysfunction (37). Given the burden of cardiovascular disease on America’s aging population, there is a need for improved understanding of the interaction between age and lifestyle and its effect on vascular decline.

Therefore, the purpose of this study is to determine if adherence to the 2010 Dietary Guidelines for Americans is independently associated with endothelial dysfunction and arterial stiffness in a cross-sectional sample of adults from the Framingham Heart Study. A secondary purpose is to determine whether diet quality moderates the effect of age on these measures of vascular health. Exploratory analyses will examine the association of individual components of the Dietary Guidelines with endothelial dysfunction and arterial stiffness.

Methods

Participants

The Framingham Heart Study is a longitudinal, community-based study of risk factors for CVD. The study was initiated in 1948 with the enrollment of 5209 adults aged 28 – 62 years (194). In 1971, 5135 children of the original cohort were enrolled, along with their spouses, in the Framingham Offspring/Spouse cohort (195). In 2002, 4095 children of the Offspring cohort were enrolled in the Framingham Third Generation cohort (196). Participants complete examinations approximately every 2-4 years, which include a detailed medical history, physical exam, clinical and laboratory testing. The current study includes dietary and vascular data collected during the seventh examination cycle of the Offspring cohort (1998 – 2001) and the first examination cycle of the Third Generation cohort (2002 – 2005).
**Dietary Assessment**

The Harvard semi-quantitative food frequency questionnaire (FFQ) (197) was mailed to participants prior to the examination and they were asked to bring the completed form to their appointment. The 126-item FFQ assesses the consumption frequency of standard servings of foods and beverages during the last year with response selections ranging from “never or less than once per month” to “6+/day.” The FFQ provides a space for participants to write-in up to three additional foods they frequently consumed that were not listed, and specifically asks for type of breakfast cereal and cooking oil regularly used. Nutrient intakes are calculated by multiplying average intake with nutrient content of individual foods, based on the United States Department of Agriculture food composition database and supplemented with other sources (198).

The 2010 Dietary Guideline Adherence Index (DGAI-2010) was applied to the FFQ data to determine the extent to which participants’ diets adhere to the 2010 Dietary Guidelines. The DGAI-2010 assesses intake of 14 food groups (fruit; dark green vegetables; orange and red vegetables; starchy vegetables; other vegetables; grains; milk; meat, protein, and eggs; seafood; nuts; legumes; sugar; variety in protein choices; and variety of fruits and vegetables) and 11 healthy choice or nutrient intake recommendations (amounts of total fat, saturated fat, trans fat, cholesterol, sodium, fiber, alcohol; and percentage of protein that is lean, milk that is low-fat, grains that are whole grains, and fruits that are whole fruits). Adherence to each category is scored on a continuous scale of 0-1, and the categories are summed and standardized to a range of 0-100 to create an overall score, with higher scores indicating greater adherence. An important component of the DGAI compared to other dietary quality assessment tools is the penalty assigned for overconsumption, which is in line with the 2005 and 2010 Dietary Guidelines emphasis on weight management. That is, the DGAI avoids assigning a higher score to individuals who meet the recommended food intakes simply through eating more. Appropriate energy levels are calculated for each participant (based on height, weight, age, sex, and physical activity estimates) and participants are penalized for consuming more than the recommended daily intake of energy-dense foods (e.g. starchy vegetables, specific protein sources, grains, meat and beans, and dairy products). Examples of diets with high and low adherence are presented in Table 4.1.
<table>
<thead>
<tr>
<th>Recommendation for 2000 kcal/day</th>
<th>Quintile 1</th>
<th>Score</th>
<th>Quintile 5</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGAI-2010²</td>
<td>40.4</td>
<td>69.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy-Specific Items</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>2.0 cups</td>
<td>0.8</td>
<td>cups</td>
<td>0.4</td>
</tr>
<tr>
<td>Dark green vegetables</td>
<td>0.2 cups</td>
<td>0.1</td>
<td>cups</td>
<td>0.3</td>
</tr>
<tr>
<td>Orange &amp; red vegetables</td>
<td>0.8 cups</td>
<td>0.4</td>
<td>cups</td>
<td>0.5</td>
</tr>
<tr>
<td>Starchy vegetables</td>
<td>0.7 cups</td>
<td>0.7</td>
<td>cups</td>
<td>1.0</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>0.6 cups</td>
<td>0.4</td>
<td>cups</td>
<td>0.8</td>
</tr>
<tr>
<td>Grains</td>
<td>6.0 ounces</td>
<td>3.7</td>
<td>ounces</td>
<td>0.6</td>
</tr>
<tr>
<td>Milk²</td>
<td>3.0 cups</td>
<td>1.5</td>
<td>cups</td>
<td>0.5</td>
</tr>
<tr>
<td>Meat³</td>
<td>3.7 ounces</td>
<td>0.0</td>
<td>ounces</td>
<td>0.0</td>
</tr>
<tr>
<td>Seafood</td>
<td>1.1 ounces</td>
<td>0.1</td>
<td>ounces</td>
<td>0.1</td>
</tr>
<tr>
<td>Nuts</td>
<td>0.6 ounces</td>
<td>0.0</td>
<td>ounces</td>
<td>0.0</td>
</tr>
<tr>
<td>Legumes</td>
<td>0.2 cups</td>
<td>0.0</td>
<td>cups</td>
<td>0.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>≤5 % energy</td>
<td>8.0</td>
<td>% energy</td>
<td>0.1</td>
</tr>
<tr>
<td>Protein variety⁴</td>
<td>≥50% of rec/each</td>
<td>0/3</td>
<td>met 50% rec</td>
<td>0.0</td>
</tr>
<tr>
<td>Fruit &amp; vegetable variety</td>
<td>≥50% of rec/each</td>
<td>3/5</td>
<td>met 50% rec</td>
<td>0.6</td>
</tr>
</tbody>
</table>

¹Examples based on actual data from two females, aged 48 years, body mass index of approximately 26 kg/m².

²Sum of all subscores, standardized to a scale ranging from 0-100.

³Penalty applied to this category for overconsumption; a score of less than 1.0 can indicate either consumption of less than the recommended amount or more than the recommended amount. In this example, underconsumption is assumed.

⁴Includes meat, seafood, and nuts.

⁵Recommendation for women is ≤1 drink/day; for men, ≤2 drinks/day.
### Table 4.1. Example of Quintile 1 and Quintile 5 Diets

(continued from previous page)

<table>
<thead>
<tr>
<th>Recommendation for 2000 kcal/day</th>
<th>Quintile 1 Daily Intake Score</th>
<th>Quintile 5 Daily Intake Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGAI-2010^2</td>
<td>40.4</td>
<td>69.6</td>
</tr>
<tr>
<td>Healthy Choice Items</td>
<td>5.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Fat</td>
<td>20-30 % energy</td>
<td>37.0 % energy</td>
</tr>
<tr>
<td>Saturated fat&lt;10 % energy</td>
<td>13.0 % energy</td>
<td>0.2</td>
</tr>
<tr>
<td>Trans fat≤1 % energy</td>
<td>3.0 % energy</td>
<td>0.0</td>
</tr>
<tr>
<td>Cholesterol&lt;300 mg</td>
<td>366.0 mg</td>
<td>0.8</td>
</tr>
<tr>
<td>Lean meat 75 % of meat</td>
<td>31.0 % of meat</td>
<td>0.2</td>
</tr>
<tr>
<td>Low-fat milk 75 % of milk</td>
<td>38.5 % of milk</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium &lt;2300 mg</td>
<td>2155.0 mg</td>
<td>1.0</td>
</tr>
<tr>
<td>Fiber</td>
<td>28 grams</td>
<td>0.5</td>
</tr>
<tr>
<td>Alcohol ≤1 drink</td>
<td>0.4 drink</td>
<td>1.0</td>
</tr>
<tr>
<td>Whole fruit 75 % of fruits</td>
<td>62.6 % of fruits</td>
<td>0.8</td>
</tr>
<tr>
<td>Whole grain 50 % of grains</td>
<td>6.7 % of grains</td>
<td>0.1</td>
</tr>
</tbody>
</table>

1^Examples based on actual data from females, aged 48 years, body mass index of approximately 26 kg/m^2.  
2^Sum of all subscores, standardized to a scale ranging from 0-100.  
3^Penalty applied to this category for overconsumption; a score of less than 1.0 can indicate either consumption of less than the recommended amount or more than the recommended amount. In this example, underconsumption is assumed.  
4^Includes meat, seafood, and nuts.  
5^Recommendation for women is ≤1 drink/day; for men, ≤2 drinks/day.
**Vascular Assessments**

Endothelial dysfunction was assessed by brachial flow-mediated dilation (FMD). Methodology and reproducibility data have been previously published (67, 199). Briefly, brachial artery diameter (mm) was imaged in the supine position with high-resolution ultrasound at rest and one minute after reactive hyperemia that was induced by 5-minute cuff occlusion of the forearm. Arterial diameter was measured offline using commercially-available edge-detection software. Brachial FMD was calculated as the percent change in brachial diameter during reactive hyperemia from the resting state (%FMD), with lower values indicating greater endothelial dysfunction. Baseline and post-deflation hyperemic flow were assessed with Doppler imaging at baseline and for 15 seconds immediately post-deflation, as described previously (200). Hyperemic mean flow ratio and deflation shear stress were calculated with the following equations: flow ratio = deflation mean flow velocity / baseline mean flow velocity and shear stress = 8 x blood viscosity (assumed to be 0.035 dyne*s/cm²) x deflation mean flow velocity / baseline diameter (200).

Arterial stiffness was assessed in the supine position with arterial tonometry as described previously (43). Briefly, blood pressure was obtained with an oscillometric device, and a tonometer recorded blood pulsations at the carotid, right brachial, right radial, and right femoral arteries. Transit distances were measured from the suprasternal notch to each recording site. Tonometry waveforms were signal-averaged offline and calibrated using cuff pressures. Carotid-femoral pulse wave velocity (PWV) was calculated from transit distances and tonometry waveforms as described previously (201), with greater velocity indicating greater arterial stiffness. Augmentation index was calculated from the carotid pressure waveform as described previously (202), with higher values reflecting greater arterial stiffness.

**Covariates**

Potential confounders of the relationship between diet and vascular health were considered in the present analysis in accordance with previous studies (43, 199). All participants underwent routine medical examination at the time of vascular assessment to obtain the following characteristics: age, sex, race, body mass index, fasting glucose, total/HDL cholesterol ratio, triglycerides, diabetes (defined as a fasting blood glucose of ≥126 mg/dL or treatment with insulin or an oral hypoglycemic agent), hypertension
(defined as systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg), or existing CVD (coronary heart disease, heart failure, stroke, transient ischemic attack, or intermittent claudication). Heart rate and blood pressure were obtained with an oscillometric device after five minutes of supine rest. Hormone replacement therapy, hypertension medication, lipid-lowering medication, and cigarette smoking (in the six hours prior to vascular testing) were determined by self-report. A variable representing the timing of a walk test in relation to the vascular assessments (before vs. after or not done) was also included.

**Statistical Analyses**

Of the 7634 participants who attended the seventh Offspring exam (n=3539) or the first Third Generation exam (n=4095), complete dietary data were available for 6020. Of these, brachial FMD data were available for 5593, flow data were available for 5135, and tonometry data were available for 5080. To maximize sample size, participants were included in analyses for which data was available.

The DGAI-2010 scores were divided into equal quintiles according to the full sample (n=6020 total, n=1204 per quintile). Means and 95% confidence intervals of participant characteristics across quintiles, adjusted for age and sex, were computed using PROC GLM in SAS. The statistical significance for trend was assessed using linear regression (PROC REG) for continuous variables and logistic regression (PROC LOGISTIC) for dichotomous variables, with DGAI-2010 entered as a continuous score.

The DGAI-2010 score and all vascular outcome variables were assessed for normality; baseline flow velocity, FMD standardized for shear stress, and PWV were positively skewed. A natural log transformation was applied to baseline flow velocity and FMD/SS, and PWV was divided into 1000 (1000/PWV). Quintile means and 95% confidence intervals of vascular characteristics, adjusted for clinical covariates (see below), were computed using PROC GLM in SAS. The statistical significance for trend was assessed using PROC GENMOD with DGAI-2010 entered as a continuous score. This procedure adjusts for the familial correlations in the present sample. First order interactions between DGAI-2010, sex, and age were assessed for each of the vascular characteristics using model 3 (described below); variables with statistically significant interactions were stratified by sex or age (< or ≥ 50 years) for further investigation.
For all vascular outcomes, three models were assessed. Model 1 included age and sex only. Model 2 included age, sex, body mass index, systolic blood pressure, heart rate, and lipid therapy; these variables were selected because they have been shown to be independent predictors of both endothelial function and arterial stiffness in the Framingham sample (43, 199). Model 3 included the variables in model 2, plus diastolic blood pressure, total/HDL cholesterol, triglycerides, glucose, diabetes, hypertension, hypertension therapy, hormone replacement therapy, prevalent CVD, smoking in the 6 hours prior to vascular testing, and completing the walk test prior to vascular testing; these variables were selected because they have been significantly associated with endothelial function or arterial stiffness in the Framingham sample (43, 199). The fully-adjusted model 3 yielded the best model fit (i.e. greatest $R^2$-squared) and was selected as the final model presented in tabular format. Differences in statistical significance between the models are discussed in the text.

Age- and sex-adjusted Spearman rank correlations between the subcategories of the DGAI-2010 and the vascular characteristics were assessed using PROC CORR. Due to the large number of correlations tested (25 subcategories x 10 vascular variables = 250), the adjusted $P$ value for statistical significance for the Spearman coefficient was 0.0002. For all other analyses, $P < 0.05$ was considered statistically significant. All analyses were conducted in SAS v9.3 (Cary, NC). Unless otherwise noted, we report adjusted means (95% CI).

**Results**

Participant characteristics according to DGAI-2010 quintile are shown in Table 4.2. Many of the characteristics were significantly associated with DGAI-2010. With increasing DGAI-2010 scores, participants were more likely to be women ($P=0.0001$), older ($P<0.0001$), non-smokers ($P<0.0001$), and have a lower BMI ($P<0.0001$), systolic and diastolic blood pressures ($P=0.004$), heart rate ($P<0.0001$), total/HDL cholesterol ($P<0.0001$), triglycerides ($P<0.0001$), and fasting glucose ($P=0.0002$). There was no significant trend for a difference across DGAI-2010 quintiles in the number of participants with diabetes, hypertension, prevalent CVD, or taking anti-hypertensive, lipid-lowering, or hormone therapy.

Vascular characteristics according to DGAI-2010 quintile are reported in Table 4.3. None of the brachial artery measures of endothelial function were related to DGAI-2010.
scores in the crude (age- and sex-adjusted only) model 1. In model 2, FMD (mm) was significantly related to diet ($P=0.042$) with participants in quintile 1 having substantially lower FMD (mm) than quintiles 2-5; however, this difference was no longer statistically significant in the fully-adjusted model 3.

Baseline mean flow velocity significantly decreased with increasing DGAI-2010 scores ($P=0.002$) and hyperemic mean flow ratio significantly increased with increasing DGAI-2010 scores ($P=0.02$) in all three models. Deflation mean flow velocity and deflation shear stress decreased with increasing dietary quintile in models 1 and 2 (all $P<0.05$), but the trend was no longer statistically significant in the fully-adjusted model 3. Carotid-femoral PWV significantly decreased with increasing dietary quintile scores in model 1 ($P<0.0001$), but was not significantly related to DGAI-2010 scores in models 2 or 3. Augmentation index significantly decreased with increasing DGAI-2010 scores ($P=0.0007$) in all three models.

We tested interactions between DGAI-2010 and both age and sex for vascular characteristics using model 3. There was a significant DGAI-2010 * age interaction for augmentation index ($P=0.047$). Stratified analyses (< or ≥ 50 years) showed that augmentation index significantly decreased with increasing DGAI-2010 scores in the older group ($P=0.045$) but not in the younger group ($P=0.36$, Table 4.4). There was a significant DGAI-2010 * sex interaction for FMD (%), but stratified analyses indicated no variation across quintile in men ($P=0.64$) nor women ($P=0.30$).

Spearman rank correlations between the vascular characteristics and the 25 individual subscores of the DGAI-2010 are displayed in Table 4.5. Similar to the results of the total DGAI-2010 score analysis, many of the subscores were significantly correlated with baseline mean flow velocity (fruit, dark green vegetables, grains, seafood, legumes, fruit/vegetable variety, saturated fat, trans fat, fiber, % lean meat, % low-fat milk, and % whole grains) and hyperemic mean flow ratio (fruit, dark green vegetables, starchy vegetables, grains, legumes, fiber, % lean meat, % whole grains). Augmentation index was significantly correlated with only four subscores (fruit, fiber, alcohol, and % whole grains), while carotid-femoral PWV was significantly correlated with six subscores (fruit, dark green vegetables, starchy vegetables, legumes, fiber, and % whole grains) despite no significant association with total DGAI-2010. Baseline brachial diameter and FMD (mm) were significantly correlated with sugar and dietary cholesterol. Similar to the total
Table 4.2. Participant characteristics according to quintile category of the DGAI-2010 (n=6020)\(^7\)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P for trend(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median DGAI-2010 score(^4)</td>
<td>43.8 (21.8-48.8)</td>
<td>52.6 (48.8-55.9)</td>
<td>58.9 (55.9-61.8)</td>
<td>64.9 (61.8-68.2)</td>
<td>72.5 (68.2-88.2)</td>
<td></td>
</tr>
<tr>
<td>Women (%)</td>
<td>36.8 (34.1, 39.5)</td>
<td>44.4 (41.6, 47.1)</td>
<td>54.7 (51.9, 57.4)</td>
<td>62.0 (59.2, 64.7)</td>
<td>72.6 (69.8, 75.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.7 (45.9, 47.5)</td>
<td>48.1 (47.3, 48.9)</td>
<td>48.3 (47.5, 49.0)</td>
<td>49.4 (48.6, 50.2)</td>
<td>51.0 (50.2, 51.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>28.1 (27.7, 28.4)</td>
<td>28.2 (27.9, 28.5)</td>
<td>27.1 (26.8, 27.4)</td>
<td>26.9 (26.6, 27.2)</td>
<td>26.7 (26.4, 27.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>121.9 (121.1, 122.8)</td>
<td>121.8 (120.9, 122.6)</td>
<td>120.8 (120.0, 121.7)</td>
<td>120.1 (119.2, 120.9)</td>
<td>120.5 (119.6, 121.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75.9 (75.3, 76.4)</td>
<td>75.5 (74.9, 76.0)</td>
<td>74.6 (74.1, 75.2)</td>
<td>74.7 (74.2, 75.2)</td>
<td>74.1 (73.6, 74.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>65.2 (64.6, 65.8)</td>
<td>64.0 (63.4, 64.5)</td>
<td>63.2 (62.6, 63.8)</td>
<td>62.1 (61.5, 62.7)</td>
<td>62.0 (61.4, 62.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>4.1 (4.0, 4.2)</td>
<td>4.0 (3.9, 4.1)</td>
<td>3.9 (3.8, 4.0)</td>
<td>3.8 (3.7, 3.9)</td>
<td>3.8 (3.8, 3.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>131.5 (126.6, 136.3)</td>
<td>126.8 (121.9, 131.6)</td>
<td>126.2 (121.4, 131.1)</td>
<td>116.9 (112.1, 121.8)</td>
<td>119.4 (114.4, 124.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>100.4 (99.2, 101.6)</td>
<td>100.4 (99.2, 101.6)</td>
<td>99.0 (97.8, 100.2)</td>
<td>97.8 (96.6, 99.0)</td>
<td>97.4 (96.2, 98.6)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5.7 (4.4, 7.1)</td>
<td>7.5 (6.2, 8.8)</td>
<td>6.0 (4.7, 7.3)</td>
<td>5.2 (3.9, 6.5)</td>
<td>6.0 (4.6, 7.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>25.8 (23.5, 28.1)</td>
<td>24.3 (22.0, 26.6)</td>
<td>24.5 (22.2, 26.8)</td>
<td>23.8 (21.5, 26.1)</td>
<td>23.5 (21.1, 25.8)</td>
<td>0.075</td>
</tr>
<tr>
<td>Hypertension medication (%)</td>
<td>19.0 (16.9, 21.0)</td>
<td>17.8 (15.8, 19.9)</td>
<td>19.6 (17.6, 21.6)</td>
<td>18.1 (16.1, 20.1)</td>
<td>18.0 (16.0, 20.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Lipid-lowering medication (%)</td>
<td>11.6 (9.8, 13.4)</td>
<td>12.7 (10.9, 14.5)</td>
<td>14.0 (12.2, 15.8)</td>
<td>12.8 (11.0, 14.6)</td>
<td>13.4 (11.6, 15.3)</td>
<td>0.19</td>
</tr>
<tr>
<td>HRT (% women)</td>
<td>15.6 (12.4, 18.8)</td>
<td>14.3 (11.4, 17.2)</td>
<td>15.4 (12.8, 18.1)</td>
<td>17.2 (14.7, 19.7)</td>
<td>15.8 (13.5, 18.1)</td>
<td>0.33</td>
</tr>
<tr>
<td>Prevalent CVD (%)</td>
<td>5.6 (4.3, 6.8)</td>
<td>5.1 (3.9, 6.4)</td>
<td>6.3 (5.1, 7.5)</td>
<td>6.5 (5.2, 7.7)</td>
<td>5.4 (4.1, 6.6)</td>
<td>0.77</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>24.6 (22.6, 26.6)</td>
<td>18.7 (16.8, 20.7)</td>
<td>13.0 (11.1, 15.0)</td>
<td>9.5 (7.5, 11.5)</td>
<td>7.4 (5.4, 9.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Smoked in prior 6 hrs (%)</td>
<td>17.7 (16.1, 19.3)</td>
<td>10.6 (9.0, 12.2)</td>
<td>7.6 (6.0, 9.1)</td>
<td>4.2 (2.6, 5.7)</td>
<td>3.3 (1.7, 4.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Physical activity score (METS)</td>
<td>37.9 (37.5, 38.3)</td>
<td>37.3 (36.8, 37.7)</td>
<td>37.5 (37.0, 37.9)</td>
<td>37.5 (37.1, 37.9)</td>
<td>38.0 (37.5, 38.4)</td>
<td>0.67</td>
</tr>
<tr>
<td>Walk test prior to testing (%)</td>
<td>15.4 (13.5, 17.4)</td>
<td>16.2 (14.3, 18.1)</td>
<td>16.0 (14.1, 17.9)</td>
<td>13.0 (11.0, 14.9)</td>
<td>14.3 (12.3, 16.3)</td>
<td>0.076</td>
</tr>
</tbody>
</table>

\(^1\)Means or percentage (95% CI) adjusted for age and sex. Age adjusted for sex only and sex adjusted for age only. DGAI-2010, Dietary Guidelines Adherence Index 2010; BP, blood pressure; HRT, hormone-replacement therapy; CVD, cardiovascular disease; METS, metabolic equivalents.

\(^2\)Derived from linear regression for continuous variables or logistic regression for dichotomous variables with DGAI-2010 as a continuous score.

\(^3\)Quintile range in parentheses; DGAI-2010 range is 0-100 possible points.
Table 4.3. Vascular characteristics according to quintile of DGAI-2010

<table>
<thead>
<tr>
<th>DGAI-2010 range</th>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P_for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.8-48.8</td>
<td>5663</td>
<td>4.16 (4.10, 4.23)</td>
<td>4.22 (4.16, 4.29)</td>
<td>4.21 (4.15, 4.28)</td>
<td>4.22 (4.15, 4.28)</td>
<td>4.21 (4.14, 4.27)</td>
<td>0.12</td>
</tr>
<tr>
<td>48.8-55.9</td>
<td>5521</td>
<td>4.33 (4.26, 4.40)</td>
<td>4.39 (4.32, 4.45)</td>
<td>4.37 (4.31, 4.44)</td>
<td>4.38 (4.31, 4.45)</td>
<td>4.38 (4.31, 4.45)</td>
<td>0.084</td>
</tr>
<tr>
<td>55.9-61.8</td>
<td>5519</td>
<td>4.11 (3.71, 4.50)</td>
<td>4.31 (3.91, 4.71)</td>
<td>4.15 (3.75, 4.56)</td>
<td>4.22 (3.82, 4.62)</td>
<td>4.10 (3.69, 4.50)</td>
<td>0.71</td>
</tr>
<tr>
<td>61.8-68.2</td>
<td>4996</td>
<td>0.97 (0.86, 1.08)</td>
<td>0.99 (0.88, 1.11)</td>
<td>0.99 (0.88, 1.10)</td>
<td>1.02 (0.90, 1.13)</td>
<td>0.99 (0.87, 1.10)</td>
<td>0.63</td>
</tr>
<tr>
<td>68.2-88.2</td>
<td>5065</td>
<td>8.02 (7.5, 8.6)</td>
<td>7.89 (7.4, 8.5)</td>
<td>7.6 (7.1, 8.2)</td>
<td>7.6 (7.1, 8.1)</td>
<td>7.5 (7.0, 8.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>5065</td>
<td>57.5 (55.0, 59.9)</td>
<td>58.4 (56.0, 60.9)</td>
<td>56.1 (53.6, 58.5)</td>
<td>57.1 (54.6, 59.6)</td>
<td>56.4 (53.9, 58.9)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>5135</td>
<td>6.6 (6.1, 7.0)</td>
<td>6.8 (6.3, 7.2)</td>
<td>6.7 (6.2, 7.2)</td>
<td>6.9 (6.4, 7.4)</td>
<td>6.9 (6.4, 7.4)</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>5062</td>
<td>4.19 (4.00, 4.39)</td>
<td>4.19 (3.99, 4.39)</td>
<td>4.04 (3.84, 4.24)</td>
<td>4.10 (3.90, 4.30)</td>
<td>4.05 (3.85, 4.26)</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td>5017</td>
<td>7.72 (7.57, 7.87)</td>
<td>7.70 (7.55, 7.85)</td>
<td>7.60 (7.46, 7.74)</td>
<td>7.62 (7.48, 7.77)</td>
<td>7.66 (7.51, 7.81)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>5291</td>
<td>11.8 (10.4, 13.3)</td>
<td>11.5 (10.0, 12.9)</td>
<td>11.0 (9.6, 12.5)</td>
<td>10.3 (8.8, 11.7)</td>
<td>10.7 (9.3, 12.2)</td>
<td>0.0007</td>
<td></td>
</tr>
</tbody>
</table>

1Means adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, heart rate, total/HDL cholesterol, triglycerides, glucose, diabetes, hypertension, hypertension therapy, lipid therapy, hormone replacement therapy, prevalent cardiovascular disease, smoking in 6 hours prior to vascular testing, walk test prior to vascular testing.

2Derived from PROC GENMOD with DGAI-2010 as a continuous score.
Table 4.4. Augmentation index stratified by age

<table>
<thead>
<tr>
<th>N</th>
<th>Age &lt; 50 years</th>
<th>Age &gt; 50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Augmentation index (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3202</td>
<td>13.1 (9.4, 16.8)</td>
<td>13.3 (9.7, 17.0)</td>
</tr>
<tr>
<td>2089</td>
<td>19.5 (17.7, 21.3)</td>
<td>18.5 (16.8, 20.3)</td>
</tr>
</tbody>
</table>

¹Means adjusted for sex, body mass index, systolic blood pressure, diastolic blood pressure, heart rate, total/HDL cholesterol, triglycerides, glucose, diabetes, hypertension, hypertension therapy, lipid therapy, hormone replacement therapy, prevalent cardiovascular disease, smoking in 6 hours prior to vascular testing, walk test prior to vascular testing.

²Derived from PROC GENMOD with DGAI-2010 as a continuous score.
Table 4.5. Spearman rank correlations between vascular characteristics and DGAI-2010 subscores

<table>
<thead>
<tr>
<th></th>
<th>Baseline Diameter</th>
<th>FMD (mm)</th>
<th>FMD (%)</th>
<th>FMD/SS Baseline Flow</th>
<th>Deflation Flow</th>
<th>Flow Ratio</th>
<th>SS</th>
<th>CF-PWV</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy-Specific Items</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>-0.025</td>
<td>-0.031</td>
<td>-0.005</td>
<td>0.007</td>
<td>-0.103(^2)</td>
<td>-0.027</td>
<td>0.080(^2)</td>
<td>-0.014</td>
<td>-0.085(^2)</td>
</tr>
<tr>
<td>Dark green vegetables</td>
<td>-0.016</td>
<td>-0.010</td>
<td>0.015</td>
<td>0.025</td>
<td>-0.098(^2)</td>
<td>-0.015</td>
<td>0.088(^2)</td>
<td>-0.007</td>
<td>-0.076(^2)</td>
</tr>
<tr>
<td>Orange &amp; red vegetables</td>
<td>0.017</td>
<td>0.017</td>
<td>-0.003</td>
<td>-0.005</td>
<td>-0.043</td>
<td>-0.006</td>
<td>0.044</td>
<td>-0.012</td>
<td>-0.020</td>
</tr>
<tr>
<td>Starchy vegetables</td>
<td>-0.011</td>
<td>-0.009</td>
<td>-0.002</td>
<td>0.002</td>
<td>0.049</td>
<td>-0.010</td>
<td>-0.060(^2)</td>
<td>-0.005</td>
<td>0.057(^2)</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>0.011</td>
<td>0.011</td>
<td>0.005</td>
<td>0.012</td>
<td>-0.018</td>
<td>-0.005</td>
<td>0.015</td>
<td>-0.006</td>
<td>-0.008</td>
</tr>
<tr>
<td>Grains</td>
<td>-0.025</td>
<td>-0.026</td>
<td>-0.014</td>
<td>-0.006</td>
<td>-0.069(^2)</td>
<td>-0.020</td>
<td>0.055(^2)</td>
<td>-0.010</td>
<td>-0.021</td>
</tr>
<tr>
<td>Milk</td>
<td>0.022</td>
<td>0.027</td>
<td>0.020</td>
<td>0.012</td>
<td>0.000</td>
<td>0.024</td>
<td>0.012</td>
<td>0.014</td>
<td>-0.028</td>
</tr>
<tr>
<td>Meat</td>
<td>0.022</td>
<td>0.019</td>
<td>-0.011</td>
<td>-0.022</td>
<td>-0.006</td>
<td>0.012</td>
<td>0.016</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Seafood</td>
<td>0.010</td>
<td>0.011</td>
<td>-0.017</td>
<td>-0.017</td>
<td>-0.058(^2)</td>
<td>-0.010</td>
<td>0.048</td>
<td>-0.012</td>
<td>-0.036</td>
</tr>
<tr>
<td>Nuts</td>
<td>0.027</td>
<td>0.031</td>
<td>0.009</td>
<td>0.008</td>
<td>0.033</td>
<td>-0.008</td>
<td>-0.044</td>
<td>-0.018</td>
<td>0.031</td>
</tr>
<tr>
<td>Legumes</td>
<td>-0.023</td>
<td>-0.020</td>
<td>0.041</td>
<td>0.031</td>
<td>-0.055(^2)</td>
<td>0.023</td>
<td>0.071(^2)</td>
<td>0.030</td>
<td>-0.063(^2)</td>
</tr>
<tr>
<td>Sugar</td>
<td>0.079(^2)</td>
<td>0.081(^2)</td>
<td>-0.022</td>
<td>-0.006</td>
<td>0.026</td>
<td>-0.003</td>
<td>-0.023</td>
<td>-0.026</td>
<td>0.026</td>
</tr>
<tr>
<td>Protein variety</td>
<td>0.040</td>
<td>0.045</td>
<td>-0.004</td>
<td>-0.010</td>
<td>-0.009</td>
<td>-0.002</td>
<td>0.006</td>
<td>-0.016</td>
<td>-0.007</td>
</tr>
<tr>
<td>Fruit &amp; vegetable variety</td>
<td>-0.003</td>
<td>-0.001</td>
<td>0.008</td>
<td>0.014</td>
<td>-0.053(^2)</td>
<td>-0.012</td>
<td>0.043</td>
<td>-0.009</td>
<td>-0.033</td>
</tr>
<tr>
<td><strong>Healthy Choice Items</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat</td>
<td>0.008</td>
<td>0.004</td>
<td>-0.023</td>
<td>-0.011</td>
<td>-0.042</td>
<td>-0.032</td>
<td>0.017</td>
<td>-0.034</td>
<td>0.015</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>-0.019</td>
<td>-0.024</td>
<td>-0.007</td>
<td>0.010</td>
<td>-0.082(^2)</td>
<td>-0.050</td>
<td>0.043</td>
<td>-0.039</td>
<td>-0.001</td>
</tr>
<tr>
<td>Trans fat</td>
<td>-0.009</td>
<td>-0.011</td>
<td>-0.003</td>
<td>0.018</td>
<td>-0.073(^2)</td>
<td>-0.036</td>
<td>0.052</td>
<td>-0.029</td>
<td>-0.036</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.057(^2)</td>
<td>-0.061(^2)</td>
<td>-0.006</td>
<td>-0.003</td>
<td>-0.049</td>
<td>-0.026</td>
<td>0.037</td>
<td>-0.001</td>
<td>-0.003</td>
</tr>
<tr>
<td>Lean meat</td>
<td>-0.017</td>
<td>-0.019</td>
<td>-0.024</td>
<td>-0.026</td>
<td>-0.088(^2)</td>
<td>-0.012</td>
<td>0.083(^2)</td>
<td>-0.003</td>
<td>-0.032</td>
</tr>
<tr>
<td>Low-fat milk</td>
<td>0.013</td>
<td>0.015</td>
<td>0.012</td>
<td>0.019</td>
<td>-0.056(^2)</td>
<td>-0.017</td>
<td>0.042</td>
<td>-0.021</td>
<td>-0.025</td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.030</td>
<td>-0.032</td>
<td>-0.001</td>
<td>0.000</td>
<td>-0.037</td>
<td>-0.023</td>
<td>0.028</td>
<td>-0.008</td>
<td>-0.012</td>
</tr>
<tr>
<td>Fiber</td>
<td>-0.016</td>
<td>-0.017</td>
<td>0.010</td>
<td>0.022</td>
<td>-0.114(^2)</td>
<td>-0.040</td>
<td>0.095(^2)</td>
<td>-0.025</td>
<td>-0.077(^2)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-0.024</td>
<td>-0.019</td>
<td>0.019</td>
<td>0.025</td>
<td>-0.015</td>
<td>-0.005</td>
<td>0.012</td>
<td>-0.001</td>
<td>-0.012</td>
</tr>
<tr>
<td>Whole fruit</td>
<td>0.030</td>
<td>0.030</td>
<td>0.012</td>
<td>0.022</td>
<td>-0.027</td>
<td>0.000</td>
<td>0.033</td>
<td>-0.005</td>
<td>-0.047</td>
</tr>
<tr>
<td>Whole grain</td>
<td>-0.008</td>
<td>-0.006</td>
<td>0.012</td>
<td>0.011</td>
<td>-0.085(^2)</td>
<td>-0.002</td>
<td>0.088(^2)</td>
<td>-0.001</td>
<td>-0.060(^2)</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age and sex. DGAI-2010, Dietary Guidelines Adherence Index 2010; FMD, flow-mediated dilation; SS, shear stress; CF-PWV, carotid-femoral pulse wave velocity; Al, augmentation index.

\(^2\)Adjusted \(P<0.05\) (Bonferroni adjustment for multiple comparisons: 0.05 / 250 correlations = 0.0002).
DGAI-2010 analyses, none of the subscores were significantly correlated with FMD (%), SS, FMD/SS, or deflation mean flow velocity.

**Discussion**

In this large, cross-sectional study we have shown that adherence to the 2010 Dietary Guidelines for Americans is significantly associated with hyperemic flow and arterial stiffness but not related to endothelial function as measured by brachial flow-mediated dilation. To our knowledge, this is the largest study to examine cross-sectional relationships between diet and vascular health and the first to report that overall diet is related to hyperemic flow. It is also the first to report an interaction between diet and age on augmentation index: we found that in adults aged 50 and older, arterial stiffness decreases with increasing diet quality, but no such relationship is present in adults younger than 50.

While the FMD procedure is widely used to assess endothelial function in nutrition research, little attention is typically given to the flow stimulus itself. However, as previously shown in the Framingham sample (200), when FMD is impaired, this may be partially indicative of a reduced stimulus and not necessarily solely due to brachial endothelial dysfunction. Under conditions of reduced flow, such as the 5-min occlusion period in the FMD test, the forearm microvasculature dilates, and when flow is restored (i.e. when the cuff is deflated), the reduced microvasculature resistance results in hyperemic flow. Hyperemic flow creates a shear stress stimulus that induces mechanical deformation of structures on the endothelial cell membranes, which is detected by the endothelial cell, stimulates production of vasodilating substances (e.g. nitric oxide, endothelial-derived hyperpolarizing factor, prostaglandin) and causes vasodilation of the vascular smooth muscle (41). However, in the presence of cardiovascular risk factors, forearm microvascular dilation during reduced flow is attenuated and the subsequent hyperemic flow is diminished (200). Shear stress and the hyperemic flow ratio are independent predictors of cardiovascular outcomes and have been shown to correlate with cardiovascular risk factors to a greater degree than FMD (203-205). However, the extent to which diet is related to these measures is unclear. Few nutrition studies utilizing FMD have measured flow (78, 134, 138, 139, 153, 164, 206-209), and none of these have reported significant treatment effects. In the present analysis, we have shown that increased adherence to the 2010 Dietary Guidelines is associated with
reduced baseline flow velocity and a greater hyperemic flow velocity ratio. As the hyperemic flow ratio is determined by both baseline and deflation flow velocity, and deflation flow velocity was not related to diet in our study, it appears that diet is primarily affecting baseline flow velocity. While part of this relationship is due to the association between DGAI-2010 and cardiovascular risk factors known to affect flow velocity (indicated by the strength of the relationship diminishing as more risk factors were included in the model as covariates), it remains statistically significant even after accounting for these risk factors. This suggests that diet may affect the microvasculature via other mechanisms than the well-known pathways of aging, atherosclerosis, and metabolic dysregulation. Examination of the subscores that appear to be driving the overall association with DGAI-2010 may be useful. We found that fruit, dark green vegetables, grains, legumes, lean meat, fiber, and whole grains were positively associated with the hyperemic flow ratio, while starchy vegetables were inversely associated. All of these variables (except starchy vegetables) plus seafood, variety of fruits and vegetables, saturated fat, trans fat, and low-fat milk were inversely associated with baseline hyperemic flow velocity. Intervention studies are needed to highlight key foods with regard to flow velocity and to identify potential mechanisms through which they exert their effects.

In the present study, diet was significantly related to augmentation index but not carotid-femoral PWV. Previous nutrition studies have primarily used PWV to assess arterial stiffness, and have reported positive associations with overall dietary patterns (high in meat/alcohol, low in fruit/dairy) (107) and increasing sodium intake (118, 119), and inverse associations with moderate alcohol consumption (109-111). The nutrition evidence for associations with augmentation index is limited: augmentation index is reported to decrease with increasing adherence to the Mediterranean diet in children (116) and decrease with consumption of non-soy legumes in adults with peripheral artery disease (210). There are mixed results with regard to moderate alcohol consumption (108, 109, 112), and no significant relationships were found with cocoa (211), milk or protein (212), and a cranberry juice cocktail (121). In the present study, we found that augmentation index decreases with greater adherence to the Dietary Guidelines, and age-stratified analyses revealed that this relationship maintained statistical significance only in the older participants (≥ 50 years). Age is the predominant risk factor for cardiovascular disease (37) and advancing age increases risk despite
control of modifiable lifestyle factors (213-216). Our results suggest that for older adults, following a diet that more resembles the 2010 Dietary Guidelines may reduce age-related arterial stiffness; in contrast, for younger adults who have yet to experience vascular decline due to age, diet may not affect arterial stiffness. Longitudinal studies of diet and vascular health and intervention studies with long-term follow-up are needed to fully understand whether diet can slow age-related vascular decline.

There is extensive evidence that FMD can be improved by dietary interventions. Individual foods and beverages that can improve FMD (%) by up to 73% include nuts (141, 142, 189, 190), flavonoid-rich chocolate (126-130), black tea (139, 140), fruit juice (133-136), and red wine (135, 137), and dietary patterns include those that are low in fat (147, 161, 164, 217), rich in unsaturated fat (78, 147, 150), based on the Mediterranean diet (148, 149, 153, 154), or rich in protein (218). However, the extent to which overall diet can maintain or impair endothelial function is unclear. To date, only one large epidemiological study has examined the cross-sectional correlation between diet and FMD. The Multi-Ethnic Study of Atherosclerosis (MESA) study conducted FMDs in over 3000 adults and found that among women (but not men), regular fish intake was positively associated with FMD (125); however, fish intake was the only component of diet assessed. The present study is the first to look at overall diet in a large, cross-sectional sample and to examine multiple components of diet in relation to FMD. Interestingly, only added sugar and total dietary cholesterol were significantly correlated with FMD (mm), and no subcomponents or the total DAGI-2010 score were significantly related to FMD (%). This is surprising given the substantial evidence from intervention studies that indicate diet is related to FMD. The null findings may be explained by the limitations of nutrition epidemiology and food frequency questionnaires in assessing habitual diet. This method relies on participants’ memory and accurate description of foods consumed during the prior year, limits responses to a predetermined list of foods and (in the Harvard version) only three non-listed items, and is susceptible to response bias (i.e., participants reporting what they consider socially acceptable rather than true intake). In contrast, intervention studies, particularly those in which food is provided to the participants, can more accurately measure consumption of the food or dietary pattern of interest, and by controlling consumption of other foods, establish the efficacy of a particular food or dietary pattern in modifying endothelial function.
Several limitations of the study must be noted. First, due to the cross-sectional, observational nature of the study utilizing single assessments of diet and vascular health, we are unable to draw conclusions about causation and related mechanisms. The Framingham cohorts are overwhelmingly white; thus, generalization to other races or ethnicities is limited. However, the use of this large, well-characterized sample enables us to examine the relationship between diet and vascular health statistical consideration of cardiovascular risk factors. In addition, the wide age range of this sample (19-89 years) allowed us to examine the relationship between diet quality and vascular health across the lifespan. Habitual diet was assessed on a single occasion with a self-reported food frequency questionnaire, which may not be representative of the diet followed earlier in life. It is possible that diet quality improved after a clinical diagnosis that had previously exacerbated arterial stiffness or endothelial dysfunction, which would mask an association in the present analysis. However, previous studies have shown that dietary changes can benefit vascular health in individuals with established cardiovascular disease, indicating that even recent dietary changes can influence arterial stiffness and endothelial dysfunction (118, 134, 138, 140, 217). Future studies should examine how habitual diet changes with time, and whether such age-related dietary shifts are associated with age-related vascular decline. Lastly, we chose to define diet quality only with respect to the 2010 Dietary Guidelines for Americans, and as such cannot draw conclusions about other dietary patterns (e.g. a Mediterranean-style diet, a low-carbohydrate diet, or a high-protein diet). Future studies are needed to determine how other dietary patterns or relative intake of macronutrients are related to arterial stiffness and endothelial dysfunction. The results of the exploratory analysis with the subscores of the DGAI-2010 suggest which aspects of habitual diet are most related to vascular decline, and can be used to guide future research.

In conclusion, we have shown that adherence to the 2010 Dietary Guidelines for Americans is significantly associated with measures of blood flow velocity and arterial stiffness but not related to brachial flow-mediated dilation in the Framingham Heart Study. Importantly, we have demonstrated that diet may be particularly important for vascular health in adults older than 50 years. Our results suggest a link between diet quality and vascular health and as such may be useful for designing interventions to treat or prevent age-related vascular decline.
CHAPTER 5. TEST-RETEST RELIABILITY OF PERIPHERAL ARTERIAL TONOMETRY IN THE METABOLIC SYNDROME

Abstract

Background – Endothelial dysfunction is an important contributor to atherosclerosis and cardiovascular disease. However, routine assessment via angiography or flow-mediated dilation is difficult due to technical limitations. Peripheral arterial tonometry (PAT) is a promising alternative method for non-invasive assessment of endothelial dysfunction.

Methods – This study assessed the test-retest reliability of PAT in adults with the metabolic syndrome (n=20) and provides sample size and power estimates for study design. Participants completed 5 PAT tests each separated by 1 week.

Results – The PAT-derived reactive hyperemia index (RHI) showed robust repeatability (intra-class correlation = 0.74). A parallel arm study powered at 0.90 would require 22 participants to detect an absolute change in RHI of 0.40 units (equal to ~25% change in this sample), whereas a crossover study would require 12 participants.

Conclusions – In conclusion, we have demonstrated that PAT can be used to assess endothelial dysfunction in adults with the metabolic syndrome as reliably as in healthy samples.
Introduction

Over the last two decades, the critical role of endothelial dysfunction in atherosclerosis has been documented in in vitro studies, animal models, longitudinal studies, and intervention trials (53). Translation of this knowledge to clinical settings has been limited by the fact that tests of endothelial function are either very invasive (angiography, intrabrachial artery infusion of acetylcholine) or difficult to standardize for efficient clinical use (flow mediated dilation, FMD, via ultrasound) (219). Peripheral arterial tonometry (PAT) has been proposed as an alternative method for assessing endothelial dysfunction (61). The PAT test is non-invasive, less operator-dependent and potentially less expensive to perform. Similar to a brachial artery FMD assessment, change in blood flow is measured before and during reactive hyperemia induced by upper forearm cuff occlusion resulting in lower forearm ischemia. A low reactive hyperemia index (RHI) has been shown to predict coronary endothelial dysfunction with 80% sensitivity and 85% specificity in a clinical setting (64). Low scores also independently predicted adverse cardiovascular events over a 7-year follow-up (220).

Before PAT can be widely adopted for clinical risk assessment, it is important to understand day to day variations. While there have been several reports of robust test-retest reliability of PAT, these studies have been conducted in healthy individuals or in patients with established disease (221-225). Given the increased risk of diabetes and cardiovascular disease in adults with the metabolic syndrome (226, 227), it is important to verify the reliability of PAT and to provide population-specific power and sample size estimates that can guide clinical trial design. Additionally, given that a reduced RHI has been associated with hyperglycemia (62, 228), it is useful to investigate if daily variations in glucose and insulin influence PAT reliability. Therefore, the purpose of the present study was to assess PAT test-retest reliability in adults with the metabolic syndrome and to use variability metrics to provide sample size and power estimates for a range of study designs. Secondary purposes were to examine the correlation between PAT scores and fasting glucose and insulin, and to determine whether PAT variability was associated with fluctuations in fasting glucose and insulin.
Methods

Participants
These data were collected as part of a study on postprandial glycemia in individuals with the metabolic syndrome (229). Men and women aged 40-65 years with a body mass index (BMI) >30 kg/m² were recruited for the study through local advertisements and the clinic volunteer roster. All participants were required to meet the criteria for the metabolic syndrome as defined by the National Cholesterol Education Program Adult Treatment Panel III (178), be in otherwise good health, and not be taking any medications known to affect glucose metabolism. Forty-one individuals were screened for the study, of which 18 failed the screening criteria and 3 withdrew from the study prior to randomization. A total of 20 participants completed the full protocol, and their characteristics at screening are reported in Table 5.1.

Protocol
As part of the postprandial study, participants underwent testing on 5 occasions, each separated by a minimum 1 week period. All tests were performed in the morning after a 12-hour fast. Endothelial function and arterial stiffness were assessed via PAT (EndoPAT, Itamar Medical Ltd, Caesarea, Israel). Vascular tests were performed in a sitting position in a quiet, dimly-lit, temperature-controlled room (70-75°F). Thimble-shaped pneumatic probes were applied to the index fingers of each hand, and an occlusion cuff connected to a rapid cuff inflator (Hokanson, Bellevue, WA) was applied to

<table>
<thead>
<tr>
<th>Table 5.1. Participant characteristics at study enrollment (n=20)</th>
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</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
</tr>
</tbody>
</table>
one forearm. Following a 10-minute rest period, PAT signals were recorded continuously during a 5-minute baseline period, a 5-minute occlusion period, and a 5-minute post-deflation period. During the occlusion period, the cuff was rapidly inflated to 250 mmHg to induce ischemia in one arm.

At the conclusion of each test, proprietary EndoPAT software calculated two indices that approximated endothelial dysfunction and arterial stiffness. The RHI was calculated as follows: the ratio of the occluded arm’s mean pulse wave amplitude at 90-150 seconds post-deflation to the mean amplitude of the same arm at baseline, divided by the same ratio from the control arm, the quotient of which is multiplied by a proprietary baseline correction factor (Itamar Medical Ltd). An alternative method of calculating RHI, designed by Framingham Heart Study researchers (fRHI), was also utilized. The fRHI analyzes the data from 90-120 seconds post-deflation, does not include the baseline correction factor, and applies a natural logarithmic transformation to the final ratio (62). Evidence from the Framingham Heart Study suggests that there is a stronger correlation between fRHI and cardiovascular risk than RHI (62). For both RHI and fRHI, lower scores indicate greater endothelial dysfunction.

Arterial stiffness was approximated by the augmentation index (AI), which is calculated from the pulse waveform collected during the baseline period, through software identification of the systolic peak ($P_1$) and reflected wave ($P_2$) inflection points. The difference between these peaks, presented as a percentage of the peak wave ($AI = (P_1 - P_2) / P_1 \times 100$), represents the degree to which arterial stiffness increases central systolic blood pressure. Since heart rate can significantly influence the pulse waveform (230), an alternative way of presenting AI is to standardize it to a heart rate of 75 bpm ($AI@75$). For both AI and AI@75, higher scores indicate greater arterial stiffness.

Fasting blood samples were obtained by finger prick and intravenous cannulisation (BD Blunt Plastic Cannula). Glucose analysis was performed using an YSI model 2300 STAT analyzer (Yellow Springs, OH) and insulin levels were measured using enzyme immunoassay kits (Alpco Diagnostics and Millipore).

The study protocol was approved by the Western Institutional Review Board (Seattle, Washington), and written informed consent was obtained from all participants prior to starting the study. All tests were completed at Glycemic Index Laboratories Inc., Toronto, Canada.
**Statistical Analyses**

All analyses were conducted using Statistical Analysis Software (SAS v9.2, Cary, NC). Variables were tested for normality and a natural log transformation was applied where appropriate. Variability in PAT metrics and metabolic parameters across the 5 visits were investigated using the mixed models procedure, with participant treated as a random factor and visit treated as a fixed effect. Test-retest reliability was primarily assessed with the intra-class correlation (ICC), calculated as $S_{b}^{2} - S_{w}^{2} / S_{b}^{2} + S_{w}^{2}$, where ‘$S_{b}^{2}$’ represents the between-subjects variance and ‘$S_{w}^{2}$’ represents within-subjects variance (231). As in previous studies, the ICC was computed using raw, non-normalized data for each individual and a mean ICC is reported for each variable (222). A second measure of variability, the coefficient of variation (CV), was appropriate for RHI given its non-negative values (232). CV was calculated as [SD / mean] x 100. Correlation and paired mean differences were also used to examine stability of each measurement over time. We examined correlations among RHI, fRHI, AI, and AI@75 and day to day fluctuations in fasting glucose and insulin. Finally, we performed power calculations to produce sample sizes for crossover and parallel-arm designs. Tables and figures depict mean ± SEM unless otherwise noted.

**Results**

Daily and overall means of PAT parameters, glucose, and insulin are displayed in Table 5.2. As expected, correlations between paired measurements (i.e. visit 1 versus visit 2; visit 2 versus visit 3) were statistically significant ($p < 0.05$) and moderate to high in strength ($r$'s ranging from 0.22 to 0.87). The CV for RHI was 23.6%, and the ICC for both RHI and fRHI was 0.74. The ICC for AI and AI@75 were 0.88 and 0.86, respectively. The primary measure of variability was the average of the absolute difference between each pair of visits, and is shown in Table 5.2. Variability in PAT was not associated with variability in glucose or insulin, and PAT results were not associated with fasting levels of glucose or insulin across the 5 visits (Figure 5.1).

Table 5.3 contains sample size calculations for both parallel arm and crossover study designs for RHI, fRHI, and AI@75 for varying magnitudes of treatment effects. For all sample sizes, $\alpha = 0.05$ and power set at 0.80 or 0.90. For example, a parallel arm study powered at 0.90 would require 22 participants to detect an absolute change in RHI of 0.40 units (equal to ~25% change in our sample), whereas a crossover study would
Table 5.2. Fasting peripheral arterial tonometry and metabolic parameters

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Grand Mean</th>
<th>Mean Variability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHI</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>fRHI</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>AI</td>
<td>14.3 ± 3.8</td>
<td>8.0 ± 3.8</td>
<td>14.4 ± 4.0</td>
<td>12.8 ± 3.9</td>
<td>11.7 ± 3.1</td>
<td>12.2 ± 1.6</td>
<td>8.6 ± 1.1</td>
</tr>
<tr>
<td>AI@75</td>
<td>14.0 ± 3.5</td>
<td>7.6 ± 3.2</td>
<td>12.3 ± 3.7</td>
<td>11.2 ± 3.3</td>
<td>10.5 ± 2.8</td>
<td>11.1 ± 1.5</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>Blood Glucose (mmol/l)</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>5.2 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Serum Insulin (mU/ml)</td>
<td>11.1 ± 1.1</td>
<td>10.3 ± 1.1</td>
<td>9.6 ± 1.1</td>
<td>11.4 ± 1.2</td>
<td>10.7 ± 1.2</td>
<td>10.6 ± 0.5</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

Data are mean ± standard error. RHI: reactive hyperemia index; fRHI: Framingham reactive hyperemia index; AI: augmentation index; AI@75: augmentation index standardized for heart rate of 75 bpm.

*Calculated as average of the absolute value of the difference in each measure between each pair of visits (e.g. |visit 1 - visit 2|, |visit 1 - visit 3|, |visit 2 - visit 3|, etc.)
Figure 5.1. Relationship between peripheral arterial tonometry-derived measures of endothelial dysfunction (reactive hyperemia index) (A,B) and arterial stiffness (augmentation index standardized for heart rate of 75 bpm) (C,D) with fasting glucose (A,C) and insulin (B,D) levels. All correlations were statistically non-significant.
Table 5.3. Sample sizes required to detect significant treatment effects in PAT variables in parallel arm and crossover study designs

<table>
<thead>
<tr>
<th>Magnitude of effect</th>
<th>RHI</th>
<th>fRHI</th>
<th>Al@75</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parallel arm</td>
<td>Crossover</td>
<td>Parallel arm</td>
</tr>
<tr>
<td></td>
<td>0.80</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>0.20</td>
<td>63</td>
<td>83</td>
<td>32</td>
</tr>
<tr>
<td>0.40</td>
<td>17</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>0.60</td>
<td>8</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>0.80</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>1.00</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

RHI: reactive hyperemia index; fRHI: Framingham reactive hyperemia index; Al@75: augmentation index standardized for heart rate of 75 bpm.

*Mean = 1.67, SD = 0.39.
†Mean = 1.67, SD = \( \sqrt{2} \times SD_{\text{within}} = 0.39. 
‡Mean = 0.24, SD = 0.27.
§Mean = 0.24, SD = \( \sqrt{2} \times SD_{\text{within}} = 0.27. 
¶Mean = 11.14, SD = 14.66.
‖Mean = 11.14, SD = \( \sqrt{2} \times SD_{\text{within}} = 11.21. \)
require only 12 participants. For AI@75, a parallel arm study powered at 0.90 would require 284 participants to detect an absolute change of 4.0 units (equal to ~50% change in our sample), while a crossover study would require 85 participants.

Discussion

In this study of adults with the metabolic syndrome, we have demonstrated excellent test-retest reliability of PAT-derived measures of endothelial function and arterial stiffness. Our results are similar or more robust to those reported in previous studies of healthy participants (221-224), coronary artery disease patients (225), and the general population (228). In contrast to large epidemiological studies that found positive associations between RHI and hyperglycemia (62, 228), we observed no relationship between RHI or fRHI and fasting glucose or insulin levels.

Brachial FMD has been used in research settings for decades to measure endothelial dysfunction. As summarized in a recent review (219), this method has been useful in estimating cardiovascular risk and evaluating interventions, but the associated costs, operator-dependence, and susceptibility to external factors have limited its use in large-scale trials and for clinical decision making. Additionally, FMD is technically challenging to complete and laboratories have individual protocols for performance and standardization. Automated edge-detection software and published methodological guidelines are helpful but substantial variability still exists. Digital PAT testing presents a promising alternative to FMD by providing a similar assessment with a lower cost, an operator-independent procedure, and, as we have shown, little day-to-day variability.

As an emerging technique, the validity, clinical significance, and prognostic value of PAT is still being established. Similar to FMD, the RHI has been shown to correlate with coronary artery vasodilation (64) and be nitric oxide dependent (233). In both the Framingham Heart Study and the Gutenberg Heart Study, the RHI was associated with traditional cardiovascular risk factors such as obesity, dyslipidemia, hypertension, diabetes mellitus, and smoking (62, 228). Low RHI has been observed in individuals with established cardiovascular disease and those at high risk compared to individuals with low to moderate risk (57, 234). Importantly, an RHI below 1.49 has been associated with a higher rate of adverse cardiovascular events over a 7-year period (220). However, studies comparing RHI and FMD indicate that they are measuring distinct components of vascular health. Three studies reported positive correlations between FMD and RHI ($r =$
Given that FMD and RHI are both associated with cardiovascular disease but do not appear to be equivocal assessments, it is plausible that assessing endothelial dysfunction in different vascular beds could substantially contribute to risk stratification. In contrast to the PAT-derived measures of endothelial dysfunction, the validity and clinical significance of PAT-derived measures of arterial stiffness have yet to be determined. Arterial stiffness is traditionally assessed via carotid-femoral pulse wave velocity or radial applanation tonometry (238-240), and both of these methods have demonstrated that greater arterial stiffness is independently predictive of cardiovascular morbidity and mortality (45, 48, 241, 242). To our knowledge, no studies have validated PAT with pulse wave velocity, while two have compared it to radial applanation tonometry with promising results (correlations of 0.68 to 0.88) (243, 244). While the PAT device automatically provides both RHI and AI for each test, neither the large epidemiological studies that have examined the association between cardiovascular disease and RHI nor the clinical trials that demonstrated the prognostic significance of RHI present any data for AI (62, 220, 228). Therefore, the clinical significance of PAT-derived arterial stiffness is unknown. The present study has shown that AI has a high degree of test-retest reliability, with an ICC of 0.86, but more work is needed to elucidate the value of this measurement.

Large population-based studies of vascular health have suggested that hyperglycemia is related to both endothelial dysfunction and arterial stiffness (43, 62). Greater glycemic variability, measured via continuous subcutaneous glucose monitoring, is also associated with endothelial dysfunction in adults with normoglycemia, hyperglycemia, and Type 2 diabetes (245). Our laboratory has demonstrated that variability in both fasting glucose and insulin are directly associated with variability in FMD in Type 2 diabetes (246). In the current study, we did not find any relationship between PAT-derived measures of endothelial dysfunction and arterial stiffness with variability of fasting glucose and insulin. This may be due to the relatively narrow range of fasting glucose (3.94 – 6.84 mmol/l) and insulin (3.0 – 22.32 mU/ml) levels that we observed, which are below the diagnostic threshold for type 2 diabetes. Notably, in our previous study of PAT reliability in young healthy adults (222), day to day change in fasting glucose levels were not significantly correlated day to day change in RHI. Sample size must be considered here, as larger epidemiological studies have sufficient power to
detect moderate correlations. Additionally, the pathophysiology of vascular health is complex and influenced by a host of factors (219). Endothelial cells in separate vascular beds have differing structural and metabolic components, which could influence how they are affected by hyperglycemia (247). It is possible that PAT is not as susceptible to variations in glycemia as FMD and carotid-femoral PWV, or that small elevations in glucose and insulin do not affect vascular health to the clinically-evident degree that substantial (i.e. diabetes-level) elevations do. Nevertheless, researchers are encouraged to consider assessing and controlling for hyperglycemia in studies of vascular health.

**Study Limitations**

Our sample size of 20 adults is relatively small, though comparable to previous studies of PAT reliability (221, 222, 225, 248). The 5 testing sessions occurred during a 5-week period, and therefore we cannot draw conclusions about PAT reliability over longer periods of time. We did not collect data on other biological or physiological parameters previously found to be related to vascular health (such as lipids, lipoproteins, and blood pressure) and cannot comment on how these variables may influence daily variation in RHI or AI. In addition, we did not collect data on medical therapy unrelated to the primary outcome of the larger clinical trial (glucose metabolism), and participants may have been taking medications known to affect vascular health (e.g. statins, anti-hypertensive medications). However, we believe that the crossover design of the study, which controls for individual differences to a greater degree than parallel arm studies, attenuates any effect that other medications may have had on the results. A key strength of our study is the metabolic syndrome sample, as no previous studies have examined PAT reliability in this population, and the five repeated measurements, whereas previous studies have included only two repeated measurements. By considering the within-subject variability that we observed, researchers will be better equipped to design adequately powered clinical trials to assess the effect of interventions on digital PAT.

**Conclusion**

Endothelial dysfunction and arterial stiffness are important components of vascular health that can be used to classify CVD risk and evaluate interventions. PAT testing is a novel approach with promising potential to provide reliable and clinically meaningful assessment of vascular health. While more research is needed to fully elucidate the clinical and prognostic significance of PAT, the current evidence indicates that PAT is a useful technique that can be incorporated relatively easily into research and clinical
laboratories. In this study, we have demonstrated that PAT can be used to assess endothelial dysfunction and arterial stiffness in adults with the metabolic syndrome as reliably as in healthy samples.
CHAPTER 6. DISCUSSION

This dissertation examined the relationship between diet and vascular risk factors and also tested the reliability of a novel method of assessing vascular health. This research utilized a variety of study designs and analytical approaches to demonstrate that nutrition is an effective strategy for reducing or moderating cardiovascular risk.

First, the Pistachio study (chapter 3) provides new evidence that pistachio consumption improves multiple cardiovascular risk factors in adults with well-controlled type 2 diabetes. CVD is the leading cause of death in this population, and diabetes is considered a CVD risk equivalent (meaning that the risk of a future cardiovascular event in an individual with type 2 diabetes is the same as someone who has already had a cardiovascular event). Therefore, aggressive prevention strategies are employed within diabetes, and treatment goals for lipids, glucose, and blood pressure are lower than in the general population (171). For many years, the primary goal of dietary modification for cardiovascular risk reduction has been to reduce total and saturated fat consumption. However, by reducing total fat consumption, individuals typically reduce consumption of healthy, unsaturated fats and always increase consumption of carbohydrates. Carbohydrates, especially highly-processed ones, raise blood sugar and triglycerides, and low-fat diets reduce HDL cholesterol. Previous studies of nuts (mixed nuts (249), walnuts (250, 251), and almonds (252)) have shown that daily consumption results in a more optimal CVD profile in adults with type 2 diabetes than low-fat diets; for the first time, we have shown that pistachio consumption has similar benefits. The American Diabetes Association recommends a DASH (Dietary Approaches to Stop Hypertension)-style diet for CVD risk management, which emphasizes fruits, vegetables, low-fat dairy, whole grains, poultry, fish and nuts (253). However, this recommendation is directed toward “patients with diabetes at risk for CVD” (page S70) and supported only by level “C” evidence (“supportive evidence from poorly controlled or uncontrolled studies; conflicting evidence with the weight of the evidence supporting the recommendation,” page S15 (171)). Our study and other recent publications (249-252) indicate that nut consumption can be particularly beneficial to cardiovascular risk reduction in type 2 diabetes, and merit consideration by the American Diabetes Association regarding a definitive recommendation for regular nut consumption.
The second study (chapter 4) provides new evidence that overall diet is related to measures of blood flow velocity, an important component of microvascular health. While there is substantial evidence that diet is related to endothelial dysfunction (primarily assessed with brachial flow-mediated dilation (FMD)), there is also evidence that changes in flow velocity and shear stress are key determinants of the FMD response (200). We observed no relationship between adherence to the Dietary Guidelines and FMD, but our results suggest that diet may be influencing endothelial function (at least partly) through changes in microvascular function. Our exploratory analysis of the DGAI subscores suggests particular food groups or nutrients that may be involved. This list includes nutrients, such as saturated and trans fat that have been shown to affect endothelial function in intervention studies (146, 147, 152). Interestingly, nut consumption per se was not associated with any conventional measures of vascular health in this cross-sectional analysis, nor did pistachio consumption specifically affect endothelial function in our Pistachio study. While the results of these two studies suggested that nuts cannot affect vascular health, several intervention studies have shown that mixed nuts, walnuts, and almonds improve endothelial function in healthy or dyslipidemic adults (141, 142, 189, 190). Further research is needed to conclusively determine if nuts can modify vascular health. Nevertheless, considering the findings from this Framingham analysis and the Pistachio study, the present work provides evidence that diet is related to cardiovascular health and can be useful in designing interventions to reduce cardiovascular risk.

The traditional cardiovascular risk factors of age, gender, dyslipidemia, hypertension, diabetes, and smoking have been successful in identifying individuals at high risk of cardiovascular events (216); however, at least 25% of individuals experiencing coronary events had no prior symptoms or traditional risk factors for CVD (254). Therefore, there is a need to identify additional characteristics that can improve risk assessment and more thoroughly evaluate the efficacy of interventions. This dissertation examined non-traditional risk factors: systemic hemodynamics and heart rate variability at rest and during acute mental stress, ambulatory blood pressure, endothelial function, and arterial stiffness. These measures are not commonly used in clinical settings due to technical challenges, patient burden and cost. Furthermore, normative values are not available for these tests, making it difficult to evaluate whether an individual’s results are normal, high, or low. However, these techniques are useful for research settings because they
enable researchers to distinguish between treatments that may have equivalent effects on traditional cardiovascular factors. For example, in the pistachio study (chapter 3), we observed no difference between the pistachio and control diets in participant weight, resting blood pressure, or even LDL cholesterol and fasting blood sugar (data not included in this dissertation), which suggests that there is no difference between the low-fat control diet and the moderate-fat pistachio diet with regard to CVD risk. However, by additionally assessing systemic hemodynamics and heart rate variability at rest and during acute mental stress as well as ambulatory blood pressure, we concluded that the pistachio diet does result in a more optimal risk profile than a low-fat diet. The Framingham study (chapter 4) also provided evidence that diet is related to measures of vascular health that are not typically measured in clinical settings. Flow-mediated dilation and carotid-femoral pulse wave velocity require specialized equipment and are technically difficult to perform; however, they are each able to predict cardiovascular risk above and beyond traditional risk factors (45-48, 57-60). Peripheral arterial tonometry (PAT) is a novel method of assessing endothelial function and arterial stiffness that is better suited to clinical application, also independently predictive of future events (220), and as the PAT reliability study shows (chapter 5), can be reliably measured in high-risk adults. By incorporating these assessments into research, scientists can better evaluate the efficacy of nutrition interventions and begin to understand the mechanisms through which diet affects vascular health. Based on emerging findings from clinical trials, it remains to be seen whether PAT is as sensitive to diet as FMD.

Recommendations for Future Research

Several recommendations for future research can be derived from the present work. Building upon the Pistachio study (chapter 3), future studies on nuts should incorporate measures of blood pressure under resting and ambulatory conditions. To our knowledge, our study is the first to assess the effects of nut consumption on 24-hour ambulatory blood pressure, and while we found a significant reduction in systolic blood pressure, this was observed in only a subset of our sample. Larger studies that include ambulatory monitoring as a primary outcome are needed to further elucidate the effects of nuts on blood pressure, in samples of type 2 diabetes, other high-risk populations, and healthy adults. Additionally, this study established the efficacy of pistachio consumption in improving multiple CVD risk factors under controlled-feeding conditions. In order to translate these findings into public health and clinical practice, it is necessary to conduct
a free-living study in which pistachios are provided or simply recommended for consumption. Outcomes should include actual consumption of pistachios, degree to which pistachios are added to the habitual diet versus replace other foods, changes in daily calories, effects on traditional risk factors (weight, cholesterol, blood sugar, blood pressure), and effects on other risk factors such as ambulatory blood pressure and vascular health.

Based on the results of the Framingham study (chapter 4), future studies on nutrition and vascular health should include measures of flow velocity. To our knowledge, our study is the first to demonstrate a relationship between diet and flow velocity; this finding should be replicated in other cross-sectional studies and can guide design of intervention studies that could show whether diet can improve measures of flow velocity and if/how changes in the flow stimulus affect FMD results. Also based on the PAT reliability study (chapter 5), researchers should consider assessing FMD and PAT simultaneously for three primary reasons: 1) PAT testing can be reliably conducted in multiple populations, 2) PAT provides measures of arterial stiffness that cannot be obtained with FMD but may be related to diet, and 3) PAT and FMD may be measuring different aspects of vascular health. Inclusion of both FMD and PAT in study protocols, as well as the other novel markers examined in this dissertation, could improve our understanding of how nutrition affects CVD risk.

**Conclusion**

In conclusion, the burden of CVD is substantial in the modern world, and there are many techniques that can be used to identify individuals at greatest risk of cardiovascular events. Dietary modification continues to be the first line of therapy recommended for risk reduction, and this dissertation demonstrates that both overall diet and specific foods such as nuts are related to vascular health. These results can be used to inform recommendations and guide future research on nutrition and vascular health.
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VITA
Katherine A. Sauder

EDUCATION

Doctor of Philosophy, Biobehavioral Health, The Pennsylvania State University 2014
Master of Science, Biobehavioral Health, The Pennsylvania State University 2011
Bachelor of Arts, Psychology, Millersville University of Pennsylvania 2006

FELLOWSHIPS

National Research Service Award, National Institute of Aging 2012-2014
Predoctoral Fellowship, American Heart Association 2012-2014
(declined in favor of competing NIH award)

AWARDS

Alumni Association Dissertation Award, The Pennsylvania State University 2014
American Society for Nutrition Travel Award 2010
NIH Office of Dietary Supplements Research Practicum Delegate 2010
Fund for Excellence in Graduate Recruitment, The Pennsylvania State University 2009

SELECTED PEER-REVIEWED PUBLICATIONS