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DEPARTMENT OF BIOLOGY

THE CORRELATION BETWEEN PULSE WAVE VELOCITY AND FLOW MEDIATED DILATION IN ADULTS WITH ENLARGED WAIST CIRCUMFERENCE

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ABSTRACT

Background: Cardiovascular disease (CVD) is the leading cause of death in the United States, and develops as the health of the arterial wall deteriorates. A greater knowledge of changes in the vasculature that precede overt disease, as determined by pulse wave velocity (PWV) and flow-mediated dilation (FMD) in individuals with enlarged waist circumference (WC), will assist in understanding the etiology of CVD and in identifying targets for risk minimization.

Methods: Cross-sectional analyses of data collected from a subsample of participants with enlarged WC (n=39) in the Habitual Diet and Avocado Trial (HAT) were conducted. Demographic information and physical measures were collected. Pulse wave velocity and pulse wave analysis were performed. Flow-mediated dilation was assessed via vascular ultrasound.

Results: This cohort of adults was 82% female. The average age at time of study was 46 ± 13 years. Mean WC was 103.9 ± 11.5 cm. A significant, weak to moderate, negative correlation was found between PWV and FMD (r= -0.37, p=0.02). However, PWV and FMD were no longer significantly correlated after adjustments for brachial SBP, central SBP, central pulse pressure, or age. PWV and FMD were significantly correlated in the subgroups above the median WC (r = -0.63, p=0.004) and above the median age (r= -0.47, p=0.04), but were non-significantly correlated in the subgroups below the medians.

Conclusions: The significant, weak to moderate, inverse association between PWV and FMD in adults with enlarged WC is similar to results found in other populations. The association was significant in subgroups above median WC and above median age, but not below. This suggests that PWV and FMD detect physiologically distinct mechanisms that converge with increasing age and adiposity.

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Chapter 1

Background and Significance

Burden of CVD

Cardiovascular disease (CVD) is a collection of chronic diseases affecting the heart and blood vessels, and is the leading cause of death in the United States (Benjamin et al., 2019). In 2018, projections estimated that more than 45.1% of adults in the US would live with CVD by 2035 (Benjamin et al., 2018). New reports suggest that this estimate has already been surpassed, with a prevalence of 48.0% in adults nationally (Benjamin et al., 2019). CVD alone is responsible for 14% of total health expenditure in the US. (Benjamin et al., 2018). Direct costs have more than doubled in just under two decades, reaching $213.8 billion in 2014 to 2015 (Benjamin et al., 2019). Costs are expected to continue to increase, especially for populations over 65 years of age (Benjamin et al., 2019).

CVD is a significant contributor to global disease burden as well. It was responsible for approximately 17.6 million deaths worldwide in 2016, and is projected to cause over 23.6 million deaths in 2030 (Benjamin et al., 2019). Similar to the US, countries like Germany and China also cite CVD as a leading cause of mortality and the largest portion of health care costs (Dornquast et al., 2016); (Bundy & He, 2016). This is likely due to the fact that many aspects of Western life, such as maintaining an unhealthy diet or a sedentary lifestyle, are primary risk factors for CVD. Communities living more traditional hunter-gatherer lifestyles, are relatively free of such risk factors. In a population of indigenous people of the Bolivian Amazon, for instance, 85% of middle-aged individuals were free of coronary artery calcium (Kaplan et al.,

2017). One study has suggested that the prevalence of CVD has decreased slightly in high-income countries between 1990 and 2015 (Roth et al., 2017), likely due to improvements in treatment and better access to those treatments. However, the most recent statistics suggest that CVD mortality increased by 21% between 2007 and 2017, largely due to failure to improve modifiable risk factors (Virani et al., 2020). CVD therefore remains a great public health burden in many regions of the world; identifying physiological risk markers for CVD is of critical importance. Robust measures are needed in order to identify high risk individuals for medical treatment and lifestyle changes, as well as to increase our understanding of vascular biology and inform therapeutic treatment targets.

Traditional risk markers and residual risk

Currently, traditional risk markers like blood pressure (BP) and low-density lipoprotein cholesterol (LDL-C) are widely used to identify individuals at risk for CVD and are classified by the FDA as surrogate endpoints for CVD (U.S. Food & Drug Administration, 2017). These markers have proven to be useful. For example, in a meta-analysis of data from just under one million adults, it was concluded that BP has strong, positive associations with mortality rates for stroke, ischemic heart disease, and other vascular diseases (Lewington et al., 2002). Antihypertensive drugs that lower and control BP are protective against CVD (Ettehad et al., 2016). After intervention, almost half as many participants in the active drug group experienced heart failure as did those in the placebo group, with relative risk of 0.51 (95% CI, 0.37 to 0.71) (Kostis et al., 1997). Comparable evidence supports the utility of LDL-C. The relationship between LDL-C and CVD risk is also strongly positive; regular use of statins, which reduces

plasma LDL-C levels, correspondingly reduces CVD risk (Piepoli et al., 2016). Because CVD is a diverse group of diseases, BP and LDL-C are just two of many factors included in risk prediction models developed by the Framingham Heart Study and the American College of Cardiology (D'Agostino et al., 2008; Goff et al., 2014).

Even after risk is assessed and appropriate preventative measures are taken, residual risk of continued disease progression or incident vascular events remains (Zanchetti, 2009). Achieving a reduction in BP or LDL-C, to target levels or below, is not satisfactorily protective for all patients. Patients often have additional risk factors, such as elevated triglycerides, that are inadequately managed even when BP and LDL-C levels are well controlled (Fruchart et al., 2008). For instance, in every category of patients (low-moderate, high, very high, and very, very high risk), there is residual risk of developing CVD even after antihypertensive medication intervention. Individuals with the highest baseline risk (defined as a 10-year CVD mortality rate greater than 20%) also have the highest residual risk after treatment (Thomopoulos, Parati, & Zanchetti, 2014). Similarly, statin therapy was shown to reduce relative risk of experiencing a cardiovascular event by 21%. However, residual risk is apparent in that 14% of participants receiving the active drug later experienced an event (Fruchart et al., 2008).

Residual risk is also conferred by subclinical markers of arterial stiffening and atherosclerosis that are often not measured in clinical settings. Evaluation of arterial stiffness by pulse wave velocity (PWV) is predictive of CVD, even with adjustment for traditional risk factors (Ben-Shlomo et al., 2014). The addition of PWV to traditional risk prediction models also improves CVD risk prediction, which suggests that PWV detects distinctive vascular abnormalities conferring risk beyond traditional measures (Ben-Shlomo et al., 2014). A second non-invasive measure that evaluates endothelial dysfunction, flow mediated dilation (FMD), is

robust in terms of risk prediction (Gokce et al., 2002). However, endothelial dysfunction is predominantly caused by traditional risk factors and therefore FMD does not significantly improve prediction beyond traditional measures (Yeboah, Crouse, Hsu, Burke, & Herrington, 2007). A greater knowledge of changes in the vasculature that precede overt disease, as determined by PWV and FMD in individuals at high risk of CVD, will assist in understanding the etiology of CVD and in identifying targets for risk minimization.

Carotid-Femoral Pulse Wave Velocity

Measurement of carotid-femoral pulse wave velocity (cfPWV) is the gold standard for non-invasive evaluation of arterial stiffness (Salvi et al., 2019). It is well established that cfPWV is strongly predictive of future vascular events and provides additional CVD risk stratification beyond that of traditional risk factors (Ben-Shlomo et al., 2014). Analysis of 2232 participants in the longitudinal Framingham Heart Study provides evidence that higher PWV is positively correlated with CVD risk, with a hazard ratio of 1.48 (95% CI 1.16 to 1.91, P=0.002) (Mitchell et al., 2010). A later meta-analysis of prospective cohort studies, including 17,653 individuals (healthy, at risk of CVD, and CVD patients), found a similar hazard ratio of 1.30 (95% CI 1.18 to 1.43, P<0.001) (Ben-Shlomo et al., 2014). PWV is also an independent predictor of vascular mortality in healthy populations (Mattace-Raso et al., 2006; Willum-Hansen et al., 2006). Visceral adiposity, another CVD risk factor, is positively associated with arterial stiffness in subjects with type 2 diabetes (Morigami et al., 2016).

PWV's ability to determine the relative elasticity of the artery is thought to underly its predictive value. Various studies confirm an inverse association between PWV and carotid artery

distensibility (Cdist) (Koivistoinen et al., 2012), as well as a positive correlation between PWV and beta-stiffness (Hwang et al., 2014). As the heart contracts, a pressure waveform is produced that travels to the periphery (Lee & Oh, 2010). Healthy, highly elastic arteries produce a slower wave, while stiff, resistant arteries increase wave speed. A meta-analysis of 19 studies shows the detrimental effects of high wave speed; the risk of future vascular events increases by 12% with a 1 m/s increase in PWV (Zhong et al., 2018) An additional consequence of reduced elasticity in the arteries is increased average BP. Due to amplified wave reflections, systolic BP increases to a greater extent than diastolic BP, leading to an increase in pulse pressure (PP) (Laurent et al., 2006). As high BP is a key CVD risk factor (Lewington et al., 2002), this added effect of arterial stiffness strongly supports the idea that PWV is useful in risk stratification.

Because PWV measures arterial stiffness, a change in PWV is likely indicative of structural changes in the artery. Arterial stiffness gradually increases with age (Lee & Oh, 2010). Over time, vascular pulsation puts pressure on the network of smooth muscle cells, collagen, and elastin fibers; alterations accumulate in the media as elastin fibers weaken and fracture, and collagen and calcium levels increase (Lee & Oh, 2010). Stiffness positively associates with carotid intima media thickness (cIMT), a marker of atherosclerosis (Krantz et al., 2011). PWV is also positively correlated with the severity of coronary artery disease (CAD), which is commonly caused by atherosclerosis (Hofmann et al., 2014). The mechanism behind this relationship is unclear. It has been suggested that high shear stress and high luminal pressure, which appear as the artery stiffens, lead to endothelial dysfunction, plaque formation, deposition of excess collagen, and subsequently atherosclerosis (Kim & Kim, 2019). However, some studies suggest that arterial stiffening and atherosclerosis have separate underlying causes (Cecelja, Sriswan, Kulkarni, Kinra, & Nitsch, 2020), and that PWV and IMT are representative of different forms

of vascular damage (Koivistoinen et al., 2012). While the exact physiological mechanism remains unclear, the predictive utility of PWV alone is convincing.

Flow Mediated Dilation

Measurement of flow mediated dilation (FMD) in the brachial artery using vascular ultrasound is the gold standard for non-invasive measurement of endothelial function (Flammer et al., 2012). FMD is negatively correlated with both CVD risk (Inaba, Chen, & Bergmann, 2010); (Shechter, Shechter, Koren-Morag, Feinberg, & Hiersch, 2014); (Ras, Streppel, Draijer, & Zock, 2013) and CVD risk factors (Celermajer, Sorensen, Bull, Robinson, & Deanfield, 1994; Maruhashi et al., 2013). Per 1% increase in FMD, the corresponding risk of CVD is reduced by 13% (RR= 0.87, 95% CI, 0.832 to 0.914) (Inaba et al., 2010). Lower FMD, indicative of impaired endothelial function and abnormal NO regulation, is independently associated with vascular disease and mortality (Gokce et al., 2002). This has been established in healthy subjects (Shechter et al., 2014) as well as in diseased populations, although a meta-analysis of 23 studies suggests that the relationship is stronger in diseased populations (Ras et al., 2013). However, FMD does not significantly improve CVD risk prediction beyond traditional measures, as it adds only about 1% accuracy to a model including all the Framingham risk factors (except HDL-C) (Yeboah et al., 2007). This suggests that endothelial dysfunction is caused by traditional risk factors.

Much of our current knowledge of normal endothelial function is thanks to a Nobel prize winning discovery by researchers Furchgott and Zawadzki. This team identified a molecule, termed endothelium-derived relaxing factor (EDRF), that causes vasodilation in the presence of

acetylcholine (Furchgott & Zawadzki, 1980). EDRF was later identified as nitric oxide (NO) (Ignarro, Buga, Wood, Byrns, & Chaudhuri, 1987). Since then, the full pathway has been elucidated. In healthy individuals, the presence of acetylcholine stimulates NO production (Furchgott & Zawadzki, 1980; Ignarro et al., 1987) via endothelial nitric oxide synthase (eNOS), from the metabolic precursor L-arginine (Palmer, Ashton, & Moncada, 1988). Release of NO from endothelial cells subsequently causes vasodilation by relaxing smooth muscle cells; this has been shown in vivo as well as in vitro (Pohl, Holtz, Busse, & Bassenge, 1986). At normal levels of expression, NO is thought to be protective against CVD because NO-mediated vasodilation increases blood flow and lowers blood pressure (Haynes, Noon, Walker, & Webb, 1993; Rees, Palmer, & Moncada, 1989). NO also inhibits processes that contribute to the development of atherosclerosis, such as migration and adhesion of leukocytes to vessel walls, LDL oxidation, and smooth muscle proliferation (Forstermann, Xia, & Li, 2017).

Endothelial dysfunction is the dysregulation of vasoactive substances that occurs as a result of low NO bioavailability (Minor, Myers, Guerra, Bates, & Harrison, 1990) and increasing age (Celermajer et al., 1994). There are a variety of factors known to cause endothelial dysfunction, although the temporal relationships between these factors are unclear. It is thought that dysregulation of lipoproteins, especially accumulation of low-density lipoprotein and apolipoprotein CIII, directly and indirectly leads to chronic inflammation (Cejkova, 2016). Dysregulation first causes endothelial activation. Activation normally occurs in order to repair injured endothelium, but becomes pathological when there is a considerable increase in endothelial monocyte adhesion in the absence of injury (Liao, 2013). Attached monocytes can then migrate into the arterial wall between endothelial cells where they settle, differentiate, and contribute to chronic inflammation (Cejkova, 2016). Secondly, dysregulation directly triggers

NF-κB signaling that turns on proinflammatory genes, and subsequently induces oxidative stress (Fruchart et al., 2008). Oxidative stress and damage occur when reactive oxygen species interact with and disable NO; low NO bioavailability then results in impaired vasodilation and increased smooth muscle tone (Csiszar, Wang, Lakatta, & Ungvari, 2008).

This reduced bioavailability of NO is a prerequisite to atherosclerosis. Structural changes follow, like thickening of the arterial wall, and remodel the damaged endothelium. Plaque accumulation then occurs, causing the lumen to shrink and causing disease severity to increase (Schoenhagen, Ziada, Vince, Nissen, & Tuzcu, 2001). A multitude of vasoactive substances are involved in the regulation of blood vessel diameter (Yanagisawa et al., 1988); (Nakashima, Mombouli, Taylor, & Vanhoutte, 1993), making it difficult to determine which pathway is primarily responsible for vessel dilation in some arteries. However, in the brachial artery specifically, flow mediated dilation has been shown to be largely NO dependent (Joannides et al., 1995). While FMD does not measure coronary endothelial dysfunction directly, brachial FMD is predictive of coronary endothelial function (Anderson et al., 1995; Teragawa et al., 2005), suggesting that dysregulation develops in both areas simultaneously. Peripheral endothelial dysfunction also independently predicts adverse cardiovascular events in populations with coronary artery disease (Heitzer, Schlinzig, Krohn, Meinertz, & Munzel, 2001). Together, these findings support FMD of the brachial artery as a representation of coronary endothelial function and therefore CVD risk.

PWV and FMD

PWV and FMD are each independent, robust measures for CVD risk stratification. PWV is suggestive of vessel structure and FMD elucidates vessel activity. The direct relationship between PWV and FMD is less clear, although there is significant overlap in the underlying mechanisms that lead to these physiologically distinct subclinical states (Figure 1). It is possible that reduced vasodilation and increased smooth muscle tone seen with endothelial dysfunction amplifies arterial stiffness; high BP and increased shear stress seen with arterial stiffness may also contribute to endothelial dysfunction. It seems likely that multiple age-related changes operate in a cyclical fashion, all amplifying each other.

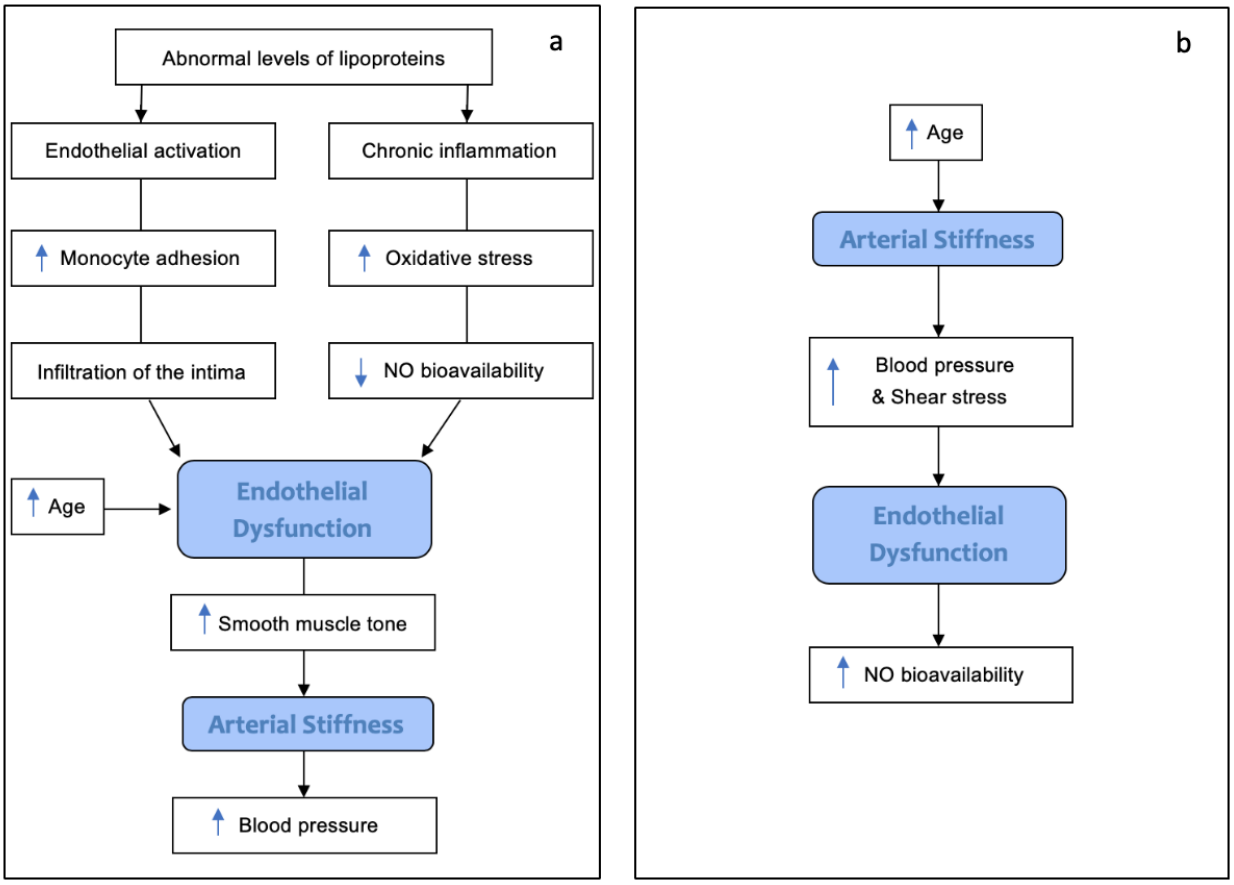


Figure 1. Possible mechanisms of CVD development a) beginning with endothelial dysfunction; b) beginning with arterial stiffness

Currently, the relationship between PWV and FMD has only been examined in cross sectional analyses. In a cohort of 135 subjects (some healthy, some with risk factors for atherosclerosis, and some with atherosclerotic disease), FMD was significantly, negatively associated ($p<0.001$) with brachial-ankle PWV ($R= -0.49$) and with heart-carotid PWV ($R= -0.36$) (Kobayashi et al., 2004). A similar negative association ($R= -0.37$, $p<0.001$) between FMD and aortic PWV was demonstrated in 101 patients with autoimmune diseases including vasculopathies and 36 healthy subjects (Soltesz et al., 2009). This finding has also been replicated in a healthy population of 100 males ($r= -0.37$, CI= [-0.57, -0.13]) (Lunder, Janic, Kejzar, & Sabovic, 2012). These moderate, negative correlations suggest that the PWV and FMD assessments are detecting separate underlying mechanisms of CVD development. Interestingly, another cross sectional analysis of this relationship in a general population of over 2000 adults found a non-significant relationship in young adults (beta= -0.02, $p= 0.11$) and a significant relationship in older adults (beta= -0.05, $p < 0.001$), although significance was lost in older adults after age and sex were accounted for (Koivistoinen et al., 2012). This again supports the idea that PWV and FMD each represent a different type of vascular damage, but additionally introduces the possibility that these mechanisms develop separately, then converge with age and with disease progression. However, because the relationship between PWV and FMD has not yet been examined prospectively, we have a limited understanding of the temporal development of both arterial stiffness and endothelial dysfunction. Additionally, the relationship has never been assessed in a cohort of adults with visceral adiposity.

Visceral Adiposity

Visceral adipose tissue (VAT), or intra-abdominal fat, is a metabolically active endocrine organ that is predictive of Metabolic Syndrome (MetS), which in turn is a risk factor for CVD (Despres & Lemieux, 2006). In women matched for abdominal subcutaneous adipose tissue mass, individuals with higher rather than lower levels of visceral adipose tissue had higher metabolic risk profiles (Ross, Freeman, Hudson, & Janssen, 2002). PWV is independently and positively correlated with visceral or android fat mass (Corrigan et al., 2016; Lu et al., 2008; Strasser et al., 2015). The association between VAT and FMD is less clear. In a cohort of 116 middle aged patients undergoing peritoneal dialysis, FMD was independently and negatively associated with visceral fat mass ($r = -0.35$, $P < .01$) (Lu et al., 2008). A recent study in 252 healthy children contradicts this, finding visceral adiposity to be positively correlated with FMD (beta (SE) 0.09 ± 0.02, $p<0.001$, $R^2 = 0.23$) (Ryder et al., 2016). This uncertainty warrants investigation in the current sample of individuals with enlarged waist circumference. There is a lack of research on the correlation between PWV and FMD in adults with visceral adiposity; because enlarged waist circumference is a significant predictor of VAT (correlation range: 0.73-0.77) (Camhi et al., 2011), the present study will provide insight into this question.

Purpose of Study

The present study was therefore focused on the association between PWV and FMD in a cohort of adults with enlarged waist circumference. Because CVD risk is positively associated with PWV and negatively associated with FMD in a variety of populations, we expected FMD to be inversely correlated with PWV in a population with enlarged waist circumference. The

secondary objective was to examine the relationships between traditional surrogate markers of CVD. We measured PWV, FMD, and other hemodynamic and anthropometric measures at one timepoint in a sample of 39 adults with enlarged waist circumference (men ≥40 inches, ≥102 cm) and women ≥35 inches, ≥88 cm)). Relationships were determined with correlational analysis.

Chapter 2

Methods

HAT Study Design

The Habitual Diet and Avocado Trial (HAT) was a randomized, controlled trial that aimed to determine the effects of consumption of one avocado per day for six months on adults with enlarged waist circumference. Endpoints were visceral adiposity (primary), metabolic syndrome markers, hepatic lipid content, and C-reactive protein (secondary), as well as concentrations of other blood markers such as insulin and cholesterol, and lifestyle factors like diet and sleep quality. This was a multi-site study including four clinics (Pennsylvania State University (Penn State Hershey and Penn State University Park), Loma Linda University, University of California at Los Angeles, Tufts University), and a coordinating center (Wake Forest University). The trial was funded by the Hass Avocado Board. At Penn State, an ancillary study examining arterial stiffness and endothelial function was conducted. This study (Protocol STUDY00009494) was approved by the Institutional Review Board of The Pennsylvania State University and is registered at ClinicalTrials.gov (Identifier: NCT03528031).

For the purposes of this thesis, I conducted cross-sectional analyses using data collected at baseline from a subsample of 39 individuals at the Penn State University Park site between August 30th, 2018 and December 19th, 2019. The 39 subjects were chosen because they were first to have all data scored and ready for analysis. Future publications will include all HAT participants from all sites. The project described was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1 TR002014.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Participants

The HAT study aimed to enroll a total of 1000 participants across sites, with approximately 250 at each clinic. Recruitment strategies included IRB-approved flyers, postings in local newspapers, advertisements on social media, and listserv emails. It was required that, at screening, all participants be ≥ 25 years of age, have a high waist circumference (defined by NCEP ATP III 2005 as ≥35 inches (≥88 cm) for women and ≥40 inches (≥102 cm) for men), and maintain a habitual diet including no more than 2 avocados per month. Individuals were excluded if they did not eat avocados, were sensitive or allergic to latex, were unable or not willing to undergo MRI scanning, had an unstable medical condition (e.g. hepatic disease, on dialysis for renal disease), were pregnant / lactating / intending to become pregnant, had gained or lost 10 lbs of body weight in the past year, were following restricted or weight loss dietary patterns, were taking unstable anti-anxiety / anti-depressive / anti-psychotic medication (defined as a dose change within the last 6 months), had taken oral steroids for longer than 7 days in the past 6 months, had elevated alcohol intake (7+ drinks/week females; 14+ drinks/week males), were participating in another clinical intervention within 30 days of baseline, or were otherwise deemed ineligible based on PI judgement. All subjects completed informed consent forms prior to participation in the intervention.

Data Collection Procedures

Participants were directed to fast for at least 8 hours prior to the visit, and they self-reported compliance to these instructions on the morning of the visit. A team of nurses and lab technicians collected participant data. Demographic information including date of birth and sex were collected via questionnaire. Physical measures were taken three times and averaged: height via stadiometer, weight via digital scale, and waist circumference at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Pulse Wave Velocity and Pulse Wave Analysis were conducted. Participants also underwent vascular ultrasounds for measurement of flow mediated dilation. A summary of baseline measurements is shown in Table 1 below.

Table 1. Data collection summary

Questionnaire	Physical measures	Pulse wave velocity	Pulse wave analysis	Vascular ultrasound
Date of birth	Height	PWV	Brachial SBP	Percent FMD
Sex	Weight	Heart rate	Brachial DBP	Baseline diameter
	Waist circumference	Pulse transit time	Central SBP	Peak diameter
			Central DBP	
			Pulse pressure	
			Augmentation pressure	
			Augmentation index	

The SphygmoCor XCEL® system

The SphygmoCor XCEL® system (AtCor Medical, Sydney Australia) was used to measure carotid-femoral pulse wave velocity (cfPWV) and to perform pulse wave analysis. The XCEL device is a newer version of SphygmocCor technology that is well validated relative to older SphygmocCor models (Hwang et al., 2014) as well as other invasive measures of PWV (Butlin & Qasem, 2016). All measurements were performed by trained personnel according to a standardized protocol.

Carotid-femoral Pulse Wave Velocity

Briefly, subjects rested for 5 minutes prior to the measurements, which were taken in the supine position. Three measurements each were obtained for PWV, heart rate, and pulse transit time; all three measurements were averaged for analysis. A cuff was placed on the subject's thigh. Throughout cuff inflation, an applanation tonometry sensor was held at the carotid artery. The XCEL device analyzes the shape of the peripheral waveforms, and subsequently determines the aortic waveform shape (Hwang et al., 2014). From this, carotid-femoral pulse wave velocity (cfPWV) is calculated (Butlin & Qasem, 2016).

PWV is the speed that the pressure wave travels through the arteries; because waveform shape is affected by the relative elasticity of the artery, PWV is indicative of arterial stiffness (Butlin & Qasem, 2016). Low PWV is desirable as elastic arteries produce a slower wave; high PWV due to arterial stiffness is cause for concern because this causes increased blood pressure. To calculate PWV, the distance between the sternal notch and the carotid artery is subtracted from the distance between the sternal notch and the femoral artery. The remaining distance is

divided by the difference between the pulse transit time from the heart to the carotid artery and the pulse transit time from the heart to the femoral artery (Butlin & Qasem, 2016). Figure 2 illustrates this calculation (Butlin & Qasem, 2016).

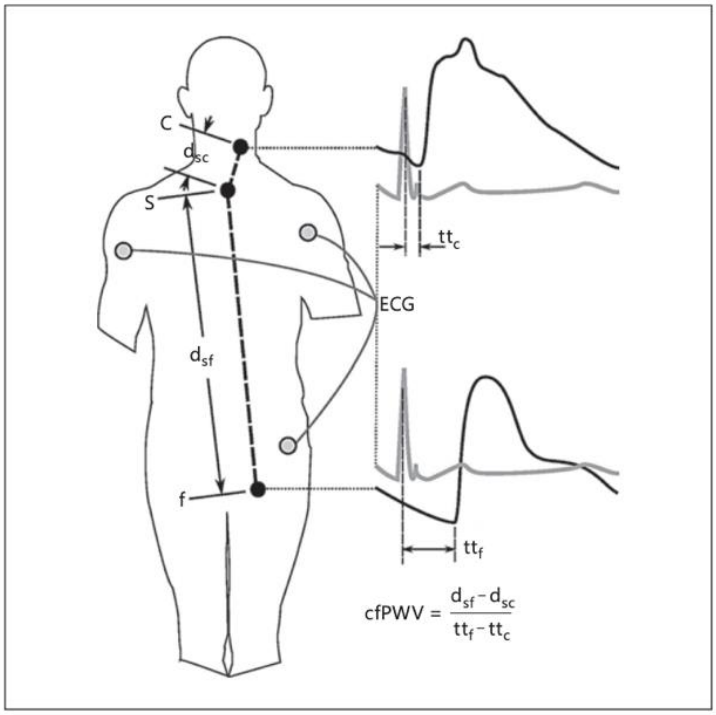


Figure 2. Formula for cfPWV calculation

Pulse Wave Analysis

Again, subjects rested for 5 minutes prior to the measurements, which were taken in the seated position. Pulse wave analysis was performed via the brachial cuff method, following published guidelines (Chobanian et al., 2003). This provided values for brachial SBP, brachial DBP, central SBP, central DBP, pulse pressure, augmentation pressure, and augmentation index (adjusted to a heart rate of 75 bpm). Measurements were each taken three times; for each variable, the second and third values were averaged and used for analysis.

Flow Mediated Dilation

To determine FMD, vascular ultrasound of the brachial artery was performed following recommended procedures (Corretti et al., 2002). All of the ultrasound examinations were completed by a single sonographer using a GE Logiq (General Electric Company, Boston MA) ultrasound imaging system with a 10-MHz linear array transducer. Continuous, longitudinal images of the brachial artery, 5 to 10 cm above the elbow on the right arm, were recorded at five frames per second during baseline (1 minute), occlusion (5 minutes), and post-deflation (2 minutes) periods. Occlusion was induced by inflation of a blood pressure cuff on the right forearm (distal to the target artery) to 250 mm Hg using an automated device (D. E. Hokanson, Inc., Bellevue, WA, USA). Cuff inflation cuts off blood flow to the hand; deflation then induces reactive hyperaemia. Reactive hyperaemia is a transient increase in blood flow that increases shear stress and subsequently increases NO release, which results in vasodilation (Flammer et al., 2012). Automated edge detection software (Brachial Analyzer; MIA, Iowa City, IA) was used to measure artery diameter in each image throughout the test. Two scorers independently determined the average diameter at baseline and the peak diameter after a period of ischemia (during deflation). I was the first scorer for all 39 scans. With baseline and peak diameters, we then calculated the FMD score, or percent change in diameter. If our values differed by more than 2%, a third scorer analyzed the scan. The two closest values were averaged for each subject.

Statistical Analysis

I analyzed data using SAS (version 9.4, SAS Institute, Cary, NC). I checked all outcome variables for normality (PROC UNIVARIATE) and made log transformations as needed to

normalize the data. I ran descriptive statistics. Pearson's correlation (PROC CORR) was used to assess the relationship between PWV and FMD. Multivariate linear regression was used to assess the relationship between PWV and FMD after adjustment for potential confounders. Each confounder (brachial SBP, brachial DBP, central SBP, central DBP, central pulse pressure, age, WC, HR, weight, BMI) was added to a separate model, with PWV as the independent variable and FMD as the dependent variable. $P<0.05$ was considered statistically significant. I also used Pearson's correlations to determine the relationships between FMD, PWV, brachial SBP, central SBP, BMI, WC, and age. I used an independent samples t-test (PROC TTEST) to compare PWV and FMD of individuals above and below the cohort's median waist circumference, as well as individuals above and below the cohort's median age.

Chapter 3

Results

Participant characteristics

The HAT study enrolled a total of 1002 participants across all the clinical sites (Figure 3). Data for PWV and FMD at baseline were only available for 39 subjects enrolled at the Penn State University Park site.

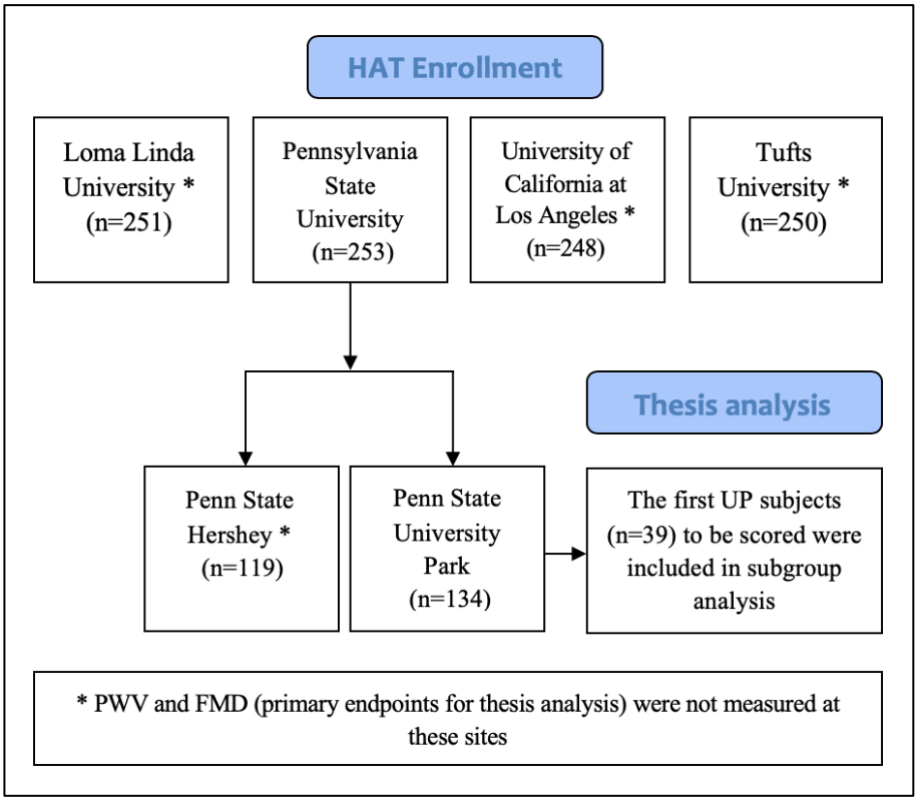


Figure 3. Participant flow

Table 2. Characteristics of the analytical sample, which were a subgroup of the HAT cohort enrolled at the Penn State University Park site (n=39)

Characteristic	*Mean ± SD*	*Range*	*Median (IQR)*
Age (years)	46 ± 13	25 – 71	45 (36 – 56)
Height (cm)	166.7 ± 8.9	147.0 – 186.5	165.5 (162.0 – 170.0)
Weight (kg)	90.5 ± 15.7	61.0 – 123.6	88.5 (79.2 – 102.0)
Waist circumference (cm)	103.9 ± 11.5	88.4 – 129.0	101.4 (93.1 – 112.3)
BMI (kg/m²)	32.5 ± 4.6	25.8 – 43.1	31.4 (28.7 – 35.8)
Brachial SBP (mmHg)	125 ± 16	98 – 169	122 (115 – 133)
Brachial DBP (mmHg)	78 ± 9	61 – 98	78 (71 – 86)
Central SBP (mmHg)	115 ± 15	91 – 154	114 (104 – 122)
Central DBP (mmHg)	79 ± 9	62 – 99	80 (72 – 87)
Central pulse pressure (mmHg)	36 ± 9	24 – 61	33 (29 – 42)
Augmentation pressure (mmHg)	10 ± 5	0 – 24	9 (7 – 14)
Augmentation index (%)[1]	23.7 ± 9.8	-4.0 – 41.0	25.0 (19.0 – 30.0)
Heart rate (bpm)	62 ± 8	48 – 80	61 (58 – 68)
PWV (m/s)	6.8 ± 1.3	3.8 – 9.7	6.7 (5.8 – 7.8)
Pulse transit time (ms)	66.1 ± 9.2	50.3 – 85.0	66.3 (58.3 – 73.7)
Baseline artery diameter (mm)	4.04 ± 0.57	3.14 – 5.61	3.86 (3.69 – 4.34)
Peak artery diameter (mm)	4.34 ± 0.58	3.54 – 5.97	4.22 (3.95 – 4.55)
FMD (%)	7.67 ± 2.76	2.89 – 13.40	7.15 (5.77 – 9.55)

[1] adjusted to HR 75

Characteristics of the subsample of University Park participants (n=39) are summarized in Table 2. This sample was 82% female (n=32 women, n=7 men). The average age at the time

of the study was 46 ± 13 years. Participant ages ranged from 25 to 71 years, with a median age of 45 years (IQR 36-56). The cohort's average waist circumference was 103.9 ± 11.5 cm, and average BMI was 32.5 ± 4.6 kg/m2.

Mean brachial SBP was 125 ± 16 mmHg, while mean central SBP was 115 ± 15 mmHg. Mean brachial and central DBPs were 78 ± 9 mmHg and 79 ± 9 mmHg, respectively. PWV was on average 6.8 ± 1.3 m/s. The cohort had a mean brachial artery diameter of 4.04 ± 0.57 mm and a mean peak diameter of 4.34 ± 0.58 mm. FMD varied widely, ranging from 2.89 % to 13.40 %; the average FMD was 7.67 ± 2.76 %.

Relationship between PWV and FMD

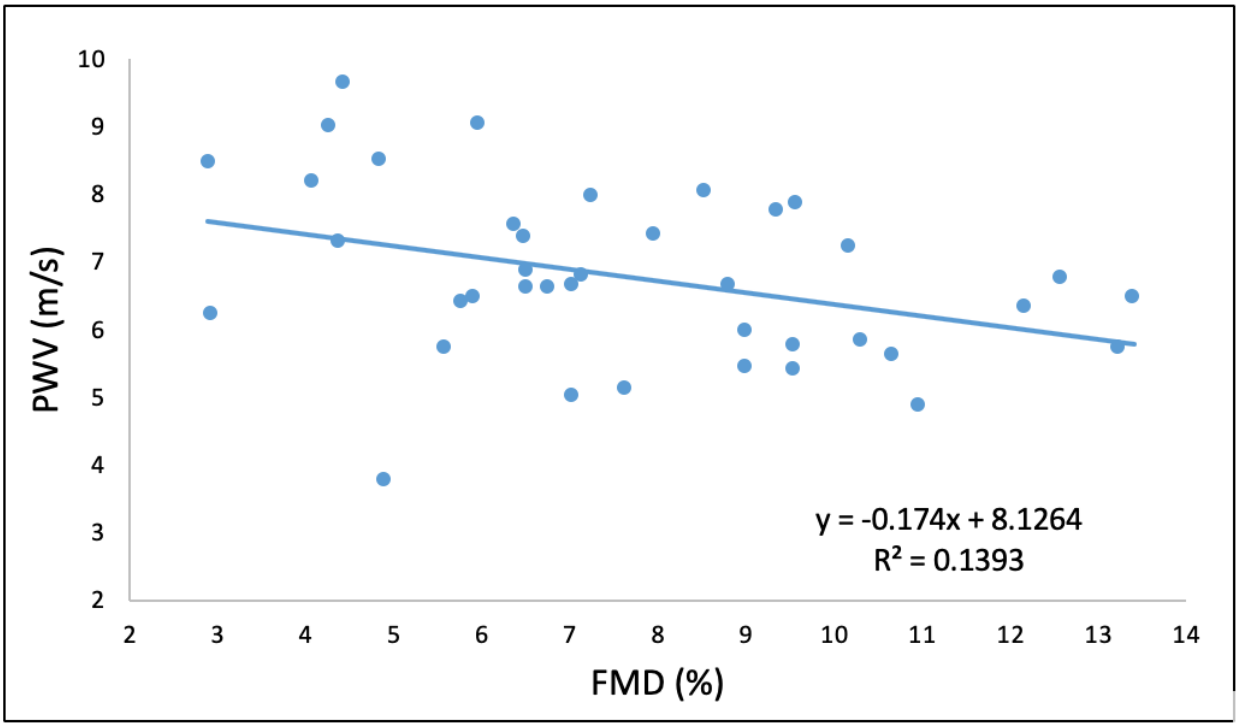


Figure 4. The association between PWV and FMD in a cohort of adults with enlarged waist circumference (n=39)

A significant, weak to moderate, negative correlation was found between PWV and FMD ($r = -0.37$, $p=0.02$), Figure 4. Higher PWV values were associated with lower FMD values. FMD explained 13.9 % of the variation in PWV ($R_2 = 0.139$).

Subgroup analysis by median WC and by median age

Table 3. Characteristics of subgroups below (n=20) and above (n=19) median WC

	WC ≤ 101.4 cm	*WC >101.4 cm*	
Characteristic	Mean ± SD	Mean ± SD	P
Age (years)	47 ± 12	45 ± 14	0.68
Height (cm)	163.0 ± 7.4	170.4 ± 8.9	0.007
Weight (kg)	78.8 ± 7.7	102.8 ± 11.9	<0.001
Waist circumference (cm)	94.7 ± 4.3	113.5 ± 8.2	<0.001
BMI (kg/m2)	29.6 ± 2.5	35.5 ± 4.4	<0.001
Brachial SBP (mmHg)	118 ± 12	133 ± 16	0.002
Brachial DBP (mmHg)	74 ± 8	83 ± 8	0.002
Central SBP (mmHg)	108 ± 11	122 ± 15	0.002
Central DBP (mmHg)	75 ± 8	84 ± 8	0.001
Central pulse pressure (mmHg)	34 ± 7	38 ± 11	0.12
Aortic pressure (mmHg)	9 ± 4	11 ± 7	0.39
Augmentation index (%)[1]	23.5 ± 7.0	23.9 ± 12.3	0.90
Heart rate (bpm)	62 ± 8	64 ± 7	0.42
PWV (m/s)	6.4 ± 1.3	7.3 ± 1.1	0.026
Pulse transit time (ms)	66.7 ± 9.6	65.4 ± 9.0	0.65
Baseline artery diameter (mm)	3.85 ± 0.37	4.24 ± 0.67	0.035
Peak artery diameter (mm)	4.16 ± 0.38	4.53 ± 0.69	0.047
FMD (%)	8.17 ± 2.88	7.15 ± 2.60	0.25

[1] adjusted to HR 75

The cohort was divided into two subgroups based on median waist circumference (WC) (101.4 cm); these groups are compared in Table 3. The mean WC of the group above the median WC (113.5 ± 8.2 cm) was significantly higher ($p<0.001$) than the mean WC of the group below median WC (94.7 ± 4.3 cm). Height ($p=0.007$), weight ($p<0.001$), and BMI ($p<0.001$) were significantly higher in subjects with a WC greater than the median. Mean age was not significantly different between groups ($p=0.68$).

All average blood pressure measures were significantly higher in those with a median WC greater than the median (all $p<0.01$). There was no difference between groups in central pulse pressure, augmentation pressure, augmentation index, heart rate, or pulse transit time (all $p>0.05$). In the subjects with a WC greater than the median, mean PWV was 7.3 ± 1.1 m/s. This was significantly higher ($p=0.026$) than mean PWV in those with a WC less than the median (6.4 ± 1.3 m/s). Baseline brachial artery diameter was significantly higher in the group above the median WC ($p=0.035$), as was peak artery diameter ($p=0.047$). However, FMD was similar between groups (7.15 ± 2.60 % above median WC, 8.17 ± 2.88 below median WC, $p=0.25$).

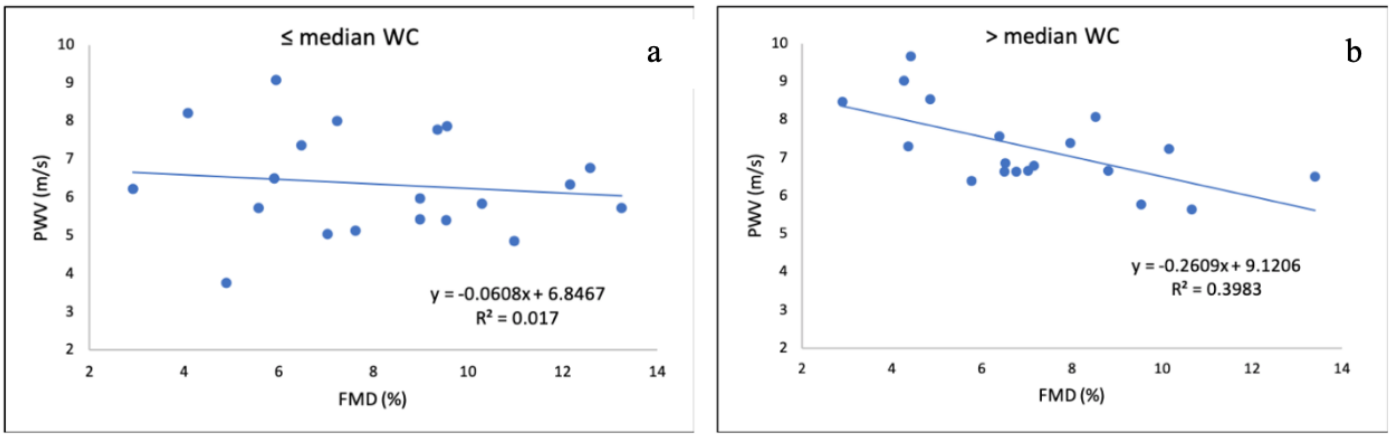


Figure 5. The association between PWV and FMD in subjects a) less than the median waist circumference (WC ≤ 101.4 cm, n=20); b) greater than the median waist circumference (WC >101.4 cm, n=19)

As shown in Figure 5, PWV and FMD were not correlated in subjects with a WC below the median (r = -0.13, p=0.58). However, in the subgroup above median WC, PWV and FMD were strongly and significantly correlated (r = -0.63, p=0.004). FMD explained 39.8 % of the variance in PWV (R_2 = 0.398).

Table 4. Characteristics of subgroups below (n=20) and above (n=19) median age

	age ≤ 45 years	*age > 45 years*	
Characteristic	Mean ± SD	Mean ± SD	P
Age (years)	35 ± 6	57 ± 7	<0.001
Height (cm)	168.7 ± 10.5	164.5 ± 6.4	0.14
Weight (kg)	94.7 ± 15.3	86.1 ± 15.2	0.086
Waist circumference (cm)	103.6 ± 11.0	104.1 ± 12.3	0.89
BMI (kg/m2)	33.3 ± 4.4	31.7 ± 4.8	0.30
Brachial SBP (mmHg)	120 ± 12	131 ± 19	0.044
Brachial DBP (mmHg)	78 ± 9	79 ± 10	0.95
Central SBP (mmHg)	110 ± 11	120 ± 17	0.036
Central DBP (mmHg)	79 ± 9	80 ± 10	0.89
Central pulse pressure (mmHg)	31 ± 5	41 ± 10	0.002
Aortic pressure (mmHg)	8 ± 4	13 ± 5	<0.001
Augmentation index (%)[1]	22.2 ± 9.6	25.4 ± 10.0	0.31
Heart rate (bpm)	64 ± 7	61 ± 8	0.24
PWV (m/s)	6.1 ± 1.0	7.6 ± 1.1	<0.001
Pulse transit time (ms)	72.4 ± 6.4	59.4 ± 6.7	<0.001
Baseline artery diameter (mm)	4.15 ± 0.70	3.92 ± 0.36	0.19
Peak artery diameter (mm)	4.48 ± 0.71	4.19 ± 0.36	0.12
FMD (%)	8.10 ± 2.23	7.22 ± 3.22	0.33

[1] adjusted to HR 75

The cohort was also separated into subgroups by median age (45 years); characteristics are summarized in Table 4. Mean age in the group above median age (57 ± 7 years) was significantly higher ($p<0.001$) than the mean age in the group above median age (35 ± 6 years). All physical measures (height, weight, WC, BMI) were similar between groups ($p>0.05$).

Mean brachial SBP was significantly higher in the group above the median age ($p=0.044$), as was mean central SBP ($p=0.036$). However, neither mean brachial DBP nor mean central DBP differed between age groups ($p>0.05$). In the group above median age, average PWV was 7.6 ± 1.1 m/s. This was significantly higher ($p<0.001$) than the average PWV in the group below median age (6.1 ± 1.0 m/s). Baseline brachial artery diameter, peak brachial artery diameter, and FMD were all similar between groups ($p>0.05$).

Augmentation index and heart rate were also not significantly different between groups ($p>0.05$). Central pulse pressure and augmentation pressure were both significantly higher in the group above median age ($p=0.002$ and $p<0.001$, respectively). Finally, pulse transit time was significantly lower in the group above median age (<0.001).

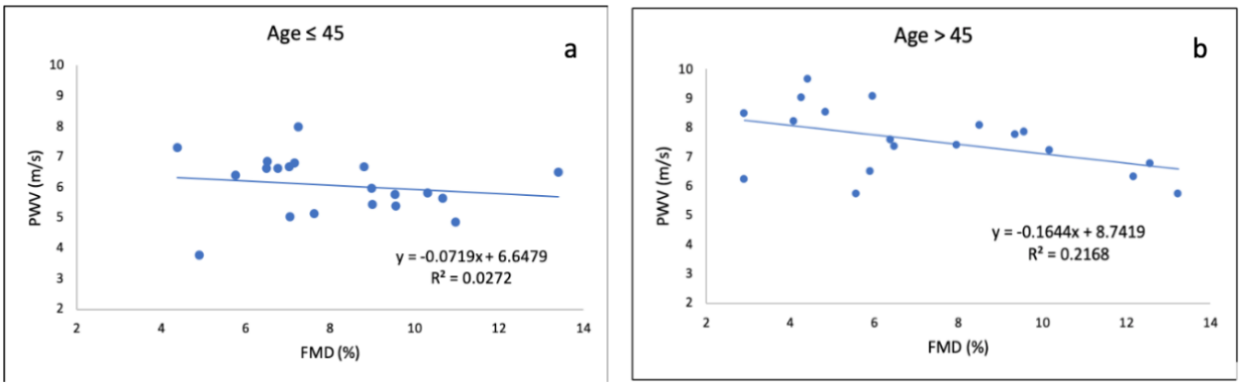


Figure 6. The association between PWV and FMD in subjects a) less than the median age (n=20); b) greater than the median age (n=19)

The correlation between PWV and FMD in the group below median age was non-significant (r = -0.17, p=0.49), Figure 6. There was a stronger, significant, inverse association between PWV and FMD in the group above median age (r = -0.47, p=0.04), and 22 % of the variation in PWV was explained by FMD (R^2 = 0.217).

Multivariate analyses

Subsequent multivariate linear regression analysis showed that PWV and FMD were no longer significantly correlated after adjustment for four separate confounders: brachial SBP (p=0.31), central SBP (p=0.22), central pulse pressure (p=0.20), and age (p=0.14). The relationship between PWV and FMD persisted, however, after adjustment for all other potential confounders (brachial DBP (p=0.041), central DBP (p=0.042), WC (p=0.031), HR (p=0.029), weight (p=0.031), BMI (p=0.020)). Adjustment for each of these confounders did increase the coefficient of determination from the original model (R^2 = 0.139): brachial DBP (R^2 = 0.323),

central DBP (R_2 = 0.333), WC (R_2 = 0.293), HR (R_2 = 0.147), weight (R_2 = 0.170), BMI (R_2 = 0.172).

Correlations with traditional CVD risk factors

Table 5. The correlation between PWV, FMD, and traditional CVD risk factors

	FMD	*Brachial SBP*	*Central SBP*	*BMI*	*WC*	*Age*
PWV	-0.37*	0.72*	0.71*	0.19	0.44*	0.61*
FMD		-0.37*	-0.33*	-0.02	-0.14	-0.31

*$p < 0.05$.

As shown in Figure 5, FMD's relationship to brachial SBP (r = -0.37) was similar to its relationship with central SBP (r = -0.33). The same can be said for PWV; its association with brachial SBP (r = 0.72) was almost identical to its association with central SBP (r = 0.71). BMI was not significantly correlated with PWV or FMD. WC and age were both significantly correlated with PWV, but not FMD.

Chapter 4

Discussion

The present analyses aimed to determine the association between PWV and FMD in a cohort of adults with enlarged waist circumference. As hypothesized, PWV and FMD were correlated in this cohort of mostly adult women with enlarged waist circumference. Subjects who had higher PWV values generally had lower FMD values. However, the relationship between PWV and FMD did not hold following adjustment for brachial SBP, central SBP, central pulse pressure, and age. Additionally, the relationship between PWV and FMD was stronger in groups above the median WC and above the median age. Together, these findings suggest that, in younger individuals with less central adiposity, PWV and FMD each assess physiologically distinct aspects of vascular health. However, with increasing age and central adiposity, there is greater similarity in the physiology; PWV and FMD assessments therefore converge.

The correlation coefficient ($r = -0.37$) for our cohort closely aligns with results from previous work in healthy males ($r= -0.373$) (Lunder et al., 2012), and in patients with autoimmune diseases ($R= -0.37$) (Soltesz et al., 2009). One study (of subjects either with risk factors for atherosclerosis or with atherosclerotic disease) showed a similar association between hcPWV and FMD ($p= -0.364$) as well as a stronger association between baPWV and FMD ($p= -0.493$) (Kobayashi et al., 2004). Each of these studies enrolled 100 or more participants. This suggests that the relationship between PWV and FMD is consistent and reproducible across populations. The weak to moderate strength of the relationship supports the idea that PWV and FMD reflect different disease mechanisms; a much stronger correlation would be expected if these measures were reflecting a single type of vascular damage.

Another subset of studies finds no significant association between PWV and FMD in healthy adults (Dhindsa, 2008), as well as in mixed population including healthy adults, those with risk factors for coronary artery disease (CAD), and those with CAD (Nigam, Mitchell, Lambert, & Tardif, 2003). These studies used smaller samples (n=40, n=32, respectively), however, and may have been underpowered to detect the relationship.

Subgroup analysis based on median WC was expected to show that the group above the median would be at increased risk for CVD, as defined by greater PWV (Watts, Bell, Byrne, Jones, & Davis, 2008). We observed that all measures of blood pressure and adiposity were significantly higher in the group above the median WC, as was PWV. Previously, greater adiposity (defined by visceral adiposity, android adiposity, WC) has been shown to predict arterial stiffness (Corrigan et al., 2016; Morigami et al., 2016; Strasser et al., 2015), which is consistent with our finding. In our cohort, PWV was significantly, moderately, positively correlated with WC; however, PWV's relationship with BMI was non-significant. This may indicate that central adiposity is a better predictor of arterial health than overall adiposity.

Given that reductions in both BMI and WC have been correlated with improved FMD (Miyazaki, 2010), FMD was expected to be significantly lower in the group above the median WC. FMD was modestly lower in the high WC group, although the difference was non-significant. In the overall cohort, FMD's relationships with BMI and WC were also non-significant. The reason for this discrepancy is unclear; although, because enlarged WC was a requirement for inclusion, there may not have been a wide enough distribution of BMI and WC in this cohort to detect a relationship with FMD. Of note, it was found that PWV and FMD were only significantly correlated in the group with a WC above the median.

In subgroup analysis based on median age, we expected to find that older participants were at increased risk for CVD, defined by greater PWV and lower FMD (D'Agostino et al., 2008; Goff et al., 2014). Measures of adiposity were similar between groups. This was unexpected, as BMI and WC have previously been correlated with age in a general population (Dobbelsteyn, Joffres, MacLean, & Flowerdew, 2001). Again, the results of our analysis likely differ because enlarged WC was a requirement for enrollment, so participants of all ages had high adiposity. Regarding hemodynamic measures, brachial SBP, central SBP, and PWV were significantly higher in the group above the median age. This finding was corroborated in the overall cohort. PWV had significant, strong, positive associations with brachial SBP, central SBP, and age. This is in agreement with the literature, as both arterial stiffness (Lee & Oh, 2010) and BP (Lewington et al., 2002) increase with age. However, the directionality of the relationship between arterial stiffness and BP is unclear. Studies have suggested that increased arterial stiffness increases average BP, due to increased pressure oscillations, substantially increasing pulse pressure (Laurent et al., 2006). High PWV in normotensive individuals is also correlated with a disproportionate increase in BP at follow-up (Kaess et al., 2012; Takase et al., 2011). Mechanistically, high BP causing arterial stiffness is more easily explained. Stress to the arterial wall with increased BP causes fragmentation of elastin and calcium deposition, reducing distensibility (Lee & Oh, 2010). Given that there is support for both pathways, it is also possible that the relationship is bidirectional.

FMD was expected to be significantly impaired in older relative to younger participants (Celermajer et al., 1994) and to be negatively correlated with age (Maruhashi et al., 2013). Surprisingly, FMD was shown to be similar between age groups, and the correlation between FMD and age in the overall cohort was non-significant. Interestingly, we then showed that the

correlation between PWV and FMD was only significant in the group above median age. Regarding blood pressure, FMD did have significant, moderate, inverse associations with both brachial and central SBP, which is in line with previous results (Celermajer et al., 1994).

Again, the relationship between PWV and FMD in the overall cohort lost significance following multivariate linear regression analysis that included any one of the following cofounders: brachial SBP, central SBP, central pulse pressure, and age. This is not surprising, as arterial stiffness is an important factor involved in BP regulation (Laurent et al., 2006), and FMD been correlated with BP (Maruhashi et al., 2013). Multiple studies also support age as a confounder. One analysis of a general population of over 2000 adults found a non-significant relationship in young adults (beta= −0.015, p= 0.111) and a significant relationship in older adults (beta −0.051, $p < 0.001$), although significance was lost in older adults after age and sex were accounted for (Koivistoinen et al., 2012). A second study found, in multivariate analysis including age among other independent variables, that PWV and FMD were not significantly correlated. However, when the analysis was limited to subjects above 70 years to lessen the effect of age, the relationship regained significance (Kobayashi et al., 2004). Over time, vascular pulsation stresses the network of smooth muscle cells, collagen, and elastin fibers; elastin fibers weaken and fracture, leading to gradual stiffening of arteries (Lee & Oh, 2010). Buildup of advanced glycosylation endproducts and calcium in the arterial walls also contributes to stiffening (Lee & Oh, 2010). Endothelial dysfunction similarly develops with age. Oxidative stress and damage occur when reactive oxygen species interact with and disable NO; low NO bioavailability then results in impaired vasodilation (Csiszar et al., 2008). Together, these changes accumulating over time make age one of the strongest predictors of vascular health.

Our analyses are limited by the relatively small sample size (n=39), and therefore may be underpowered to detect the true effect of traditional CVD risk factors on the relationship between PWV and FMD. For the same reason, our findings may be disproportionately affected by the data distribution. Standard deviations for mean values in older adults and in those with higher WC tended to be larger. Additionally, based on the scatterplots (Figures 5 and 6), there are more data points on either end of the distribution; these are likely driving the relationship. Future research should use a larger sample size. The cohort was also comprised mostly of women; results may not be generalizable to men. Additionally, these subjects were not well characterized metabolically. Lipid, lipoprotein, and glucose level data were unavailable, but these factors have previously been shown to affect CVD risk (D'Agostino et al., 2008). Future work should use populations with an even distribution of men and women to look at sex differences, and should include analysis of metabolic profile.

Regarding FMD, there was a lack of significance between subgroups separated by median age and median WC for FMD. Also, correlations between FMD and traditional CVD risk factors (BMI, WC, age) were unexpectedly non-significant. This may be due to the largely female cohort, and to lack of control for the menstrual cycle. In premenopausal women, FMD tends to be enhanced in the late follicular phase of the menstrual cycle (by approximately 1.5% relative to other phases) due to increased estrogen levels in the bloodstream (Adkisson et al., 2010). This may have resulted in over-estimation of FMD in some of the younger women in our cohort. Future studies should control for the effects of the menstrual cycle on FMD either by excluding women or by requiring that women be assessed in the first seven days of the menstrual cycle.

Diet and lifestyle interventions also should be conducted to determine changes in PWV and FMD following changes in behavioral CVD risk factors. Because CVD is a heterogenous class of diseases, treatment of a single risk factor will not eliminate risk; residual risk will remain. Analysis of the effect of behavioral risk factors on arterial stiffness and endothelial dysfunction will provide holistic preventative measures to lower CVD risk that are more cost effective than medications.

Finally, because no prospective cohort studies looking at the relationship between PWV and FMD have been published, much of the literature on the temporal development of arterial stiffness relative to endothelial dysfunction is speculative. Arterial stiffness may precede and trigger endothelial dysfunction in some individuals; endothelial dysfunction may cause arterial stiffness in others. This cannot be fully examined with cross sectional analyses.

In conclusion, the weak to moderate, inverse correlation between PWV and FMD in adults with enlarged WC suggests that arterial stiffness and endothelial dysfunction are physiologically distinct processes. Because this relationship was only significant in older subjects and in those with higher WC, arterial stiffness and endothelial dysfunction likely converge and occur simultaneously as age and adiposity increase. PWV and FMD are therefore clinically relevant across populations, but are likely better predictors of CVD and CVD risk factors in older adults with high adiposity. Cross sectional analysis, however, does not provide sufficient evidence to explain the temporal relationship between these two subclinical states; prospective analysis is needed to develop a clear understanding of how they progress over time.

Appendix A

Consent Form

Habitual Diet and Avocado Trial (HAT)
Informed Consent Form to Participate in Research
Penny Kris-Etherton, PhD, RD, Principal Investigator
The Pennsylvania State University, University Park

Introduction

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. The investigators listed above are in charge of the study at Penn State University, University Park. You are being asked to take part in this study because you have an increased waist circumference, which is one of the risk factors for metabolic syndrome, and this study is testing an intervention that may have an impact on decreasing your risk of health complications. Your participation is voluntary. Please take your time in making your decision as to whether or not you wish to participate. Ask the study staff to explain any words or information contained in this informed consent document that you do not understand. You may also discuss the study with your friends and family.

Why is this study being done?

The purpose of the Habitual Diet and Avocado Trial (HAT) is to determine whether eating avocados has an impact on decreasing body fat in adults who are at higher risk of becoming overweight or obese. Consumption of avocado has been linked to a variety of health benefits, but much of this research has been for short periods of time and in small samples.

How many people will take part in the study?

One thousand (1,000) people at 5 research sites will take part in this study, including approximately 250 people at two Penn State University sites.

What is involved in the study?

The study is divided into two parts:

- Screening: If you agree to participate, study personnel will first check that you are qualified by doing the tests listed below. This is called screening. Some of the tests will be repeated during the study.
- At the end of the screening visit, if you meet all the study requirements, you will be randomized into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. You will have an equal chance of being placed in either group.

- If you are randomized to Group A, you will be provided with one avocado to eat each day. You will pick the avocados up at the clinic every two weeks. You will be provided with information about how to choose, store and ripen avocados, along with ideas about how to incorporate avocados into your daily eating habits. You will be asked to eat 1 avocado every day.

- If you are randomized to Group B, you will be asked to follow your usual diet. You can eat up to two avocados per month, but we will not provide avocados to you until the end of the study.
- In both Group A and Group B, you will visit the clinic every month for six months for a follow-up visit.

Below is a list of all the procedures that happen during the study. Some of these tests will be repeated during the study.

- **Review medical history:** During screening and at the 6 month visit, you will be asked questions about your general medical history.

- **EKG:** You will have a 12-lead EKG performed at screening, which is used to detect abnormal heart rhythms.

- **Physical measures:** We will measure your height at the baseline visit only. We will measure your waist at screening, baseline, 3 and 6 month visits. We will measure your weight at the baseline visit, 3 month visit and 6 month visit. We will measure your blood pressure at all study visits except screening (baseline, 1 month and monthly thereafter).

- **MRI** (picture) of your abdomen: MRI (also called Magnetic Resonance Imaging) is painless and safe and gives a better picture than an X-Ray.
    - You will lie on a table that moves you in and out of a tunnel. You will hear loud noises. You must lie very still so that a picture can be taken. Tell your study doctor if you have a history of claustrophobia (fear of closed spaces). There is no radiation involved.
    - There is no fasting required before the MRI.
    - This will be done at screening and the 6 months visit.
- **Blood draw:** A needle will be used to draw small amount of blood from a vein in your arm or hand for routine tests. For each visit, about 30 mL (millimeter) (2 tablespoons) will be drawn. If you stay on the study for the whole duration a total of about 90 mL (millimeter) (6 tablespoons) will be drawn. Sometimes a blood test may need to be repeated with your permission if the initial blood draw is unsuccessful. If this happens the total amount of blood drawn will be more than this.

  You will have blood drawn during the baseline visit and at the 3 month and 6 month visits. Your blood will be used to check your blood cholesterol, triglycerides, glucose, insulin, high sensitivity C-reactive protein (hsCRP), and fatty acid levels, collect a DNA sample.
- **Diet Diary:** A study team member will call you to ask you what you ate in the past 24-hours. You will be asked to provide a detailed description of the type and amount of food eaten including the time at which the food item was consumed and related details. You will be provided with instructions on how to complete this 24 hour recall during the screening visit. We will ask you to do this at the screening visit, 2 month visit, 4 month visit and 6 month visit, for a total of 4 recalls.
- **Other Questionnaires:** We will also ask you other questions about your diet and other parts of your daily life and health behaviors. We will ask you about your avocado consumption. We will ask you some of these questionnaires at each study visit.
- **Pulse Wave Analysis (PWA) and Pulse Wave Velocity (PWV):** At week 0 and at 26 weeks you will undergo a test that measures your blood pressure and pulse wave forms. If you use tobacco products, you will be asked to refrain from use that day until the test has been completed.

The PWA measurement is very similar to a routine blood pressure measurement. Prior to the measurement, you will be asked to sit with your feet flat on the ground and rest for at least 5 minutes. A blood pressure cuff will be placed on your upper arm against your skin. The cuff will inflate, then deflate for 5 seconds, and then partially reinflate. It is important that you remain still during this measurement. After 1 minute of rest, the procedure will be repeated twice, for a total of 3 measurements at each time point this test is performed. Repeated measurements are used to increase accuracy.

For the PWV measurement, we will ask you to lay flat on a hospital bed without a pillow. A thin cuff will be placed on your upper leg. We will gently place a hand held probe against an artery in your neck. This probe will measure the pressure waves of the blood in your artery. Once a good waveform is obtained, the blood pressure cuff on your leg will inflate to measure the pressure waveforms in that artery. Having these simultaneous measurements allows the device to calculate the speed at which blood is traveling through your arteries. The PWV test will be performed three times with at least one minute rest between measurements at each of the time points this test is performed.

- **FMD:** At week 0 and 26 weeks you will undergo FMD testing. If you use tobacco products, you will be asked to refrain from use on the day of testing until the test is complete. This test shows the health of your arteries and will be performed using an ultrasound machine. Ultrasound is often used to see images of babies in the womb. We will use ultrasound to measure the diameter of an artery in your upper arm, before and after the inflation of a blood pressure cuff on the forearm. In most people, this procedure produces dilation (opening up) of the artery. The purpose of this test is to assess the effects of avocados on blood vessel function.

  FMD test will be performed on the brachial artery in your right arm and takes about 30 minutes. The following procedure is used:

  1. In a private room, you may be asked to remove your shirt and put on a hospital gown. (You will not be asked to remove any clothing below the waist). You will lie quietly on a bed in a quiet, darkened room.
  2. Your right arm will be extended at a 45-degree angle from your shoulder and rest on some foam cushion supports. A blood pressure cuff will be placed on your forearm.
  3. A research assistant will place 3 EKG electrodes (stickers) on your upper chest and stomach.
  4. You will be asked to rest for 10 minutes.
  5. A technician, trained in medical ultrasound, will sit at the head of the bed. The technician will apply ultrasound gel on your arm and will place an ultrasound probe (which looks like a microphone) on that arm.
  6. An image of the blood vessels in your arm will be viewed on the ultrasound equipment next to the bed. The technician may need to move the probe over a small area of your upper arm to obtain the clearest image. The image of the artery will be videotaped for 5 minutes.
  7. Next, the blood pressure cuff on your lower arm will be tightly inflated (to a pressure of 250 mmHg) and it will remain inflated for 5 minutes.
  8. While the cuff remains inflated, the technician will continue to record the image of the artery in your upper arm. At the end of 5 minutes, the cuff will be deflated and images

will be captured and recorded for an additional 2.5 minutes. It is very important throughout the recording that you rest quietly and keep your arm as still as possible.

- **Acute stress testing:** At week 0 and week 26 you will undergo acute stress testing. If you use tobacco products, you will be asked to refrain from smoking for two hours prior to testing until the test has been completed. You will also be asked to refrain from exercising on the day of testing and you will be asked not to eat or brush your teeth for at least two hours prior to this test. We will schedule your visits to be at a consistent time of day for week 0 and week 26. These tests will require approximately two hours of your time at each visit. Your blood pressure and heart rate will be monitored during stress task testing, using a blood pressure monitor with an arm cuff. During this stress testing, seven adhesive sensors will be placed on your neck and chest. Three of them are circular sensors used for EKG assessment. Four are strips of adhesive tape that will be placed around your neck and chest. The bands have a mild adhesive on them and will stick to your skin. If you find the sensors uncomfortable, the researcher will loosen them for you. These sensors are used to measure changes in electrical activity in your heart so that cardiac output (the amount of blood that your heart pumps every minute) can be measured.

  These measurements will be done while you rest and while you perform tasks that you may find to be stressful, including one physical challenge (immersing the foot in icy water for 2.5 min). The full details of the tasks will be explained at the first visit (one involves delivering a speech and the other involves mental arithmetic). Your blood pressure and heart activity will be recorded as you do the tasks. You will be monitored during the tasks and your performance will be evaluated. Audio and video images of your performance will be collected and stored without any identifying information (only the date and your study ID number will be marked on the recording). After your second testing session, a research assistant will explain the purpose of these tasks. After the rest period and at 4 other times during this visit, you will be asked to give a saliva sample (5 total saliva samples at each of 2 visits). The saliva will be used to measure hormones which change in response to stress. You will also be asked to complete brief questionnaires about your relationships and your mental and emotional responses to the tasks.

How long will I be in the study?

You will be in the study for six (6) months. You can stop participating at any time. If you decide to stop participating in the study, we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences.

What are the risks of the study?

The risk of harm or discomfort that may happen as a result of taking part in this research study is not expected to be more than in daily life or from routine physical or psychological examinations or tests. You should discuss the risk of being in this study with the study staff.

Blood Draws: During the blood draw, you may experience discomfort, swelling, bruising and/or bleeding where the needle is inserted. Occasionally, some people become dizzy, lightheaded or feel faint. Infection may occur on rare occasions.

MRI: While no significant risks have been found from the use of MRI scans, you may be bothered by the MRI machine noise and by feelings of being closed in (claustrophobia).

Pulse Wave Analysis and Pulse Wave Velocity: There are no known risks associated with the Pulse

Wave Analysis (PWA) and Pulse Wave Velocity (PWV) measurements. The sensation of pressure from the blood pressure cuff or hand-held probe may be uncomfortable. There is a possibility for red blotching or mild bruising (petechiae) appearing on the skin above and below the location of the blood pressure cuff. Studies indicate that petechiae are rare (occurring in less than ½ of 1% of patients) and it is typically not uncomfortable and does not require treatment.

FMD: There are no known risks associated with ultrasound used with FMD testing. However, because the blood pressure cuff on your right forearm is inflated tightly, it is likely that your hand and arm below the blood pressure cuff will experience "pins and needles" (tingling and pricking sensations) while the cuff is inflated and for a few minutes after it is released. This feeling is similar to what you feel when your hand or foot "fall asleep." During the 5 minutes that the blood pressure cuff is inflated on your forearm, your arm could become numb and we will ask you not to move it. This might be moderately painful. However, any discomfort or numbness should go away within minutes of cuff deflation and there are no known long-term risks associated with this test. There is a possibility for red blotching or mild bruising (petechiae) to appear on the skin above and below the location of the blood pressure cuff. Studies indicate that petechiae are rare (occurring in less than ½ of 1% of patients) and it is typically not uncomfortable and it does not require treatment. There are no risks associated with measurement of blood pressure, heart rate, or EKG as long as you are not allergic to adhesive tape. Temporary redness at the site of the electrode placement is possible.

All video tapes from the ultrasound will have no personal identifying information associated with them and will be stored in a locked closet indefinitely since there is no indication on the tape of who the subject is.

Acute Stress Testing: During the stress tasks (being asked to perform a speech and mental arithmetic) when being videotaped, you may be nervous or feel uncomfortable. Both of these "stress" situations may cause a rise in blood pressure and heart rate. You will be monitored at all times while doing the tasks and if any unsafe blood pressure is noted (not anticipated), the procedure will be discontinued.

The foot cold pressor task may result in moderate discomfort but should go away quickly after the foot is taken out of the water. Although rare, some people experience dizziness and a drop in blood pressure after the foot cold pressor task. If this happens to you, inform research staff. There is a small risk of vasovagal reaction (passing out) during the cold pressor. In our experience this is very rare (one case in 20 years of conducting these studies). A published paper reporting on outcomes of 4500 cold pressor tests estimated the risk of fainting as 1.6% of cold pressor administrations (Kiviniemi et al 2010 The American Journal of Cardiology). In the rare case in which this happens, we will act quickly to invert you so that your head is below your heart and the test will be discontinued. Your blood pressure and symptoms will be monitored until symptoms subside and blood pressure returns to baseline. You should not perform the cold pressor test if you have a condition called Reynaud's syndrome, a condition in which your hand or other body parts have a painful reaction to cold temperatures. If you have Reynaud's, you experience extreme pain in response to cold (such as taking items out of the freezer) and your skin may turn white or gray in response to cold exposure. If you think that you have Reynaud's syndrome, please discuss it with the researchers and the cold pressor test will not be conducted.

The sensor bands, applied to your neck and chest, may cause a slight irritation of the skin – this should go away rapidly once the bands are removed. There is a risk of mild discomfort with the removal of the adhesive sensors and redness at the site where the sensors have been placed is common, but temporary. It is possible that you could be allergic to the adhesive used in the sensors.

Other Possible Risks:

If you are randomized to Group A, avocados are generally recognized as safe and are consumed by adults in the general population on a regular basis. There is a slight risk that you may have an unknown allergy to avocados and may therefore have an allergic reaction. There is a chance that you could experience gastrointestinal (GI) upset. There is a chance that you could injure yourself when cutting or removing the pit from the avocado. We will provide instructions and training regarding how to cut an avocado, remove the pit, and peel an avocado. Because we are asking you to add the avocados to your diet, there is also a risk that you could gain weight.

In addition, there is a slight risk of a breach of confidentiality. We will do our best to protect your confidential information. There also may be other side effects that we cannot predict. You should tell the research staff about all the medications, vitamins and supplements you take and any medical conditions you have. This may help avoid side effects, interactions and other risks.

Pregnant women are excluded from participation in this study because this is a dietary intervention and modifications to diet without physician input are not recommended. Sexually active women of childbearing potential must use a reliable method of birth control while participating in this study. Reliable methods of birth control are: abstinence (not having sex), oral contraceptives, intrauterine device (IUD), DepoProvera, tubal ligation, or vasectomy of the partner (with confirmed negative sperm counts) in a monogamous relationship (same partner). An acceptable, although less reliable, method involves the careful use of condoms and spermicidal foam or gel and/or a cervical cap or sponge. We encourage you to discuss this issue further with your physicians if you have any questions.

Taking part in this research study may involve providing information that you consider confidential or private. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to have access to research records, will be made to keep your information safe.

Are there benefits to taking part in the study?

You may or may not receive benefit from participating in this study. We hope the information learned from this study will benefit other people in the future.

What other choices are there?

This is not a treatment study. Your alternative is to not participate in this study.

What are the costs?

All study costs, including the avocados and procedures related directly to the study, will be paid for by the study. Costs for your regular medical care, which are not related to this study, will be your own responsibility.

Will your research records be confidential?

The results of this research study may be presented at scientific or medical meetings or published in scientific journals. Your identity and/or your personal health information will not be disclosed unless it is authorized by you, required by law, or necessary to protect the safety of yourself or others. There is always some risk that even de-identified information might be re-identified.

Participant information may be provided to Federal and other regulatory agencies as required. The Food and Drug Administration (FDA), for example, may inspect research records and learn your identity if this study falls within its jurisdiction.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U.S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies) and
- The Office of Research Protections

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this Web site at any time.

Will you be pair for participating?

For your time and participation in the study you will receive monetary compensation of $500, prorated as follows and paid at the completion of your participation in the study:
Completion of baseline measurements: $100
Completion of week 12 measurements: $125
Completion of week 26 measurements: $275
Total for completion of the study = $500

For completion of the vascular measurements (FMD and Acute Stress Testing), you will receive $50 pro-rated as follows and paid at the completion of your participation in the study:
Completion of baseline measurements = $10
Completion of 26 week measurements = $40
Total for completion of vascular measurements = $50

For completion of the acute stress testing measurements you will receive $100 pro-rated as follows and paid at the completion of your participation in the study:
Completion of baseline measurements: $30
Completion of 26 week measurements: $70
Total for completion of stress testing measurements = $100

If you are a Penn State employee, you will be asked to provide your name and Penn State ID number and payment will be provided by direct deposit via the payroll system. If you are not a Penn State employee, you will be paid by check and your Social Security Number must be collected for tax reporting purposes. The compensation that you receive for participation in this study is taxable income. Total payments within one calendar year that exceed $600 will require the University to report these payments to the IRS annually.

Who is sponsoring this study?
This study is being sponsored by the Hass Avocado Board. The sponsor is providing money, avocados or other support to Wake Forest University Health Sciences and The Pennsylvania State University to help conduct this study. The researchers do not, however, hold a direct financial interest in these sponsors or the foods being studied.

Who is sponsoring this study?
In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

For more information on medical treatment for research related injuries or to report a study related illness, adverse event, or injury you should call Dr. Penny Kris-Etherton at 814-863-2923.

What are my rights as a research study participant?
Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences. The investigators also have the right to stop your participation in the study at any time. This could be because it is in your best medical interest, your condition worsened, new information becomes available, you had an unexpected reaction, you failed to follow instructions, or because the entire study has been stopped.

You will be given any new information we become aware of that would affect your willingness to continue to participate in the study.

Whom do I call if I have questions or problems?
For questions about the study or in the event of a research-related injury, contact the study investigator, Penny Kris-Etherton at 814-863-2923.

The Institutional Review Board (IRB) is a group of people who review the research to protect your rights. If you have a question about your rights as a research participant, or you would like to discuss problems or concerns, have questions or want to offer input, or you want to obtain additional information, you should contact the Chairman of the IRB at (336) 716-4542.

You will be given a copy of this signed consent form.

Signatures
I agree to take part in this study. I authorize the use and disclosure of my health information as described in this consent and authorization form. If I have not already received a copy of the Privacy Notice, I may request one or one will be made available to me. I have had a chance to ask questions about being in this study and have those questions answered. By signing this consent and authorization form, I am not

releasing or agreeing to release the investigator, the sponsor, the institution or its agents from liability for negligence.

Participant Name (Printed):_____

Participant Signature: _____ Date: _____Time:_____ am pm

Person Obtaining Consent (Printed):_____

Person Obtaining Consent:_____ Date:_____ Time:_____ am pm

Appendix B

FMD Brachial Diameter Scoring Protocol

These rules were adapted from the Vascular Tools Manual, the Framingham Heart Study Scoring routines, personal communications with Dr. Vita's lab, and our experiences scoring difficult scans.

Basic Rules:

- Readers should sit straight in front of the screen, not at an angle.
- Baseline and Deflation from a single visit should be measured in the same sitting.
- Always check calibration at the beginning of scoring. If you have not scored the image before, the latest calibration result is always memorized from the last scan you scored.
- Use the same size and location of the ROI during both the baseline and deflation images.
- Measure vessel diameter at the M line. The M Line is defined as the line between the adventia and intima structures. If you have to use the I line, make that decision in ALL scans for that subject. Review with PI if you think the I line is justified).
- Experience with scoring helps you understand the software. Feel free to practice several times before you actually collect data.

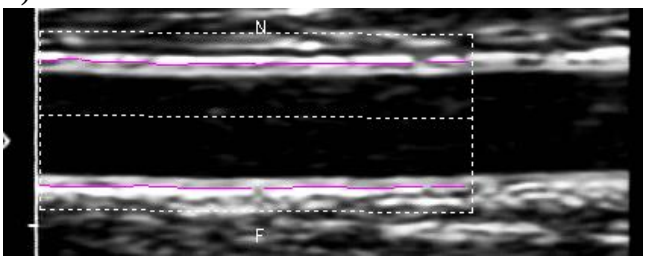
General Steps to for scoring:

1. Open the *Brachial Analyzer for Research* program.
2. Open the participant's file.
3. You will be prompted to calibrate. Calibration for the purpose of brachial diameter scoring must be completed on a frame that contains a vessel image (not Doppler). The files begin with Doppler images, so you must click "Finish" to get out of the calibration procedure, navigate forward through the scan to a vessel image, and then re-calibrate as described in step 4.
4. On the top toolbar, click Action → Calibrate. The vertical axis is on the right side of the screen. Locate the hash marks that are numbered. Use the mouse to place the bulls-eye markers on the numbered marks on the axis. The line will turn green when it is perfectly straight. This distance is 1 cm, which is equal to 10 millimeters. Verify that the marker distance box by the action menu says 10 millimeters. Then click "Finish".
5. On the FMD Brachial Diameter Scoring Form, fill out the table in the "General Information" section so you know when each condition starts and stops. Play the video, with your mouse hovering over the stop button. Focus on the event labels and stop when they change from Doppler to 2D baseline, baseline to inflate, inflate to deflate, deflate to Doppler, Doppler to 2D deflation.
6. Navigate through the file until you find a frame with a clear image and clear vessel borders that can be used for training (aka initializing/defining the region of interest). It is best to select a frame in the BASELINE condition; you can select a frame the DEFLATION condition, but never train on a frame from the INFLATE condition. You may want to play through the entire file once or twice to determine which section of the vessel has the clearest borders for the majority of the scan. The exact same section of the vessel must be scored for both BASELINE and DEFLATION, so make sure it is a section that is clear for both conditions.
7. On the top toolbar, click Action → Initialize (Define ROI). The Training Box ROI size and placement can be changed by right clicking on the circles and moving the mouse. ROI size determination will be based on quality of image, in general the ROI should be between 6–11mm, and larger is good if the quality will allow it. If images are poor quality, a smaller box can be used (3.5 – 4.0 mm). Move box

until you are satisfied that the ROI includes the longest segment with visible borders, and click "Next".

   a. Note: Training is the most critical part of successful scoring. It depends greatly on the quality of the image (which at this point, you have no control over). This is where experience comes in, allowing you to judge what modifications will work and what will not. You can make several training attempts before you actually collect data.

8. Pink measuring lines will appear on the screen. You can adjust the placement of these lines by clicking with the mouse (click where you want the line to go; do not click on the line and drag it to the proper place). The software is finicky and may not comply with where you want to put it. Do not worry too much because the line will likely move when you go on to the next step. In some scans, the pink line is much thinner than the M-line, so it will appear at the top or bottom of the M-line. It is not necessary that the thin pink line be in the middle of the thick M-line; it is necessary that it be consistently on the M-line (or I-line if it is being scored).



9. Select "Proceed" If you are satisfied with the location of the pink lines. They may move, so if you are not satisfied after clicking "Proceed", go back to step one or two and make adjustments to the ROI position or line placement. You may also decide to start over and train on a different frame.
10. Make note of the training frame and the corresponding confidence. Training frames should have a confidence of at least 70%, meaning that at least 70% of the image within the box has scorable borders. If the confidence is lower than 70%, re-train on a different vessel section or frame or use a different size ROI box (following the guidelines on box size in step 7 above).
11. When you are satisfied with the training frame, click "Proceed to Launch". The software will analyze all of the frames based on the rules you just defined in this training frame. Observe the frames as they are analyzed and determine whether the program is correctly defining the borders for nearly all frames. If the pink line is deviating far from the M-line (or I-line), stop ■ the analysis and retrain on a new frame or using a new ROI. It is acceptable if the lines "wiggle" a bit, but "jumps" on many frames are a problem.
    a. You will be prompted to close the Results window for scans with more than 1000 frames. Click "OK" to close it for analysis, and re-open it when analysis is complete (View → Analysis Results).
12. When the analysis has completed and you are satisfied with the general results, you must reject unnecessary frames (this includes Doppler and INFLATE frames). To reject these frames you want to exclude, go to the "results" window, you will see an Excel-like window. There you can click and highlight the frames you want to reject, and then click reject. You will observe that the data curve is adjusted according to your modifications. **Be sure not to reject the vessel diameter frames at Cuff-out, which appear after INFLATE and before the DEFLATE Doppler frames**. These frames are expected to have the same diameter as baseline and are a QC check for probe movement.
13. Omit marginal data by rejecting all frames with confidence less than 30%. In the Results window, click on the tab called "Quality Control". Set "Confidence Threshold" to 30% (do not use the Trend Threshold). This rejects all frames that the program identifies 30% or less of the M-line (or I-line) within the ROI for a given frame.
14. You must now save the file ***three times*** so that you can calculate baseline and deflation data properly. We place the ROI box and analyze the diameters in both baseline and deflation in the same file to make sure the placement is the same, but we must separate the conditions so that the software can correctly calculate the average baseline diameter and peak deflation diameter.
    a. On the top toolbar, click File → Save As and add _All to the end of the file name, then save.

b. The _All file will remain open. Reject all DEFLATE frames (including pre-Doppler Cuff-out frames) and keep all BASELINE FRAMES. On the top toolbar, click File → Save As and replace "_All" with "_Baseline."
c. Re-open the _All file. Reject all BASELINE frames and keep all DEFLATE frames. On the top toolbar, click File → Save As and replace "_All" with "_Deflation".

15. You will then go through the Baseline and Deflation files individually, rejecting and/or editing frames as necessary.
    a. Rejecting individual frames: You can reject frames that contain unscorable vessel images. These vessels usually do not have distinct lines. To reject these frames you want to exclude, go to the "results" window, you will see an Excel-like window. There you can click and highlight the frames you want to reject, and then click reject. You will observe that the data curve is adjusted according to your modifications. **Do not reject** outliers where the measuring lines are accurate and frames that look good but are different in diameter from other frames. Always go back and look at the accuracy of the measuring lines. *If you find that you need to reject more than ~10% of the frames, retrain on another frame or another section of the vessel.*
    b. Editing individual frames: Less manual editing is desirable. For most images, the program is more accurate than you are. You can edit frames that contain scorable vessel images. These vessels usually do have a distinct M-line, but the program failed to place a measuring line appropriately. To edit these frames, click on the icon that is a shadow of a person. Here you can edit the placement of the pink line. Do your best to correctly adjust the measuring lines to measure the M line - neighboring frames can usually be a good reference. Once you have completed your editing, select "Finish". *If you find that you need to edit more than ~5% of the frames, retrain on another frame or another section of the vessel.*
16. When you are satisfied with the scoring for both Baseline and Deflation, save the files with your changes and fill out the FMD Brachial Diameter Scoring Form. Most data needed for the data collection form can be found on the "results" screen.
17. ***NOT SURE IF THIS STILL WORKS***: If you do not remember the frame that you trained on, you can click on the "Report" button in the Results window. The top table contains study information, and you can scroll down to the row that states "Frame-initialized".

FMD SCORING FORM – BRACHIAL DIAMETER

Participant ID _____ **Scorer** _____ **Scoring Date** _____

GENERAL INFORMATION

	Baseline		Occlusion	Deflation		
	Doppler	Brachial	Inflation	Cuff-out	Doppler	Brachial
Frame						
Time						

Overall Quality of Scan on scale of 1-5 (1 = unscorable, 5 = very clear) Near wall: _____ Far wall: _____

Training frame: _____ !! Near wall scoring: M line _____ I line _____

Confidence: _____ Far wall scoring: M line _____ I line _____

Location of ROI (approximate) ROI box points: _____, _____

ROI Box Dimensions (mm): _____x_____

BASELINE MEASUREMENTS

Average Diameter: _____ ± _____ Minimum Diameter: _____ ± _____

MSE Poly Fit _____ Maximum Diameter: _____ ± _____

DEFLATION MEASUREMENTS

Diameter at Cuff-out: _____ _____ _____ _____ _____ Average: _____

(pre-doppler)

Difference between average diameter at baseline and average diameter at cuff-out: _____

(must be less than ±0.2 mm)

1st Peak Diameter: _____ ± _____ Time: _____ Frame # _____

Did you visually review the peak frame? _____ Time to peak from cuff-out (seconds) _____

60-second Diameter: _____ ± _____ Time: _____ Frame # _____

There will be 5 frames at 60sec. Choose the first one unless it was rejected

FMD CALCULATIONS

Peak diameter – baseline average = dilation / baseline average = change x 100 = % change

_____ – _____ = _____ / _____ = _____ x 100 = _____

BIBLIOGRAPHY

Adkisson, E. J., Casey, D. P., Beck, D. T., Gurovich, A. N., Martin, J. S., & Braith, R. W. (2010). Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. *Exp Biol Med (Maywood), 235*(1), 111-118. doi:10.1258/ebm.2009.009186

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., . . . et al. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol, 26*(5), 1235-1241. doi:10.1016/0735-1097(95)00327-4

Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., . . . Wilkinson, I. B. (2014). Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol, 63*(7), 636-646. doi:10.1016/j.jacc.2013.09.063

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., . . . Stroke Statistics, S. (2019). Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation, 139*(10), e56-e528. doi:10.1161/CIR.0000000000000659

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., . . . Stroke Statistics, S. (2018). Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation, 137*(12), e67-e492. doi:10.1161/CIR.0000000000000558

Bundy, J. D., & He, J. (2016). Hypertension and Related Cardiovascular Disease Burden in China. *Ann Glob Health, 82*(2), 227-233. doi:10.1016/j.aogh.2016.02.002

Butlin, M., & Qasem, A. (2016). Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel), 4*(4), 180-192. doi:10.1159/000452448

Camhi, S. M., Bray, G. A., Bouchard, C., Greenway, F. L., Johnson, W. D., Newton, R. L., . . . Katzmarzyk, P. T. (2011). The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. *Obesity (Silver Spring), 19*(2), 402-408. doi:10.1038/oby.2010.248

Cecelja, M., Sriswan, R., Kulkarni, B., Kinra, S., & Nitsch, D. (2020). Association of pulse wave velocity and intima-media thickness with cardiovascular risk factors in young adults. *J Clin Hypertens (Greenwich)*. doi:10.1111/jch.13812

Cejkova, S., Kralova-Lesna, I., Poledne, R. (2016). Monocyte adhesion to the endothelium is an initial stage of atherosclerosis development. *Cor et Vasa, 58*(4), 419-425.

Celermajer, D. S., Sorensen, K. E., Bull, C., Robinson, J., & Deanfield, J. E. (1994). Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol, 24*(6), 1468-1474. doi:10.1016/0735-1097(94)90141-4

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jr., . . . National High Blood Pressure Education Program Coordinating, C. (2003). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension, 42*(6), 1206-1252. doi:10.1161/01.HYP.0000107251.49515.c2

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., . . . International Brachial Artery Reactivity Task, F. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol, 39*(2), 257-265. doi:10.1016/s0735-1097(01)01746-6

Corrigan, F. E., Kelli, H. M., Dhindsa, D. S., Heinl, R. E., Mheid, I. A., Hammadah, M., . . . Quyyumi, A. A. (2016). Abstract 14115: Changes in Body Fat Distribution Predict Arterial Health. *Circulation, 131*.

Csiszar, A., Wang, M., Lakatta, E. G., & Ungvari, Z. (2008). Inflammation and endothelial dysfunction during aging: role of NF-kappaB. *J Appl Physiol (1985), 105*(4), 1333-1341. doi:10.1152/japplphysiol.90470.2008

D'Agostino, R. B., Sr., Vasan, R. S., Pencina, M. J., Wolf, P. A., Cobain, M., Massaro, J. M., & Kannel, W. B. (2008). General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation, 117*(6), 743-753. doi:10.1161/CIRCULATIONAHA.107.699579

Despres, J. P., & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature, 444*(7121), 881-887. doi:10.1038/nature05488

Dhindsa, M., Sommerlad, S.M., DeVan, A.E., Barnes, J.N., Sugawara, J., Ley, O., Tanaka, H. (2008). Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. *J Appl Physiol, 105*, 427–432. doi:doi:10.1152/japplphysiol.90431.2008.

Dobbelsteyn, C. J., Joffres, M. R., MacLean, D. R., & Flowerdew, G. (2001). A comparative evaluation of waist circumference, waist-to-hip ratio and body mass index as indicators of cardiovascular risk factors. The Canadian Heart Health Surveys. *Int J Obes Relat Metab Disord, 25*(5), 652-661. doi:10.1038/sj.ijo.0801582

Dornquast, C., Kroll, L. E., Neuhauser, H. K., Willich, S. N., Reinhold, T., & Busch, M. A. (2016). Regional Differences in the Prevalence of Cardiovascular Disease. *Dtsch Arztebl Int, 113*(42), 704-711. doi:10.3238/arztebl.2016.704
10.3238/arztebl.2016.0704

Ettehad, D., Emdin, C. A., Kiran, A., Anderson, S. G., Callender, T., Emberson, J., . . . Rahimi, K. (2016). Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet, 387*(10022), 957-967. doi:10.1016/S0140-6736(15)01225-8

Flammer, A. J., Anderson, T., Celermajer, D. S., Creager, M. A., Deanfield, J., Ganz, P., . . . Lerman, A. (2012). The assessment of endothelial function: from research into clinical practice. *Circulation, 126*(6), 753-767. doi:10.1161/CIRCULATIONAHA.112.093245

Forstermann, U., Xia, N., & Li, H. (2017). Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. *Circ Res, 120*(4), 713-735. doi:10.1161/CIRCRESAHA.116.309326

Fruchart, J. C., Sacks, F., Hermans, M. P., Assmann, G., Brown, W. V., Ceska, R., . . . Zimmet, P. (2008). The Residual Risk Reduction Initiative: a call to action to reduce residual vascular risk in patients with dyslipidemia. *Am J Cardiol, 102*(10 Suppl), 1K-34K. doi:10.1016/S0002-9149(08)01833-X

Furchgott, R. F., & Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, 288*(5789), 373-376. doi:10.1038/288373a0

Goff, D. C., Jr., Lloyd-Jones, D. M., Bennett, G., Coady, S., D'Agostino, R. B., Sr., Gibbons, R., . . . Wilson, P. W. F. (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol, 63*(25 Pt B), 2935-2959. doi:10.1016/j.jacc.2013.11.005

Gokce, N., Keaney, J. F., Jr., Hunter, L. M., Watkins, M. T., Menzoian, J. O., & Vita, J. A. (2002). Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation, 105*(13), 1567-1572. doi:10.1161/01.cir.0000012543.55874.47

Haynes, W. G., Noon, J. P., Walker, B. R., & Webb, D. J. (1993). Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens, 11*(12), 1375-1380. doi:10.1097/00004872-199312000-00009

Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., & Munzel, T. (2001). Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation, 104*(22), 2673-2678. doi:10.1161/hc4601.099485

Hofmann, B., Riemer, M., Erbs, C., Plehn, A., Navarrete Santos, A., Wienke, A., . . . Simm, A. (2014). Carotid to femoral pulse wave velocity reflects the extent of coronary artery disease. *J Clin Hypertens (Greenwich), 16*(9), 629-633. doi:10.1111/jch.12382

Hwang, M. H., Yoo, J. K., Kim, H. K., Hwang, C. L., Mackay, K., Hemstreet, O., . . . Christou, D. D. (2014). Validity and reliability of aortic pulse wave velocity and augmentation index determined by the new cuff-based SphygmoCor Xcel. *J Hum Hypertens, 28*(8), 475-481. doi:10.1038/jhh.2013.144

Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E., & Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A, 84*(24), 9265-9269. doi:10.1073/pnas.84.24.9265

Inaba, Y., Chen, J. A., & Bergmann, S. R. (2010). Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging, 26*(6), 631-640. doi:10.1007/s10554-010-9616-1

Joannides, R., Haefeli, W. E., Linder, L., Richard, V., Bakkali, E. H., Thuillez, C., & Luscher, T. F. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation, 91*(5), 1314-1319. doi:10.1161/01.cir.91.5.1314

Kaess, B. M., Rong, J., Larson, M. G., Hamburg, N. M., Vita, J. A., Levy, D., . . . Mitchell, G. F. (2012). Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA, 308*(9), 875-881. doi:10.1001/2012.jama.10503

Kaplan, H., Thompson, R. C., Trumble, B. C., Wann, L. S., Allam, A. H., Beheim, B., . . . Thomas, G. S. (2017). Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. *Lancet, 389*(10080), 1730-1739. doi:10.1016/S0140-6736(17)30752-3

Kim, H. L., & Kim, S. H. (2019). Pulse Wave Velocity in Atherosclerosis. *Front Cardiovasc Med, 6*, 41. doi:10.3389/fcvm.2019.00041

Kobayashi, K., Akishita, M., Yu, W., Hashimoto, M., Ohni, M., & Toba, K. (2004). Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. *Atherosclerosis, 173*(1), 13-18. doi:10.1016/j.atherosclerosis.2003.10.013

Koivistoinen, T., Virtanen, M., Hutri-Kahonen, N., Lehtimaki, T., Jula, A., Juonala, M., . . . Kahonen, M. (2012). Arterial pulse wave velocity in relation to carotid intima-media thickness, brachial flow-mediated dilation and carotid artery distensibility: the Cardiovascular Risk in Young Finns Study and the Health 2000 Survey. *Atherosclerosis, 220*(2), 387-393. doi:10.1016/j.atherosclerosis.2011.08.007

Kostis, J. B., Davis, B. R., Cutler, J., Grimm, R. H., Jr., Berge, K. G., Cohen, J. D., . . . Applegate, W. B. (1997). Prevention of heart failure by antihypertensive drug treatment in older persons with isolated systolic hypertension. SHEP Cooperative Research Group. *JAMA, 278*(3), 212-216. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9218667

Krantz, M. J., Long, C. S., Hosokawa, P., Karimkahani, E., Dickinson, M., Estacio, R. O., . . . Havranek, E. P. (2011). Pulse wave velocity and carotid atherosclerosis in white and Latino patients with hypertension. *BMC Cardiovasc Disord, 11*, 15. doi:10.1186/1471-2261-11-15

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., . . . European Network for Non-invasive Investigation of Large, A. (2006). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J, 27*(21), 2588-2605. doi:10.1093/eurheartj/ehl254

Lee, H. Y., & Oh, B. H. (2010). Aging and arterial stiffness. *Circ J, 74*(11), 2257-2262. doi:10.1253/circj.cj-10-0910

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R., & Prospective Studies, C. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet, 360*(9349), 1903-1913. doi:10.1016/s0140-6736(02)11911-8

Liao, J. K. (2013). Linking endothelial dysfunction with endothelial cell activation. *J Clin Invest, 123*(2), 540-541. doi:10.1172/JCI66843

Lu, Q., Cheng, L. T., Wang, T., Wan, J., Liao, L. L., Zeng, J., . . . Li, K. J. (2008). Visceral fat, arterial stiffness, and endothelial function in peritoneal dialysis patients. *J Ren Nutr, 18*(6), 495-502. doi:10.1053/j.jrn.2008.05.006

Lunder, M., Janic, M., Kejzar, N., & Sabovic, M. (2012). Associations among different functional and structural arterial wall properties and their relations to traditional cardiovascular risk factors in healthy subjects: a cross-sectional study. *BMC Cardiovasc Disord, 12,* 29. doi:10.1186/1471-2261-12-29

Maruhashi, T., Soga, J., Fujimura, N., Idei, N., Mikami, S., Iwamoto, Y., . . . Higashi, Y. (2013). Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart, 99*(24), 1837-1842. doi:10.1136/heartjnl-2013-304739

Mattace-Raso, F. U., van der Cammen, T. J., Hofman, A., van Popele, N. M., Bos, M. L., Schalekamp, M. A., . . . Witteman, J. C. (2006). Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation, 113*(5), 657-663. doi:10.1161/CIRCULATIONAHA.105.555235

Minor, R. L., Jr., Myers, P. R., Guerra, R., Jr., Bates, J. N., & Harrison, D. G. (1990). Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest, 86*(6), 2109-2116. doi:10.1172/JCI114949

Mitchell, G. F., Hwang, S. J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., . . . Benjamin, E. J. (2010). Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation, 121*(4), 505-511. doi:10.1161/CIRCULATIONAHA.109.886655

Miyazaki, S., Hiasa, Y., Takahashi, T., Tobetto, Y., Chen, H… Ohtani, R. (2010). Waist circumference reduction is more strongly correlated with the improvement in endothelial function after acute coronary syndrome than body mass index reduction. *J. Cardiol., 55*, 266—273.

Morigami, H., Morioka, T., Yamazaki, Y., Imamura, S., Numaguchi, R., Asada, M., . . . Inaba, M. (2016). Visceral Adiposity is Preferentially Associated with Vascular Stiffness Rather than Thickness in Men with Type 2 Diabetes. *J Atheroscler Thromb, 23*(9), 1067-1079. doi:10.5551/jat.33399

Nakashima, M., Mombouli, J. V., Taylor, A. A., & Vanhoutte, P. M. (1993). Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J Clin Invest, 92*(6), 2867-2871. doi:10.1172/JCI116907

Nigam, A., Mitchell, G. F., Lambert, J., & Tardif, J. C. (2003). Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. *Am J Cardiol, 92*(4), 395-399. doi:10.1016/s0002-9149(03)00656-8

Palmer, R. M., Ashton, D. S., & Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature, 333*(6174), 664-666. doi:10.1038/333664a0

Piepoli, M. F., Hoes, A. W., Agewall, S., Albus, C., Brotons, C., Catapano, A. L., . . . Monique Verschuren, W. M. (2016). 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Rev Esp Cardiol (Engl Ed), 69*(10), 939. doi:10.1016/j.rec.2016.09.009

Pohl, U., Holtz, J., Busse, R., & Bassenge, E. (1986). Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension, 8*(1), 37-44. doi:10.1161/01.hyp.8.1.37

Ras, R. T., Streppel, M. T., Draijer, R., & Zock, P. L. (2013). Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol, 168*(1), 344-351. doi:10.1016/j.ijcard.2012.09.047

Rees, D. D., Palmer, R. M., & Moncada, S. (1989). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci U S A, 86*(9), 3375-3378. doi:10.1073/pnas.86.9.3375

Ross, R., Freeman, J., Hudson, R., & Janssen, I. (2002). Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *J Clin Endocrinol Metab, 87*(11), 5044-5051. doi:10.1210/jc.2002-020570

Roth, G. A., Johnson, C., Abajobir, A., Abd-Allah, F., Abera, S. F., Abyu, G., . . . Murray, C. (2017). Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol, 70*(1), 1-25. doi:10.1016/j.jacc.2017.04.052

Ryder, J. R., Dengel, D. R., Jacobs, D. R., Jr., Sinaiko, A. R., Kelly, A. S., & Steinberger, J. (2016). Relations among Adiposity and Insulin Resistance with Flow-Mediated Dilation, Carotid Intima-Media Thickness, and Arterial Stiffness in Children. *J Pediatr, 168*, 205-211. doi:10.1016/j.jpeds.2015.08.034

Salvi, P., Scalise, F., Rovina, M., Moretti, F., Salvi, L., Grillo, A., . . . Parati, G. (2019). Noninvasive Estimation of Aortic Stiffness Through Different Approaches. *Hypertension, 74*(1), 117-129. doi:10.1161/HYPERTENSIONAHA.119.12853

Schoenhagen, P., Ziada, K. M., Vince, D. G., Nissen, S. E., & Tuzcu, E. M. (2001). Arterial remodeling and coronary artery disease: the concept of "dilated" versus "obstructive" coronary atherosclerosis. *J Am Coll Cardiol, 38*(2), 297-306. doi:10.1016/s0735-1097(01)01374-2

Shechter, M., Shechter, A., Koren-Morag, N., Feinberg, M. S., & Hiersch, L. (2014). Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease. *Am J Cardiol, 113*(1), 162-167. doi:10.1016/j.amjcard.2013.08.051

Soltesz, P., Der, H., Kerekes, G., Szodoray, P., Szucs, G., Danko, K., . . . Szekanecz, Z. (2009). A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. *Clin Rheumatol, 28*(6), 655-662. doi:10.1007/s10067-009-1118-y

Strasser, B., Arvandi, M., Pasha, E., Haley, A. P., Stanforth, P., & Tanaka, H. (2015). Association between visceral fat mass, arterial stiffness measures and cardiovascular health status in middle aged adults. *Atherosclerosis, 241*(1), e165.

Takase, H., Dohi, Y., Toriyama, T., Okado, T., Tanaka, S., Sonoda, H., . . . Kimura, G. (2011). Brachial-ankle pulse wave velocity predicts increase in blood pressure and onset of hypertension. *Am J Hypertens, 24*(6), 667-673. doi:10.1038/ajh.2011.19

Teragawa, H., Ueda, K., Matsuda, K., Kimura, M., Higashi, Y., Oshima, T., . . . Chayama, K. (2005). Relationship between endothelial function in the coronary and brachial arteries. *Clin Cardiol, 28*(10), 460-466. doi:10.1002/clc.4960281004

Thomopoulos, C., Parati, G., & Zanchetti, A. (2014). Effects of blood pressure lowering on outcome incidence in hypertension: 3. Effects in patients at different levels of cardiovascular risk--overview and meta-analyses of randomized trials. *J Hypertens, 32*(12), 2305-2314. doi:10.1097/HJH.0000000000000380

U.S. Food & Drug Administration. (2017). FDA Facts: Biomarkers and Surrogate Endpoints. Retrieved from https://www.fda.gov/about-fda/innovation-fda/fda-facts-biomarkers-and-surrogate-endpoints

Virani, S. S., Alonso, A., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., Carson, A. P., . . . Stroke Statistics, S. (2020). Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. *Circulation*, CIR0000000000000757. doi:10.1161/CIR.0000000000000757

Watts, K., Bell, L. M., Byrne, S. M., Jones, T. W., & Davis, E. A. (2008). Waist circumference predicts cardiovascular risk in young Australian children. *J Paediatr Child Health, 44*(12), 709-715. doi:10.1111/j.1440-1754.2008.01411.x

Willum-Hansen, T., Staessen, J. A., Torp-Pedersen, C., Rasmussen, S., Thijs, L., Ibsen, H., & Jeppesen, J. (2006). Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation, 113*(5), 664-670. doi:10.1161/CIRCULATIONAHA.105.579342

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., . . . Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature, 332*(6163), 411-415. doi:10.1038/332411a0

Yeboah, J., Crouse, J. R., Hsu, F. C., Burke, G. L., & Herrington, D. M. (2007). Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation, 115*(18), 2390-2397. doi:10.1161/CIRCULATIONAHA.106.678276

Zanchetti, A. (2009). Bottom blood pressure or bottom cardiovascular risk? How far can cardiovascular risk be reduced? *J Hypertens, 27*(8), 1509-1520. doi:10.1097/HJH.0b013e32832e9500

Zhong, Q., Hu, M. J., Cui, Y. J., Liang, L., Zhou, M. M., Yang, Y. W., & Huang, F. (2018). Carotid-Femoral Pulse Wave Velocity in the Prediction of Cardiovascular Events and Mortality: An Updated Systematic Review and Meta-Analysis. *Angiology, 69*(7), 617-629. doi:10.1177/0003319717742544

ACADEMIC VITA

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EDUCATION

The Pennsylvania State University, University Park, PA
Schreyer Honors College
Bachelor of Science in Biology, Vertebrate Physiology option
Minor in Psychological Science
Graduation: May 2020

Relevant Courses

Biology - Biodiversity, Function & Development of Organisms, Molecules & Cells, Ecology, Medical Embryology, Mammalian Physiology, Neurobiology, Functional Neuroscience (with corresponding labs)
Psychology – Intro to Psychology, Abnormal Psychology, Social Psychology, Cognitive Development, Child Psychopathology
Chemistry – Chemical Principles 1 & 2, Organic Chemistry 1 & 2 (with corresponding labs)
Biochemistry – General Biochemistry 1 & 2
Physics – Introductory Physics 1 & 2
Mathematics – Intro to Biostatistics (with lab), Calculus and Biology 1 & 2

RESEARCH EXPERIENCE

Research Internship, Children's Hospital of Philadelphia Summer 2019
Amplified Musculoskeletal Pain Syndrome (AMPS) Program, Division of Rheumatology
Supervisor: Dr. Sabrina Gmuca, MD, MSCE

- Abstracted medical records contributing to a database characterizing AMPS presentation & prognosis
- Worked on a retrospective study of disordered eating in AMPS patients; created a database, wrote inclusion/exclusion criteria, performed chart reviews

Undergraduate Research Assistant
Vascular Health and Interventions Laboratory May 2018 – May 2020
Advisors: Professor Kristina Petersen, Professor Sheila West

- Contributed to the Habitual Diet and Avocado Trial (HAT), studying the effects of unsaturated fatty acids from avocados on cardiovascular disease risk markers
- Completed a thesis aiming to determine the relationship between cardiovascular disease risk markers (pulse wave velocity and flow-mediated dilation) in cross sectional analysis of adults with enlarged waist circumference

Laboratory of Developmental Neuroscience 2017-2018
Advisor: Professor Suzanne Scherf

- Contributed to a study using computer-based training to improve social skills in autistic children
- Recruited participants, scheduled participant visits, entered data

TEACHING EXPERIENCE

Undergraduate Teaching Assistant 2018-2019
Biochemistry and Molecular Biology 1, for Dr. James Howell
Intro to Anthropology, for Dr. Douglas Bird

WORK EXPERIENCE

Concierge & Cashier, Merchandise Department Summer 2017
Sesame Place Theme Park, Langhorne, PA

Assistant Camp Staff Member Summer 2017
CCNS Preschool, Washington Crossing, PA

VOLUNTEERING

Distinguished member, Global Medical/Dental Brigades (2017 – May 2020)

- Traveled to Ghana (May 2018) and Honduras (March 2019)*;* Held medical clinics, helped build pipe system to improve clean water access
- Planned to travel to Greece (May 2020) to hold medical clinic for Syrian refugees, trip cancelled due to COVID-19

Member, Apollo Benefiting THON (2016 - May 2020)

- Student run organization fundraising for pediatric cancer research

CareMate, Saint Mary Medical Center (Summer 2017)

Member, Rules and Regulations THON Committee (2016-17)

HONORS & SCHOLARSHIPS

Evan Pugh Scholar's Award (2018-19, for academic excellence)

The President's Sparks Award (2017-18, for academic excellence)

The President's Freshmen Award (2016-17, for academic excellence)

Penn State Provost's Award (2016-20, $16,000)

Bristol-Myers Squibb Company Scholarship (2016-20, $8,000)

PUBLICATIONS

Pianucci, L., Sonagra, M., Greenberg, B.A., **Priestley, D.R**., & Gmuca, S. Disordered Eating Among Adolescents with Chronic Pain: The Experience of a Pediatric Rheumatology Subspecialty Pain Clinic. *J Pediatr Psychol*. Submitted for publication.

ADDITIONAL SKILLS

REDCap Database

SAS Programming