EVALUATION OF THE RELATIONSHIP BETWEEN CAROTID PERIVASCULAR ADIPOSE TISSUE AND ARTERIAL HEALTH

EVALUATION OF THE RELATIONSHIP BETWEEN CAROTID PERIVASCULAR ADIPOSE TISSUE AND ARTERIAL HEALTH

By

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A Thesis Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements for

the Degree Masters of Science in Kinesiology

McMaster University MASTER OF SCIENCE (2015)

Hamilton, Ontario (Kinesiology)

TITLE: EVALUATION OF THE RELATIONSHIP BETWEEN CAROTID PERIVASCULAR ADIPOSE TISSUE AND ARTERIAL HEALTH

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NUMBER OF PAGES: xv, 88

Abstract

Perivascular adipose (PVAT) has been hypothesized to influence arterial health, where an excess can lead to pathogenesis of atherosclerosis and other arterial pathologies. A novel assessment of carotid PVAT is the use of carotid extra media thickness (EMT) ultrasonography. Currently, there is a lack of research to demonstrate the relationship between carotid EMT and existing measures of arterial health, notably, central pulse wave velocity, and carotid distensibility and intimal media thickness. In the current cross sectional study, 81 participants of younger recreationally active (ages 23.2 ± 2.5 years), younger sedentary (ages 26.4 ± 7.2 years), older healthy (ages 70.3 ± 5.4 years) and older adults with coronary artery disease (CAD) (ages 67.9 ± 8.7 years) were recruited. Resting measures of central arterial stiffness was examined through the assessment of aPWV, while measures of local carotid stiffness were examined through carotid distensibility. Aortic PWV was calculated using an accepted direct distance method (80% of carotid to femoral direct distance) and time difference between the feet of the carotid and femoral waveforms. Carotid intima-media thickness (IMT), a measure of the inner arterial walls, and carotid extra media thickness (EMT), a measure of carotid PVAT, were assessed through B-mode ultrasound images and a semi-automated edge tracking software. Carotid EMT, IMT, and aPWV were significantly greater in older adults than in younger adults ($p < 0.05$). No difference in carotid EMT was found between younger recreationally active (0.47 ± .08 mm) and sedentary adults (0.46 ± .06 mm). There were also no differences in carotid EMT between the older healthy (0.58 ± .06 mm) and older adults with CAD (0.54 ± 0.08 mm). Carotid EMT was also significantly correlated with age (r =0 .500), waist circumference (r = 0.521), aPWV (r =0.431), carotid distensibility (r = -0.364 and IMT (r = 0.404). Despite significant

correlations, carotid EMT was not an independent predictor of aPWV, carotid distensibility and IMT. Because of the lack of predictive power in measures of arterial stiffness and carotid IMT, there is a potential that carotid EMT may be an independent vascular disease marker. Future investigations should involve carotid EMT in longitudinal studies to evaluate the potential marker for a more comprehensive cardiovascular risk assessment.

Acknowledgements

I would like to thank my supervisor, Dr. Maureen MacDonald for all her guidance, care and support throughout my undergraduate and graduate experience. I was her first student to accept from the Medical Radiation Sciences program and that was a risk, knowing that my background in ultrasound was different from the expertise of a kinesiology/physiology student. There were numerous times when I had last minute presentations, written submissions, grants where you would be willing to make sure that I was ready and prepared. Your care for not only academic work, but also skill development, career advancement and life was incredible and shows that you care about all of your students, treating us as life-long friends, not just another student or number. I would like to extend my appreciation to my committee members, Dr. Peter Keir and Dr. Ada Tang for their guidance and expertise. I would also like to thank all the participants of the study. Without your help and voluntary participation in this study, we would not be able to further our understanding of the human physiology and look for ways to help advance technology for our health benefit.

I would like to thank the members of the Vascular Dynamics Laboratory for their assistance in recruitment, data collection and academic insight. Without your hard work, the completion of the study would not be possible. Special thanks goes out to Jason Au for all of his clever ideas, meticulous attention to detail and guidance as my “in lab supervisor”. It has been an honour to work with all of you in the lab and I hope to continue our connections in the future.

I would like to thank my family, friends and my life partner, Amy Lu for all their support in my academic journey. Having gone through these past two years with you to go through tough times together solidifies my confidence heading into our future.

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LIST OF ALL ABBREVIATIONS

aPWV	Aortic Pulse Wave Velocity
BMI	Body mass index
CAD	Coronary Artery Disease
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EMT	Extra media thickness
IMT	Intima-media thickness
LDL	Low density lipoprotein
MAP	Mean arterial pressure
MMP	Matrix metalloproteases
NO	Nitric Oxide
PA	Physical activity
PP	Pulse pressure
PTT	Pulse transit time
PVAT	Perivascular Adipose Tissue
SBP	Systolic blood pressure
VSMC	Vascular smooth muscle cell
WC	Waist circumference

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Cardiovascular disease (CVD) is a pathological disorder of the heart and blood vessels, and is the leading cause of death around the world [1]. Specifically, atherosclerosis and hypertension, dominate other pathologies as major contributors to death and disability, with a projected highest ranking for mortality by 2020 [2]. Hence, the study and analysis of the human cardiovascular system is vital.

While blood pressure, family history of cardiovascular disease, age, ~~LDL~~Low Density Lipoprotein-cholesterol, and male sex are generally recognized as traditional risk factors for the determination of cardiovascular health, arterial stiffness has also been shown to be an independent predictor of CVD [3]. Arterial stiffness can be described as the reduced ability for an artery to vasodilate and vasoconstrict in response to changes in blood pressure [119]. Changes in the stiffness of arteries can be measured non-invasively in humans using several different techniques including carotid femoral aortic pulse wave velocity (aPWV) and carotid artery distensibility [4]. There are, however, limitations to all techniques currently used in the assessment of central arterial stiffness, which impact the feasibility of incorporating these ~~and incorporation of~~ assessments into routine clinical practice. The existing barriers to the inclusion of measurements of arterial stiffness in the prediction of CVD risk include technical limitations of assessment methods well as a lack of consensus with respect to the impact of blood pressure on the measurement of arterial stiffness [4, 5]. As such, it would be valuable to develop alternative methods of assessing arterial stiffness that are technically feasible and independent of blood pressure.

In the area of carotid artery structure and function, it has been suggested that an excess of perivascular adipose tissue (PVAT) may be involved in the pathogenesis of atherosclerosis and other arterial pathologies [7]. Previous studies have determined that, in the human heart, increased periventricular adipose tissue is associated with increased ventricular contractile stiffness [8-10]. Existing indices of carotid artery health include intima media thickness (IMT) and carotid artery stiffness. As adipose tissue surrounds all conduit arteries of the body, and carotid artery stiffness and IMT has been shown to be predictive of cardiovascular mortality [12], it is critical to examine the relationship between carotid PVAT and carotid artery health.

Carotid artery extra-media thickness (EMT) is a method that provides an estimation of the thickness of the adventitial layer and PVAT of the carotid arteries in humans [13]. Currently, there is a lack of research examining the relationship between carotid EMT and existing measures of arterial health. This literature review will provide information on the current methods of evaluating arterial health including arterial stiffness and carotid IMT, the impact of aging and physical activity on arterial health and explore the use of carotid EMT as a potential alternate or complementary indicator of the arterial health in humans.

1.2 Arterial Anatomy

Arteries are tubular structures that carry blood from the heart to all the tissues of the human body [14]. Structurally, arteries consist of three distinct layers: the intima, the media and the adventitia. The intimal layer, the most inner layer, includes the endothelium and the internal elastic lamina, which borders between the intima and media [15]. The medial layer consists of smooth muscle cells that arrange circularly around the blood vessel, capable of

vasoconstriction and vasodilation [14]. The outermost layer of the artery is the adventitia, which is composed of collagen, elastin and adipose tissue [14]. Histologically, an artery is primarily made up of collagen and elastin, which give an artery stability, resilience and compliance [16]. During heart cycles, the pulsatile flow from systole increases the pressure in, and diameter of, the artery. Elastin provides the artery with the ability to expand and recoil artery back to form, while collagen provides strength to prevent the artery from rupturing at high pressures [17]. The following (figure 1) is a structural diagram of the different layers of the arterial wall:

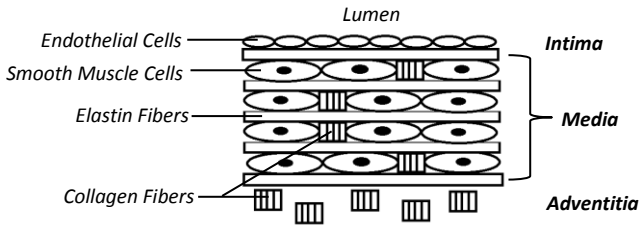

Figure 1. Structural diagram of the arterial walls and layout of elastin and collagen fibers, to demonstrate the spread of fibers within the arterial wall. Elastin provide elastic properties while collagen provide structural support.

Structurally, the elastin fibers and smooth muscles in the media run parallel to each other [45]. In the adventitia layer, collagen fibers are arranged in large bundles in a helical structure [120]. Not all arteries are equal in terms of elastin and collagen composition, as we see more elastin in the proximal aorta, but more collagen in peripheral arteries distally [45].

1.3 Arterial Stiffness

Elastin and collagen play an important role in arterial wall stiffness as elastin bears most of the wall stress during low and normal pressures, while collagen plays a protective role in higher pressures [19]. There is a dynamic process of production and degradation of elastin and collagen, and in normal circumstances, this balance is held stable [16]. However, stimulation by inflammatory processes or hypertension can lead to excessive production of collagen and decreasing amounts of normal elastin [20-21]. In arterial stiffening, there also is an increased presence of disorganized and dysfunctional collagen with broken and ineffective elastin [16]. Ultimately, an imbalance of increased collagen and decreased elastin leads to increased vascular stiffness.

At a molecular level, arterial stiffness is influenced through the activity of matrix metalloproteases (MMPs) [28]. MMPs are categorized into collagenase, elastase, and gelatinase, depending on their function. Normally, MMPs help regulate the elastin and collagen content through degradation of these fibers [121]. Tissue inhibitors of metalloproteases (TIMPs) counter these processes by providing a regulatory mechanism to inhibit the degradation of elastin and collagen [29]. Protein expression, interactions with other MMPs, and increased blood flow are contributors to upregulation of MMP expression. Injury and inflammation also contribute to the increase production of MMPs, which in turn degrade elastin and collagen content [16].

In addition to structural contributions from elastin and collagen, arterial stiffness is also influenced by endothelial and vascular smooth muscle cell (VSMC) signaling. VSMC tone can be modified through shear stress from blood flow [22] and through paracrine mediators such as angiotensin II and nitric oxide [23]. In normal endothelial function, shear stress promotes nitric

oxide production, which then induces relaxation of the VSMC. Endothelial dysfunction is defined as the imbalance of vasodilation and vasoconstriction processes that are influenced by the endothelial cells [122] and endothelial dysfunction may be linked to increases in arterial stiffness [24]. A reduction of nitric oxide from endothelial dysfunction can lead to the stiffening of the arterial wall [25. Angiotensin II has been shown to increase VSMC tone and it has been demonstrated that angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockade both can decrease arterial stiffness [27][26].

1.3.1 Arterial Stiffness and Aging

Simply put, the walls of our arteries get stiffer as we age [30]. It has been well established in the literature that arteries in the human body stiffen with age, with increases in stiffness observed predominantly in the central, elastic arteries [31-32]. At a cellular level, the balance between MMPs and TIMPs is altered, inducing increased MMP gene expression or increased secretion of MMP's by inflammatory cells [33]. In aging, this chronic imbalance leads to a modified elastin to collagen ratio, thereby promoting stiffening of the arterial wall [34]. Previous research using rodent models, demonstrated a decrease in elastin and a two-fold increase in collagen in 30 month-old animals compared to 6-month old animals [35]. Further comparisons in this study of the same age groups have shown increases in MMP-2 content and activity, without concomitant changes in TIMPs.

Aging is also related to endothelial dysfunction [34]. In rodent models, older rats (8-12 months old) demonstrate a decreased flow mediated nitric oxide production compared to a younger group (2-3 months old) [38]. In human models, endothelium-dependent vasodilation

decreases with aging in both normotensive and hypertensive adults [39]. Angiotensin II is also found to be increasingly abundant in endothelial cells with aging [40]. As angiotensin II has been shown to increase VSMC tone, and increases arterial stiffness, this suggests that endothelial dysfunction, decreased nitric oxide availability, and increased angiotensin II are all present with aging.

1.3.2 Arterial Stiffness in Population with Coronary Artery Disease

Coronary Artery Disease (CAD) is a chronic condition where lesion formation and arterial reformation leads to plaque development and ultimately, stenosis or occlusion of the arteries that feed the heart [41]. In a prospective study [42], aortic pulse wave velocity (aPWV), a measure of arterial stiffness, was found to be an independent predictor of CAD. This was a large study in the Netherlands comprised of 2835 participants, with complete data sets for 2265. Also, aPWV was also found to improve the prediction of cardiovascular disease when added to models including risk factors (smoking, body mass index, brachial blood pressures, and diabetes), pulse pressure and measurement of atherosclerosis using common carotid intimal-media thickness (cIMT). In another study, aPWV was found to be significantly increased in groups with CAD involving multiple vessels compared to groups with single or two-vessel disease [43]. In the Thai population, arterial stiffness was also shown to be an independent predictor of CAD and improves the prediction beyond the traditional risk factors [37].

Although arterial stiffness measurements have the potential to predict coronary artery disease risk, when compounded with other clinical states, arterial stiffness may not have such a big impact in predictive power. In a study looking at the relationship between arterial stiffness

and coronary artery disease risk in individuals with metabolic syndrome [36], it was found that brachial-ankle PWV was not a significant determinant of the number of diseased coronary arteries using multiple linear regression analyses. However, in the group without metabolic syndrome, brachial-ankle PWV was a significant determinant of the number of diseased coronary arteries in their study population. These different predictive capacities in different populations indicate that more research is required with respect to the utility of inclusion of arterial stiffness in models of CVD risk prediction.

1.3.3 Arterial Stiffness and Physical Activity

It has been well-established in the literature that exercise and physical activity are associated with a reduced risk of CVD [123-124]. Specifically with respect to arterial stiffness, previous research has provided evidence that habitual aerobic exercise can potentially reduce the effects of age-related increases in aPWV in older males [125]. Another study also described the lack of differences in aPWV between active pre-menopausal and active post-menopausal women [126], demonstrating that exercise can potentially attenuate the effects of age on arterial stiffness. Although studies suggest that moderate intensity aerobic exercise can reduce arterial stiffness [127-129], evidence suggests that even light intensity aerobic exercise can be beneficial for older adults [130].

Although the current literature appears to support the notion that aerobic exercise can lower arterial stiffness, there is controversy over the effects of resistance exercise training on arterial stiffness. There are previous studies that demonstrate an increase in arterial stiffness with high intensity resistance training [131-132]. Other studies have also provided evidence

that resistance exercise training has no influence on arterial stiffness [133-134]. A combination of resistance and aerobic training, however, appears to decrease arterial stiffness [135].

1.3.4 Measurement of Arterial Stiffness

There are a variety of different non-invasive techniques used to assess arterial stiffness in humans. These methods include measurement of PWV, local arterial stiffness, and augmentation index. Each technique is derived based on different theories, different assumptions, and different uses, depending on the investigation. Each method also has different benefits, special control requirements and limitations. As new discoveries are made, the measurement of arterial stiffness is ever changing and new recommendations are agreed upon within the scientific community, to enhance to robustness of these measurement tools.

1.3.4.1 Aortic Pulse Wave Velocity

Theoretical models of the human central aorta are useful to assess arterial stiffness [44]. The current accepted model of the central aorta consists of a simple elastic tube that enables the propagation of a pulse wave that travels along the tube [45-46]. A pulse wave can be defined as a pressure wave generated by the arterial pressure pulse [47]. The speed of the travelling pulse wave or PWV is linked to the elastic modulus of the arterial wall through the Moens-Korteweg equation (equation 1).

$$PWV = \sqrt{\frac{E\,h}{2\,R\,\rho}}$$

Equation 1. Moens-Korteweg equation defining Pulse Wave Velocity, where E is Young's modulus, h is the wall thickness, R is the radius of the arterial lumen and ρ is the density of blood.

In the equation, E is Young's modulus, h is wall thickness, R is the radius of the artery, and ρ is the density of blood [48]. Since it has been established that PWV is dependent on the elasticity of the arterial walls, measurement of arterial PWV is an accepted index of arterial stiffness.

Aortic pulse wave velocity (aPWV) is currently the "gold standard" technique to measure central aortic stiffness, representing the stiffness of the aorta [55]. Specifically, aPWV has been shown to be an independent predictor of cardiovascular disease in the general population and in populations with clinical conditions such as hypertension [56], coronary artery disease (CAD) [57], and diabetes [58]. The recommended cut-off value for aPWV in the prediction of cardiovascular disease is 10 m/s [50].

Aortic PWV is typically determined from the transit time between the pulse at the carotid and the femoral arteries [49]. The pulse transit time is determined by the time difference between the arrival of pressure waveforms at the sites of the common carotid and common femoral pulse points [53]. Specifically, the anatomical location of these pulse points would be at the right common carotid artery and at the right common femoral artery. The foot of each arterial pressure waveform, defined as the arrival of the pulse, is used to determine the pulse transit time (PTT). A schematic representing typical arterial pressure w waveforms at each of the sites is shown in Figure 2.

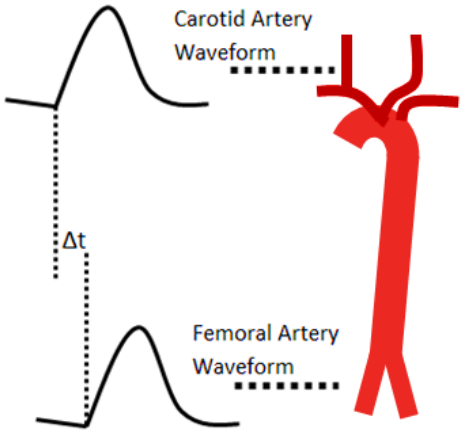

Figure 2. Measuring aPWV with the time difference (Δt) between the feet~~oot~~ of the right carotid artery and right femoral artery waveforms ~~each waveform~~

A surface distance measurement is taken to estimate the path length distance between the two pulse measurement sites [54]. This measurement can be done with a measuring tape, although a caliper is recommended, as body surface contours can potentially introduce error on a tape measure. Based on the current consensus, 80% of the distance between the sites is then divided by the pulse transit time [44]. The calculation for aPWV is shown in equation 2.

$$aPWV = \frac{direct\ distance\ (m)\ x\ 0.8}{pulse\ transit\ time\ (s)}$$

Equation 2. Calculation for aPWV using the carotid-femoral approach, where the 80% of the direct distance is divided by the time difference between the arrival of each pulse.

Research previous to this consensus took a different approach to measuring the distance between the two sites. The anatomical distance between the two sites is not simply a straight direct measurement, as the arteries and aorta go through different directions and bifurcations

[50]. Because it is technically difficult to obtain a pulse non-invasively at the aortic arch, the carotid pulse is taken as a surrogate. This led to the subtraction method, where the distance between the common carotid arterial pulse site and the aortic arch [44]. Two common methods of the subtraction methods have been used [137]:

1. Distance between the sternal notch and femoral artery subtracted by the distance between the sternal notch and carotid artery.
2. Total distance between the sternal notch, umbilicus and femoral artery is subtracted by the distance between the sternal notch and carotid artery.

Because of the inaccuracies of surface markers to estimate arterial anatomy, the use of magnetic resonance imaging have determined that in humans, the carotid to aortic arch distance is affecting the aPWV calculation by approximately 3% [136]. The standard suggested was to take the 80% of the direct measurement between the carotid and femoral pulse site [44]. Evidence from the expert consensus demonstrated an overestimation of 0.4%, but was the most accurate method over the subtraction methods.

Consensus standards have been established to standardize the measurement of PWV to enable comparisons between studies and to control for physiological changes that may alter the accuracy of the measurement [50]. The following are the recommended controls for measuring aPWV [51-53]:

- The environment must be quiet with consistent room temperature.
- Measurements are taken in the supine position after 10 minutes of quiet rest with no talking or sleeping during the measurement

- The right common carotid and right common femoral arteries are the sites to perform the measurement
- Refrain from meals, caffeine or smoking 3 hours prior to the measurement.
- A single data set must be taken for at least 10 heart cycles, with the mean pulse transit time obtained from this data set.
- Obtain the mean of two data sets. If the difference between these two means is greater than 0.5 m/s, perform a third measurement and obtain the median value from all three measures.
- When performing repeated measures, taking the measurement during the same time of day is preferred.

There are some limitations to the use of PWV in assessing arterial stiffness. The femoral pressure waveform may be difficult to obtain due to patient large body habitus [51] or in instances of arterial disease; the pressure wave may be delayed, which introduces inaccuracies in the PTT [44]. Other limitations include the distance measurement, where abdominal obesity or large bust sizes in women can introduce error [51]. Investigators must be aware that small distance inaccuracies can alter the absolute value of aPWV [54]. When comparing different data sets from different studies, it is important to note whether what distance measurement method was used. Repeated measures of the same method are accepted in intervention studies, but comparing data that used different distance measurement techniques would introduce critical errors [44].

1.3.4.2 Local Arterial Stiffness Measures

The stiffness properties of an artery can also be defined in terms of pressure and diameter changes [59]. When comparing the stiffness of two arteries, a stiffer artery will require more pressure to achieve the same increase in diameter. The measurement of pressure and diameter change to represent arterial stiffness is derived from the Bramwell-Hill model that uses the Moens-Korteweg equation to demonstrate the relationship between PWV and arterial volume with blood pressure [60]. This model, along with advances in measurement tools, led to the development of techniques to assess local arterial stiffness of the common carotid artery [62-63].

Measurements of common carotid artery compliance and distensibility are derived from the models that equate changes in arterial volume and blood pressure to PWV [64]. Using brightness mode (b-mode) ultrasonography, the common carotid artery can be imaged to determine the changes in diameter throughout a heart cycle [61]. With the assumption that an artery is cylindrical in shape, the cross sectional area can be derived from the lumen diameter obtained from two dimensional ultrasound [65]. Local arterial blood pressure at the site of the diameter measurement can be estimated through a combination of calibrated beat-by-beat brachial or radial artery blood pressure measurement and local carotid artery blood pressure wave form determination [66]. One assumption of this method is that diastolic blood pressure is consistent throughout the arterial tree [67]. This assumption permits the estimation of local carotid artery blood pressure through measurement of blood pressure in the digit and forecasting of pressure in the carotid artery [69]. Combining measurements of carotid blood pressure and carotid diameters, compliance and distensibility can be calculated with the following equations (Equation 3 & 4.) [70]:

$$Compliance = \frac{\pi(r_{systolic})^2 - \pi(r_{diastolic})^2}{Pressure_{systolic} - Pressure_{diastolic}}$$

Equation 3. The Calculation of Carotid Compliance, where the change in the cross-sectional area of the artery is divided by the change in blood pressure.

$$Distensibility = \frac{Compliance}{\pi(r_{diastolic})^2}$$

Equation 4. The Calculation of Distensibility as a Function of Compliance, where the change in cross-sectional area of the artery is divided by the product of the change in blood pressure and diastolic cross-sectional area.

In Equation 3, "r" represents the radius of the carotid artery. The difference between the calculation of compliance and distensibility is that distensibility includes the control for diastolic arterial cross sectional area [71]. It is important to acknowledge that there are many variations in human anatomy. Since sizes of carotid arteries can vary [72], the initial baseline diameters are not reflected in compliance. The distensibility of two arteries may be different, even though the compliance may be the same, which may potentially indicate arterial remodeling [138].

Although carotid compliance and distensibility are accepted measures of local carotid arterial stiffness, there are some assumptions in the model upon which the measurements are based. First, due to limitations of ultrasonography, the physical measurement of the artery is limited to a single dimension diameter measurement [73]. To determine cross-sectional area, it is assumed that the arterial lumen is a perfect circle [74]. With regards to the carotid blood pressure, in addition to diastolic pressure assumptions, the procedure requires the measurement of carotid pressures on the contralateral carotid to the ultrasound measurement

[63]. Due to the limitations to the measurement technique of simultaneous carotid diameter and voltage measurements, this is a necessary assumption that the blood pressures are the same bilaterally. Previous research on the carotid distensibility methodology performed non-simultaneous measurements of ultrasonography and tonometry readings to ensure that the same location was recorded [139]. However, since blood pressure is a variable measure, it is recommended to assess blood pressure and diameter changes simultaneously [140].

Despite these limitations, the measurements of carotid compliance and distensibility have been accepted in the scientific community and have been used widely in studies examining arterial stiffness in humans [44]. In cross-sectional studies, carotid compliance and distensibility have been negatively correlated to aPWV [75-76] indicating that a less compliant or less distensible artery can potentially be equated to a stiffer artery.

1.3.4.3 Augmentation Index

Arterial pressure waveforms are generated from the heart, with a forward wave and then preceded by a reflected wave [50]. The forward wave is generated from ventricular ejection, while the reflected wave is the result of distal peripheral resistance. Typically, a waveform at an elastic artery would not see the reflected wave until later in diastole, while a stiffer artery would see the reflected wave early. The resulting wave is a combination of the forward and reflected wave, which augments the pressure wave detected. This augmented section is quantified as the augmentation pressure (first point of inflection to the peak of the waveform). The peak of the waveform refers to the systolic pressure while the foot of the

waveform refers to the diastolic pressure. The difference between the systolic and diastolic pressure is referred to as the pulse pressure (PP). To calculate augmentation index (AIx), the augmentation pressure is divided by the PP. AIx is usually displayed as a percentage as it is dimensionless [141]. Figure 3 below demonstrates a typical arterial waveform and the measure of augmentation index.

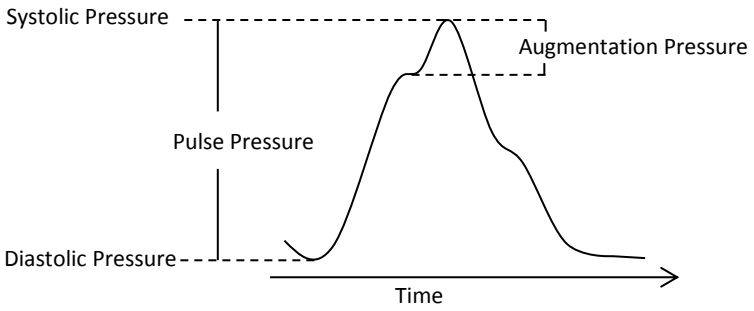

Figure 3. Arterial Pressure Waveform demonstrating the different components to calculate AIx. The difference between the systolic pressure and the first inflection is noted as the augmentation pressure. The pulse pressure is defined as the difference between systolic and diastolic pressure. AIx is calculated as the augmentation pressure divided by the pulse pressure.

The non-invasive assessment of arterial pressure waveforms are typically performed with applanation tonometry over a more accessible artery, namely the carotid or radial artery. To assess central aortic waveforms, the waveforms taken at the carotid or radial artery would undergo a transfer function, which are based on previous research [143]. For the carotid artery, and it is recommended that a transfer function is not necessary since the carotid artery and the aortic arch are anatomically very close and their pressure waveforms are very similar [142].

The use of a transfer function is a limitation to the use of pressure waveforms as transfer functions introduce error in obtaining absolute pressure values [144]. Another limitation of using the transfer function is the inaccuracy of the AIx. The transfer function relies on the use of central systolic blood pressure to calibrate the arterial waveform taken at the radial artery [146]. Unfortunately, transfer functions using blood pressure measurements do not accurately convert AIx as it is more reliant on higher frequency pressure signals [144]. Despite these limitations, central AIx have shown to be an independent predictor for mortality [64] and cardiovascular disease [145]. The use of applanation tonometry to measure augmentation index is also considered to be simple to perform and well tolerated by the individual [50]. Recently, the American Heart Association published a scientific statement that does not recommend the use of AIx in assessing central arterial stiffness [147]. Although the use of AIx has been shown to be useful and effective, there is not enough evidence in the literature to demonstrate the use of AIx across different populations. The statement from the AHA, recommends the analysis of the amplitudes of the forward and reflected waves. This refers to the reflection magnitude, which is the ratio of the amplitude of the forward and reflected pressure waves [148].

1.4 Carotid Artery Intima Media Thickness

The examination of the pathophysiology of the human carotid artery stems primarily from the technical difficulties associated with non-invasive examinations of the coronary arteries [77]. The coronary arteries are the major arteries that supply the heart with blood flow for nutrient and waste exchange and atherosclerotic development and stenosis in the coronary arteries have detrimental effects on the heart's ability to function [78]. Correlations between

coronary arterial atherosclerosis and carotid artery atherosclerosis have been observed in previous studies [79-81]. Because the carotid artery has been more technically feasible to image, measures of the carotid artery function and pathogenesis have been adopted as a surrogate to the coronary artery of the heart [82].

The intimal and medial layers of the artery are the most inner layers and this is where atherosclerotic development typically occurs [83]. The atherosclerotic process begins with endothelial cell damage and the subsequent accumulation of white blood cells, cholesterol and triglycerides in between the intima and medial layers, characterized as “fatty streaks” [84]. These fatty streaks can later calcify and harden, forming plaques in the arterial wall. Plaque development increases arterial stiffness [85] and decrease arterial compliance and distensibility [86]. Atherosclerosis can lead to acute cardiovascular diseases, such as myocardial infarction and stroke [87].

The theoretical basis of the measurement of the carotid intimal media thickness is that the atherosclerotic development in carotid arteries begins between the intima and media layers [88]. A carotid IMT can range from 0.5 mm to greater than 1.0 mm [91], and has been associated to increasing age [92]. Interestingly, although carotid IMT is an independent predictor of cardiovascular disease and mortality [94-95], carotid IMT does not predict arterial stiffness. In a study of 366 participants (ages 30-80 years), carotid IMT did not predict arterial stiffness measured as aPWV and AIx in diabetic and non-diabetics [93]. The investigators recognized that although carotid IMT is positively correlated to aPWV and AIx, this relationship is lost when adjusting for age, gender, and heart rate.

1.4.1 Carotid Artery IMT and Aging

From the evidence presented in past research, carotid IMT increases as we age [166-168]. In a large cross-sectional study, 289 participants in Japan were recruited, ranging from ages 21 to 98 [169]. Carotid IMT, plaque occurrence, and traditional cardiovascular disease risk factors were obtained. The study found a strong correlation (r = 0.83, $p < 0.05$) between carotid IMT and age. With participants over 50 years old, only age was independently related to carotid IMT. The mean carotid IMT was also found to increase with each increasing decade.

From the measurement of carotid IMT, the concept of vascular age was popularized [170]. The concept of vascular age utilizes multiple factors to calculate an individual's risk of cardiovascular disease [171]. These factors include age, body mass index, blood pressure, hypertension, cholesterol, fasting glucose, diabetes, smoking, medical history of cardiovascular disease, and carotid IMT [172]. In a value-based approach to calculate vascular age, a combination of race, sex and carotid IMT is used [170]. Some studies have suggested the use of vascular age to communicate cardiovascular disease risk to patients [173-175]. Although the use of carotid IMT in calculating vascular age may be a useful tool in cardiovascular risk assessment, but there is no commonly accepted calculation method to implement in clinical use [171].

1.4.2 Carotid Artery IMT and CAD

The literature has established a positive relationship between carotid IMT and the progression of CAD, where participants with a higher number of stenosed vessels were found with thicker carotid IMT [87,149-150]. In a study involving 558 participants (ages 40-81) with

suspected CAD [151], carotid IMT was found to be an independent predictor of CAD. Participants with CAD had significantly greater IMT than participants without CAD, regardless whether the diseased involved one, two or three vessels. They also established that over 95% of participants with carotid IMT greater than 1.15 mm had significant coronary artery stenoses.

Although thicker carotid IMT is seen in individuals with more severe coronary artery stenosis compared to healthy individuals, the correlation between carotid IMT and coronary artery percent stenosis is weak (r = .23-.27) [152-153]. In a cross-sectional study of 350 participants [152], a large overlap in IMT values was found between subjects with and without coronary artery stenosis. The study suggested that there is no definite cutoff point to define whether someone has a coronary artery stenosis. This contrasts to an established cutoff in carotid IMT to define atherosclerosis, where values greater than or equal to 0.9 mm is considered to have abnormal IMT [154-155].

1.4.3 Carotid Artery IMT and Physical Activity

The relationship between physical activity and carotid IMT has been well investigated in the literature [156-158]. Several studies investigating the differences in sedentary behavior versus active lifestyle demonstrated increases in IMT in individuals who lead a less active lifestyle [159-161]. In a large population study, 614 healthy men and women (ages 30-60 years) participated in physical activity and carotid IMT assessments, with three years between baseline and post measures [162]. The initial baseline measurement of carotid IMT demonstrated a positive relationship between the proportion of time spent in sedentary activity and increases in carotid IMT. It was found, after 3 years, participants with periods of

vigorous activity demonstrated a significantly lower carotid IMT compared to those who is only performed light to moderate levels of activity. Interestingly, this relationship was observed in participants with initially higher periods of light to moderate activity.

Although physical activity may reduce carotid IMT in adults, there is a lack of association between physical activity in childhood and carotid IMT in adulthood. In a prospective study in Finland, 1603 participants (ages 9-15 years) were followed for 21 years [163]. Although carotid IMT was not related to childhood physical activity, the study suggests that aortic IMT may be a more sensitive marker for early atherosclerosis in children. Other studies, have also found controversial results where data showed no influences of physical activity on carotid IMT in healthy participants [156, 164-165]. It has been suggested that the controversial data could potentially be due to the error introduced by the use of self-report questionnaires used to evaluate physical activity [157].

1.4.4 Measurement of Carotid Artery IMT

The use of b-mode ultrasonography to delineate the carotid IMT has been widely recognized and accepted [176]. The intimal media layers can be visualized and quantified, thereby indicating the atherosclerotic development in carotid arteries [89]. Sonographically, the intima appears as a bright white layer, while the media layer is a dark black layer [90]. The media layer is bordered by the bright white adventitia layer that enables delineation of the media border (Figure 3).

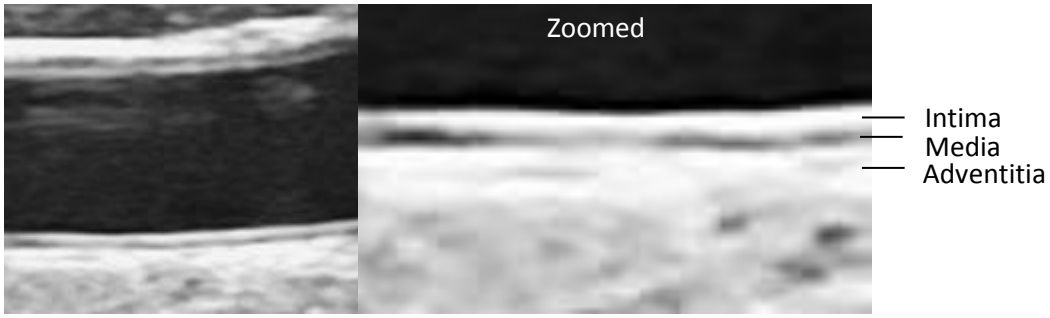

Figure 4. Ultrasound Image of the IMT. Defined in this image is the intima is defined as the bright layer, deep to that a dark media layer, followed by a bright adventitia layer.

Although anatomically the carotid IMT can be seen in the near-wall and far-wall on ultrasound, the far-wall measurement has been used more frequently due to the higher quality and resolution in the produced images [176]. Specifically, when imaging the common carotid artery, the mean measurement over a 10 mm length of IMT should be acquired at end diastole and the measurement location should be free of plaque [177].

The measurement of carotid arterial wall can also be performed through the use of computed tomography (CT) [178] or magnetic resonance imaging (MRI) [179]. One of the limitations to the ultrasound approach of measuring carotid IMT is the single dimension view that is obtained. With CT or MRI, a three dimension view of the carotid can be obtained. However, the dose of ionizing radiation is still a concern with CT imaging and the high costs of MRI imaging make ultrasound imaging a more feasible method to obtain carotid IMT data [178].

1.5 Carotid Artery Perivascular Adipose Tissue (PVAT)

Surrounding most conduit arteries is adipose tissue that borders next to the adventitia or PVAT [99]. Adipose tissue has been theorized to play an important role in stiffness of the cardiovascular system [7]. In the heart, studies have shown an increased thickness of epicardial fat is associated with an increased instance of atherosclerosis [7, 100-101]. PVAT of the thoracic aorta, in a study of 286 patients with possible coronary artery disease, was associated with coronary atherosclerosis, where PVAT volume and number of coronary plaque were significantly correlated [102]. In a study of 1414 African Americans, thoracic aorta PVAT was found to be associated with coronary arterial calcification [103] thereby indicating that increased PVAT, may be associated with increased CVD risk [104-106].

While the detailed cellular mechanisms of the effects of PVAT on arterial pathophysiology are not well understood, PVAT seems to contribute to arterial endothelial function through signaling both relaxation and constriction factors of vascular smooth muscle [107]. Specifically PVAT releases nitric oxide to act on the vascular smooth muscle to relax [108], while PVAT also releases angiotensin II to induce vasoconstriction [109]. In addition, PVAT contributes to arterial stiffness through the release of inflammatory factors, such as interleukin-6, that have been associated with endothelial dysfunction [110].

As an important component of the arterial wall, the carotid adventitia layer contributes greatly to arterial health through the presence of adventitial fibroblasts [96]. Adventitial fibroblast can be activated to produce nitric oxide to stimulate vasorelaxation. Upon vascular injury, adventitial fibroblasts can also migrate into the intima and media complex and contribute to atherosclerotic development [98]. Structurally, adipose tissue also exists within the adventitia layer, making the adventitia and perivascular adipose tissue complex more

homogeneous [112]. This histologic homogeneity between the adventitia and PVAT, presents challenges to the use of ultrasound imaging as a method to quantify and measure the adventitia layer and the PVAT [97]. The carotid adventitia is composed mainly of collagen fibers [98], which appear bright and echogenic on B-mode imaging. With the media layer, the inner border appears darker or hypoechoic, allowing for contrast differentiation between the media and adventitia layer [13]. Unfortunately, the outer border around the adventitia layer does not provide a good contrasting border. Hence, identifying and measuring the adventitia layer and PVAT as discrete structures non-invasively continues to be a challenge.

1.5.1 Carotid Artery Extra Media Thickness (EMT)

Although PVAT can be imaged through modalities such as computed tomography [113] or magnetic resonance imaging [114], ultrasound could potentially provide a more feasible tool for use in research and in clinical settings. Compared to other imaging techniques, ultrasound is more readily available, cheaper and does not involve ionizing radiation [115].

In 2009, Dr. Michael Skilton and his team developed a new technique that could potentially quantify PVAT around the common carotid arteries with the use of ultrasonography imaging [13]. By positioning the ultrasound probe on the lateral side of the neck, the ultrasound beam penetrates through the internal jugular vein before reaching the common carotid artery. Through this acoustic window, the jugular vein acts as a contrasting border, as the lumen is typically darker than the adventitia layer of the carotid artery [116]. The bright layer seen through this ultrasound imaging technique will encompass three different components: the carotid adventitia, carotid PVAT and the jugular vein. Together, these three layers are termed

the extra-media thickness (EMT) [117]. Figure 4 demonstrates the ultrasound appearance of the carotid EMT.

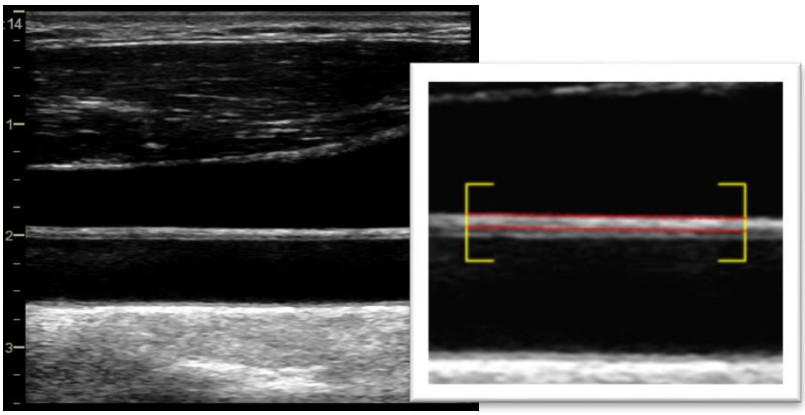

Figure 5. Carotid EMT Ultrasonography Delineated by the Red Region of Interest. The semi-automated software highlights the 1 centimeter region of interest between the yellow brackets.

Carotid EMT ultrasonography is a novel measurement with the potential to represent a quantitative assessment of PVAT of the carotid arteries and perhaps be used as an indicator of arterial health in conjunction with, or in place of assessments of arterial stiffness and carotid IMT. Recent studies have demonstrated the potential for EMT to provide additional information to that available from carotid IMT [13]. Carotid EMT has also been shown to be associated with excessive weight gain in infancy [118], and epicardial and pericardial fat in adults [116]. In a cross-sectional study with older adults (ages 53-68 years), carotid EMT is thicker in obese adults and adults with type 2 diabetes compared to the healthy group [117]. Carotid EMT can potentially provide information on the carotid PVAT, which may translate to information on arterial health.

1.6 Objectives and Hypothesis

There are two objectives to the current study. The first objective was to determine whether PVAT, as measured by carotid EMT, is different in cohorts of individuals with expected varying degrees of arterial health. Specifically, the cohorts included younger recreationally active adults, younger sedentary adults, older adults and older adults with coronary artery disease. For this objective, we hypothesize that in younger adults, the sedentary group will have thicker carotid EMT than the recreationally active group. Similarly in the older adults, we predict that older adults with coronary artery disease will have thicker carotid EMT than the healthy older adults. Overall, we predict that older adults will have thicker carotid EMT than younger adults.

Our second objective focused on an examination of the relationship between carotid PVAT, as measured by carotid EMT and arterial health. The aim of this objective was to determine how carotid EMT contributes to existing models that predict arterial stiffness and carotid IMT in individuals with an expected range of arterial health. We hypothesized that carotid EMT will be an independent predictor of aortic pulse wave velocity (aPWV), carotid compliance, distensibility and IMT will explain additional variance in existing models used to predict arterial health indices.

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CHAPTER 2: EVALUATION OF THE RELATIONSHIP BETWEEN CAROTID PERIVASCULAR ADIPOSE TISSUE AND ARTERIAL HEALTH

2.1 Introduction

Cardiovascular disease, and more specifically, atherosclerosis and hypertension, dominate other pathologies as major contributors to death and disability, with a projected number one ranking for mortality by 2020 [1]. Hence, the study and analysis of the human cardiovascular system is vital. In terms of research examining of carotid artery structure and function, many previous studies have focused on the assessment of the thickness of the inner layers of the arterial wall, termed the intima media thickness (IMT) [2], while the assessment of the outer adventitia layer and the surrounding adipose tissue has been neglected. Most arteries in the human body are directly surrounded by adipose tissue and it is hypothesized that an excess of perivascular adipose tissue (PVAT) may be involved in the pathogenesis of atherosclerosis and other arterial pathologies [3]. Previous studies have determined that, in the human heart, increased periventricular adipose tissue is associated with increased ventricular contractile stiffness [4-6]. As adipose tissue surrounds all conduit arteries of the body, and carotid artery stiffness and IMT has been shown to be predictive of cardiovascular mortality [7], we aimed to examine the relationship between carotid PVAT and carotid artery health.

The development and assessment of the carotid extra-media thickness (EMT) technique is in its infancy and further research is required to evaluate this method of assessment of the thickness of the adventitial layer and PVAT of the carotid arteries in humans. The first objective of this study was to determine whether PVAT, as measured by carotid EMT, was different in cohorts of individuals with expected varying degrees of arterial health. Specifically, the cohorts

included younger recreationally active adults, younger sedentary adults, older adults and older adults with coronary artery disease. Previous research tells us that factors, such as lack of physical activity [8] and presence of CAD, can have detrimental effects on our arterial health [9-10]. Hence, investigations examining PVAT in individuals with varying arterial health profiles can fill in this knowledge gap.

Currently, there is a lack of research to demonstrate the relationship between carotid EMT and existing measures of arterial health, notably, central pulse wave velocity, and carotid distensibility and IMT. Therefore, to fill in the gap of knowledge, current study will also examine the relationships between carotid EMT and these existing measures of arterial health. The aim of this objective was to determine how carotid EMT contributes to existing models that predict arterial stiffness and carotid IMT in individuals with an expected range of arterial health.

2.2 Methods

2.2.1 Participants

Eighty one participants in total of four different populations were recruited from across Ontario, Canada to take part in this cross-sectional observational study. The four populations included: younger recreationally active adults (n= 26, age = 23.2 ± 2.5 years old), younger sedentary adults (n= 25, age = 26.4 ± 7.2 years old), older healthy adults (n= 15, age = 70.3 ± 5.4 years old), and older adults with coronary artery disease (CAD) (n= 15, age = 67.9 ± 8.7 years old).

For the younger recreationally active group the inclusion criteria included males and females ages 18 to 50 years old, who participate in at least 150 minutes of moderate to

vigorous intensity physical activity per week according to the Canadian Physical Activity Guidelines. Any participants in this group with pre-existing diagnosed cardiovascular disease or any other diagnosed conditions were excluded. The inclusion criteria for the younger sedentary adults group were ages 18 to 50 years old that scored less than 600 MET-min/week on the International Physical Activity Questionnaire [11]. Any participants in this group with pre-existing diagnosed cardiovascular disease or any other diagnosed conditions were excluded. The inclusion criteria for the older health adult group included those aged 65 years or older. Any participants in this group with pre-existing diagnosed cardiovascular disease were excluded from the study. The inclusion criteria for the older adult with CAD group were previous CAD event that was defined as the patient having at least one of the following: angiographically documented stenosis >=50% in at least one major coronary artery; myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft surgery; and positive exercise stress test determined by a positive nuclear scan or symptoms of chest discomfort accompanied by ECG changes of greater than 1 mm horizontal or down sloping ST-segment depression. The exclusion criteria are as follow: smoking within 3 months, non-cardiac surgical procedure within 2 months, myocardial infarction or coronary artery bypass graft within 2 months, percutaneous coronary intervention within 1 month, New York Heart Association class II-IV symptoms of heart failure [12], documented valve stenosis, documented severe chronic obstructive pulmonary disease, symptomatic peripheral arterial disease, unstable angina, uncontrolled hypertension, uncontrolled atrial arrhythmia or ventricular dysrhythmia, and any musculoskeletal abnormality that would limit lying down supine for a 2 hour testing session.

2.2.2 Study design

Participants were invited for a single visit to the Vascular Dynamics Laboratory in the Department of Kinesiology at McMaster University. Prior to the visit, relevant medical history and current medical condition information was obtained through questionnaire to ensure the participants met the inclusion and exclusion criteria. Participants were instructed to be fasted and refrain from caffeine intake 8 hours and from strenuous physical activity 24 hours prior to the visit. If participants regularly took medication for specific medical conditions, the participants were instructed to take their medication as usual. The visit began with an overview of the study, and written, informed consent was then obtained. Anthropometric measures were then performed and recorded. After resting supine for 10 minutes, vascular measures were conducted. The entire testing session took approximately 1 hour, in a quiet, temperature controlled environment.

2.2.3 Anthropometric measures

Participants were instructed to wear light clothing and requested to remove their shoes for the anthropometric measures. Body mass was measured to the closest 0.1 kg using a digital scale (Detecto Scales, FHD Series, Webb City, Missouri, USA). Supine height was measured to the closest 0.5 cm. Waist circumference was measured to the closest 0.5 cm in the supine position [13]. Tape measure was used at the border of the superior iliac crest on the right side of the body following the end of a normal expiration.

2.2.4 Resting heart rate and blood pressure

After anthropometric measures were taken, participants were asked to rest supine for 10 minutes. Following this rest period, four sets of brachial blood pressure measurements were taken using an automated sphygmomanometer (Dinamap Pro 100, Critikon LCC, Tampa, Fla, USA). Each set of measurements provided supine brachial systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP). The first set of blood pressure measurement was not included in the calculation as recommended [14]. The average of the last three measurements was recorded and averaged. Continuous heart rate was measured with a single-lead electrocardiograph (Powerlab model ML795; ADInstruments, Colorado Springs, CO, USA). Reconstructed continuous measurements of brachial blood pressure were taken using an automated finger cuff and calibration system (Finometer MIDI, Finapres Medical Systems BV, Amsterdam, The Netherlands). Continuous measurements of heart rate and blood pressures were obtained simultaneously using a data acquisition system (Powerlab model ML795; ADInstruments, Colorado Springs, CO, USA), with a corresponding software program (Labchart 7; ADInstruments Inc., Colorado Springs, CO, USA).

2.2.5 Vascular measures

Pulse Wave Velocity

The measurement of central arterial stiffness was obtained through the measurement of aortic pulse wave velocity (aPWV). This was done through the acquisition of arterial pressure waveforms over the right carotid and right femoral arteries using simultaneous applanation tonometry (model SPT-301; Millar Instruments, Houston, TX, USA). The tonometry signals were

continuously post-processed with a band pass filter (5-30 Hz) to identify the foot of the waveforms (Powerlab model ML795; ADInstruments, Colorado Springs, CO, USA). Physiologically, the foot of each waveform represents end diastole, where the sharp rise of the waveform occurs.

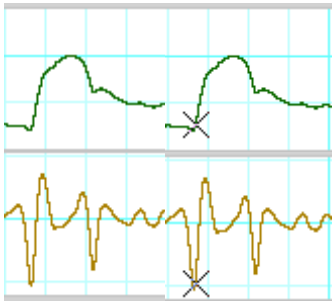


Figure 1. Tonometry Waveforms and the Corresponding Filtered Waveform

After a band pass filter, the minimum point represents the foot of the waveform obtained from the tonometer. The time difference between the two minimum points of the filtered carotid and femoral signals was denoted as the pulse transit time (PTT). Two sets of 10 waveforms were taken, and used to calculate the average PTT for each set. The equation for aPWV is as follows:

$$aPWV = \frac{direct\ distance\ (m)\ x\ 0.8}{pulse\ transit\ time\ (s)}$$

The distance between pulse measurement sites was estimated using a direct surface measurement between the two arterial pulse points (right carotid and femoral arteries), using a non-elastic tape measure. The aPWV was calculated using the average PTT for each set, producing two aPWV values for each participant. If both aPWV calculations were greater than 0.5 m/s in difference, a third set of 10 waveforms was taken. The final aPWV was calculated

with the average PTT of either the initial two sets in the case of agreement or three sets of waveforms in the case a third was required.

Carotid Compliance and Distensibility

Common carotid arterial stiffness was estimated from the determination of both carotid compliance and distensibility. The calculation of carotid compliance and distensibility are as follows:

$$Compliance = \frac{\pi(r_{systolic})^2 - \pi(r_{diastolic})^2}{Pressure_{systolic} - Pressure_{diastolic}}$$

$$Distensibility = \frac{Compliance}{\pi(r_{diastolic})^2}$$

where r represents the radius of the common carotid artery. From the above equations, the required variables include the size of the artery and the blood pressure. To obtain the size of the right common carotid artery, b-mode ultrasound images were taken with a 12 MHz linear transducer (12L-RS Transducer; GE Medical Systems, Horten, Norway) with a high resolution ultrasound machine (Vivid Q; GE Medical Systems, Horten, Norway). The transducer was placed on the right neck to image the right common carotid artery. Simultaneously, applanation tonometry was performed on the left common carotid artery, over the point of greatest pulsation. A finger cuff measurement system that reconstructs brachial blood pressure (Finometer MIDI, Finapres Medical Systems BV, Amsterdam, The Netherlands) was used to estimate brachial blood pressure. With the participant rested in a supine position, the diastolic and mean arterial pressures of all conduit arteries are assumed to be consistent throughout the arterial tree [15]. With waveform information from the applanation tonometry and

reconstructed brachial blood pressure, carotid artery systolic blood pressure can be calculated by equating the minimum and mean waveform value to the diastolic and mean arterial pressure respectively [16]. A total of 10 heart cycles of simultaneous ultrasound images, applanation tonometry waveforms and finger cuff blood pressure measurements were obtained and analyzed.

Carotid Intima Media Thickness

The measurement of carotid intima media thickness was performed using b-mode ultrasonography on the right common carotid artery. A 30 second video loop was obtained with triggering via single lead echocardiography and the images were saved in the DICOM (Digital Image and Communications in Medicine) format in a picture archiving and communication system (EchoPAC Clinical Workstation System 110.0.2; GE Medical Systems, Horten, Norway). The far-wall IMT of the common carotid artery was determined using a semi-automated edge tracking software [AMS (Artery Measurement System) Image and Data Analysis; Gothenburg Sweden] to analyze the frames acquired from the ultrasound imaging. Using the differences in brightness intensities, or acoustic impedance, the software detected the different edges of the lumen to intima interface and media to adventitia interface to delineate the intima-media thickness. With a selection of a region of interest, each frame was analyzed and the intima-media borders were displayed and calculated. An average distance between 100 points across the region of interest displayed a mean IMT measurement for that specific image frame. A total of 10 end-diastolic frames were measured and the average of these 10 values each representing 100 individual IMT data points (10 frames x 100 IMT measurements/frame = 1000 IMT measures per data set) was reported for the common carotid IMT measurement.

Carotid Extra Media Thickness

B-mode ultrasonography (frequency: 13 MHz, frames rate: 40 FPS, depth: 3.0 cm) of the right neck region in which the acoustic window included from superficial to deep, the jugular vein lumen, venous wall, perivascular adipose tissue, common carotid adventitial, media, intima and lumen. One 30 second b-mode image loop was obtained and then each end-diastolic frame was extracted and compiled in one DICOM file (Sante DICOM Editor 3.1.13, Santescroft, Athens, Greece). This extracted file went through post-processing by inverting the gray scale of every frame. By inverting the shades of gray, the EMT is now dark and anechoic, allowing the edge tracking software to easily detect the borders (AMS Image and Data Analysis). A total of 10 end diastolic frames were compiled and measured in a region of interest with 100 points. The average of these points per frame was taken for each image. The measurement of the total 10 frames was averaged to produce the EMT measurement reported in this study.

2.2.6 Statistical analysis

Statistical analyses were performed with SPSS software (SPSS 20. IBM, Armonk, NY, USA). All statistical analyses were performed with a minimum criterion alpha level of $p \leq 0.05$ to determine statistical significance. To determine differences in carotid EMT between different groups of expected varying degrees of age and vascular health. We deployed a one way, between group analysis of variance with Tukey post hoc tests. To verify the statistical power in performing the ANOVA test, power calculation software (G*Power, University of Düsseldorf, Germany) was used

To test the day-to-day repeatability of the novel measure of carotid EMT, 6 younger sedentary males were involved with a baseline measure and asked to return 12 weeks after for a second measure. Participants were asked to maintain their regular sedentary states, with no change to their daily activities. Baseline and post-12 week measures were analyzed and compared with coefficients of variation.

To determine if EMT is a predictor of arterial stiffness, specifically aPWV and carotid distensibility, and carotid IMT, a multiple regression analysis with a backward model was performed. If the resulting model involved more than one independent predictor, a forward model was performed to determine how much of the variance each variable explained in predicting the two measures of arterial stiffness and carotid IMT. To determine if EMT explains additional variance in a model that predicts arterial stiffness and carotid IMT with currently well-established variables, a multiple regression analysis was performed with a blocked entry method. The first block involved variables that predict arterial stiffness, specifically age, sex, body mass index, brachial blood pressures and carotid IMT. These factors are included because they influence and affect arterial stiffness [17]. In the model that predicts carotid distensibility, brachial blood pressures were excluded because these variables are involved in the calculation of carotid distensibility. In modeling IMT, only age, sex, body mass index, and brachial blood pressures were used, as these factors influence the carotid IMT [18]. The next block involves all the previously mentioned variables and the inclusion of EMT.

2.3 Results

2.3.1 Participant Characteristics

The participant characteristics and anthropometric measures are reported in Table 1. Between the younger recreationally active and sedentary group, there were no group mean differences in age, height, body mass index, or resting HR and brachial artery blood pressures. Between the older healthy and adults with CAD groups, there were no average differences in age, weight, height, or resting heart rate, and brachial artery blood pressures. There were differences in age and brachial artery blood pressures between the younger and older groups.

2.3.2 Vascular Outcome Measures

The vascular outcome measures are reported in Table 2. There were differences in aPWV [$F(3,77) = 27.16$, $p < 0.001$] , carotid compliance [$F(3,76) = 6.90$, $p < 0.001$], distensibility [$F(3,76) = 10.56$, $p < 0.001$] and IMT [$F(3,77) = 91.83$, $p < 0.001$]. Specifically, aPWV was faster in the older versus younger groups, but there were no differences between each group within an age range (Figure 1). Similarly, carotid distensibility was elevated in the older versus younger groups, with no differences between each group within the age range (Figure 2). Finally, carotid IMT was thicker in the older groups compared to the younger groups with no differences between each group within the age range (Figure 3).

The carotid EMT measurements are reported in Table 2. Post-hoc power analysis was performed to determine if the study was sufficiently powered to answer the questions posed. From the data collected, the effect size was 0.646, alpha = 0.05, n = 81 and a total of four groups. The power calculated was 0.999, demonstrating that the probability of making a

false positive (type II error) will be <0.1%. Because EMT is a new technique in measuring perivascular adipose tissue, we conducted a small day-to-day repeatability measurement on 6 participants. The average coefficient of variability of the 6 participants was 0.071 (Table 3).

Carotid EMT measurement was different between age groups [$F(3,77) = 10.808$, $p < 0.001$]. Carotid EMT was found to be elevated in older adults compared to younger adults. There were no group differences in carotid EMT between younger recreationally active and sedentary adults. There were also no group differences in carotid EMT between older healthy and older adults with CAD (Figure 4).

2.3.3 Associations

Pearson's correlation analyses were conducted to determine the relationship between carotid EMT, anthropometric measures and the other vascular outcome measures. Carotid EMT was significantly correlated with age ($r = 0.5$, $n = 81$, $p < 0.001$), waist circumference ($r = 0.521$, $n = 44$, $p < 0.001$), aPWV ($r = 0.431$, $n = 81$, $p < 0.001$), carotid IMT ($r = 0.404$, $n = 81$, $p < 0.001$), and carotid distensibility ($r = -0.364$, $n = 80$, $p < 0.001$) (Table 4).

In addition, we performed a multiple regression analyses to determine if carotid EMT is an independent predictor of current measures of arterial stiffness and carotid IMT. In a backwards model to predict aPWV (Table 5), the final model included age, brachial systolic blood pressure and sex (Table 6). These variables predicted aPWV, $F(3,76) = 61.011$, $p < 0.001$, $R^2 = 0.707$. With carotid distensibility (Table 7), the final model resulted in only age as an independent predictor (Table 8). Age significantly predicted carotid distensibility, $F(1,79) =$

34.428, $p < 0.001$, $R^2 = 0.306$. Lastly, the final model for predicting carotid IMT (Table 9) resulted in only age as an independent predictor, $F(1,79) = 248.561$, $p < 0.001$, $R^2 = 0.761$ (Table 10).

We also wanted to determine if assessment of carotid EMT can add additional information to the current models of predicting arterial health as represented by arterial stiffness and carotid IMT. Specifically, the block entry method was used to determine if EMT can explain any additional variance in existing prediction models. In the model for aPWV (Table 11), the variables included in the model were age, sex, body mass index, brachial systolic, diastolic and mean arterial pressure, and IMT. This model was significant in predicting aPWV, $F(7,72) = 25.27$, $p <0.001$, $R^2 = 0.711$. The additional of EMT into the model explained an additional of 0.3%, however, the F change was not significant ($p = 0.391$). In the model to predict carotid distensibility (Table 12), the variables of age, sex, body mass index, and IMT were included. This model was significant, $F(4,75) = 8.536$, $p <0.001$. The addition of EMT explains an additional 0.9% of the variance, which was not significant ($p = 0.324$). In the final model to predict carotid IMT (Table 13), the variables of age, sex, body mass index, and brachial systolic, diastolic and mean arterial pressure were included. This model was significant, $F(6,79) = 35.718$, $p < 0.001$. The addition of EMT explains an additional 0.01% of the variance, which was not significant ($p = 0.552$).

Table 1: Participant Characteristics

	Younger Recreationally Active Adults	Younger Sedentary Adults	Older Healthy Adults	Older Adults with CAD
Gender	13/13 (M/F)	25 (M)	8/7 (M/F)	15 (M)
Age, yrs	23.2 ± 2.5*	26.4 ± 7.2*	70.3 ± 5.4	67.9 ± 8.7
Height, m	1.72 ± .08	1.77 ± .09	1.69 ± .10†	1.75 ± .06
Mass, kg	68.4 ± 13.0	81.0 ± 20.7**	80.8 ± 15.7	84.6 ± 16.4**
Resting Heart Rate, bpm	61.8 ± 9.2‡	62.9 ± 10.7‡	59.0 ± 9.1	52.9 ± 8.4
Resting Systolic Blood Pressure, mmHg	115.6 ± 9.5*	112.5 ± 9.3*	128.4 ± 19.3	130.9 ± 22.9
Resting Diastolic Blood Pressure, mmHg	67.2 ± 5.4‡	67.4 ± 4.4‡	73.1 ± 12.1	76.1 ± 10.0
Resting Mean Arterial Pressure, mmHg	86.5 ± 4.4*	85.3 ± 4.8*	94.3 ± 13.2	99.3 ± 13.6
Body Mass Index, kg/m^2	23.1 ± 2.6	25.7 ± 5.3	28.3 ± 4.9†	27.7 ± 5.2†
Waist Circumference, cm	73.5 ± 6.6*	-Not Collected-	92.1 ± 10.8	94.9 ± 12.2

Values are represented as means ± SD.

*Significantly different than Older Healthy and Older Adults with CAD

**Significantly different than Younger Recreationally Active Adults

†Significantly different than Younger Sedentary Adults

‡Significantly different than Older Adults with CAD

Table 2: Vascular Measures

	Younger Recreationally Active Adults	Younger Sedentary Adults	Older Healthy Adults	Older Adults with CAD
Aortic PWV (m/s)	5.74± 1.17*	6.76 ± 0.78*	9.61 ± 2.61	9.95 ± 2.57
Carotid Distensibility, $mmHg^{-1}$	$6.2e^{-3} \pm 2.1e^{-3}$*	$6.0e^{-3} \pm 2.9e^{-3}$*	$3.4e^{-3} \pm 1.3e^{-3}$	$3.2e^{-3} \pm 1.1e^{-3}$
Intimal Media Thickness, mm	0.50 ± .05*	0.48 ± .09*	0.79 ± .10	0.84 ± 0.11
Extra Media Thickness, mm	0.47 ± .08*	0.46 ± .06*	0.58 ± .06	0.54 ± 0.08

Values are represented as means ± SD.

*Significantly different than Older Healthy and Older Adults with CAD

Table 3: Pearson's Correlations with EMT

	r	n	Significance (p)
Age	.500	81	<0.001
Body Mass Index	.251	81	.012
Waist Circumference	.521	44	<0.001
Resting Heart Rate	-.231	80	.02
Resting Systolic Blood Pressure	.247	80	.014
Resting Diastolic Blood Pressure	.289	80	.005
Resting Mean Arterial Pressure	.316	80	.002
Aortic PWV	.431	81	<0.001
Carotid Compliance	-.358	80	0.001
Carotid Distensibility	-.364	80	<0.001
Intimal Media Thickness	.404	81	<0.001

Table 4: Day-to-Day Repeatability for Carotid EMT

Participant	Day 1 EMT	Day 2 EMT	Mean	Standard Deviation	CV
1	0.463888	0.423984734	0.443936445	0.028216	0.063559
2	0.445719	0.489968825	0.467843725	0.03129	0.06688
3	0.521874	0.576730516	0.549302346	0.038789	0.070616
4	0.536827	0.556822272	0.546824682	0.014139	0.025856
5	0.515528	0.522048047	0.518787964	0.00461	0.008887
6	0.40512	0.531176281	0.468147999	0.089135	0.1904
				Average	0.071033

Table 5: Backward Multiple Regression Model Predicting aPWV

Model	Predictors	Beta	t	p	F	ANOVA sig.	R^2
1	EMT	.064	.864	.391	22.126	.000	.714
	Age	.591	4.204	.000			
	Sex	-.147	-2.215	.030			
	BMI	-.056	-.753	.454			
	SBP	.319	3.115	.003			
	DBP	-.071	-.576	.566			
	MAP	.106	.732	.467			
	IMT	-.029	-.216	.829			
2	EMT	.065	.887	.378	25.620	.000	.714
	Age	.567	6.815	.000			
	Sex	-.146	-2.221	.029			
	BMI	-.055	-.746	.458			
	SBP	.317	3.129	.003			
	DBP	-.066	-.553	.582			
	MAP	.101	.712	.479			
3	EMT	.062	.848	.399	30.125	.000	.712
	Age	.573	6.974	.000			
	Sex	-.145	-2.223	.029			
	BMI	-.059	-.809	.421			
	SBP	.313	3.115	.003			
	MAP	.048	.460	.647			
4	EMT	.066	.908	.367	36.497	.000	.711
	Age	.577	7.113	.000			
	Sex	-.148	-2.282	.025			
	BMI	-.052	-.731	.467			
	SBP	.343	4.479	.000			
5	EMT	.061	.850	.398	45.772	.000	.709
	Age	.569	7.102	.000			
	Sex	-.141	-2.211	.030			
	SBP	.328	4.461	.000			
6	Age	.599	8.356	.000	61.011	.000	.707
	Sex	-.143	-2.234	.028			
	SBP	.328	4.468	.000			

Table 6: Forward Multiple Regression Model Predicting aPWV

Model	Predictors	Beta	t	p	F	ANOVA sig.	R^2	R^2 Change
1	Age	.766	10.527	.000	110.818	.000	.587	.587
2	Age	.586	7.995	.000	84.636	.000	.687	.100
	SBP	.364	4.973	.000				
3	Age	.599	8.356	.000	61.011	.000	.707	.019
	SBP	.328	4.468	.000				
	Sex	-.143	-2.234	.028				

Table 7: Backward Multiple Regression Model Predicting Carotid Distensibility

Model	Predictors	Beta	t	p	F	ANOVA sig.	R^2
1	EMT	-.110	-.992	.324	7.024	.000	.322
	Age	-.553	-2.617	.011			
	Sex	-.011	-.114	.910			
	BMI	-.061	-.572	.569			
	IMT	.084	.427	.671			
2	EMT	-.110	-.997	.322	8.894	.000	.322
	Age	-.555	-2.663	.009			
	BMI	-.058	-.567	.572			
	IMT	.087	.446	.657			
3	EMT	-.114	-1.039	.302	11.918	.000	.320
	Age	-.477	-4.230	.000			
	BMI	-.059	-.580	.563			
4	EMT	-.119	-1.098	.276	17.863	.000	.317
	Age	-.494	-4.561	.000			
5	Age	-.553	-5.868	.000	34.428	.000	.306

Table 8: Forward Multiple Regression Model Predicting Carotid Distensibility

Predictor	Beta	t	p	F	ANOVA sig.	R^2
Age	-.533	-5.868	.000	34.428	.000	.306

Table 9: Backward Multiple Regression Model Predicting Carotid IMT

Model	Predictors	Beta	t	p	F	ANOVA sig.	R^2
1	EMT	-.039	-.598	.552	35.718	.000	.776
	Age	.841	11.451	.000			
	Sex	-.026	-.446	.657			
	BMI	-.031	-.480	.633			
	SBP	.065	.732	.466			
	DBP	-.144	-1.357	.179			
	MAP	.168	1.340	.184			
2	EMT	-.039	-.602	.549	42.100	.000	.776
	Age	.838	11.532	.000			
	BMI	-.028	-.436	.664			
	SBP	.069	.775	.441			
	DBP	-.143	-1.358	.179			
	MAP	.171	1.372	.174			
3	EMT	-.040	-.629	.531	51.040	.000	.775
	Age	.835	11.616	.000			
	SBP	.066	.752	.454			
	DBP	-.148	-1.418	.161			
	MAP	.166	1.343	.183			
4	Age	.815	12.582	.000	64.219	.000	.774
	SBP	.071	.816	.417			
	DBP	-.154	-1.486	.142			
	MAP	.163	1.332	.187			
5	Age	.827	13.078	.000	85.780	.000	.772
	DBP	-.149	-1.440	.154			
	MAP	.208	1.893	.062			
6	Age	.834	13.140	.000	125.879	.000	.766
	MAP	.078	1.235	.221			
7	Age	.872	15.766	.000	248.561	.000	.761

Table 10: Forward Multiple Regression Model Predicting Carotid IMT

Predictor	Beta	t	p	F	ANOVA sig.	R^2
Age	.872	15.766	.000	248.561	.000	.761

Table 11: Block Entry Multiple Linear Regression Model for aPWV

Model	Predictors	Beta	t	P	F	ANOVA sig.	R^2	R^2 Change
1	Age	.628	4.691	.000	25.270	.000	.711	.711
	Sex	-.147	-2.221	.029				
	BMI	-.053	-.713	.478				
	SBP	.311	3.056	.003				
	DBP	-.063	-.517	.607				
	MAP	.110	.763	.448				
	IMT	-.037	-.278	.782				
2	Age	.591	4.204	.000	22.126	.391	.714	.003
	Sex	-.147	-2.215	.030				
	BMI	-.056	-.753	.454				
	SBP	.319	3.115	.003				
	DBP	-.071	-.576	.566				
	MAP	.106	.732	.467				
	IMT	-.029	-.216	.829				
	EMT	.064	.864	.391				

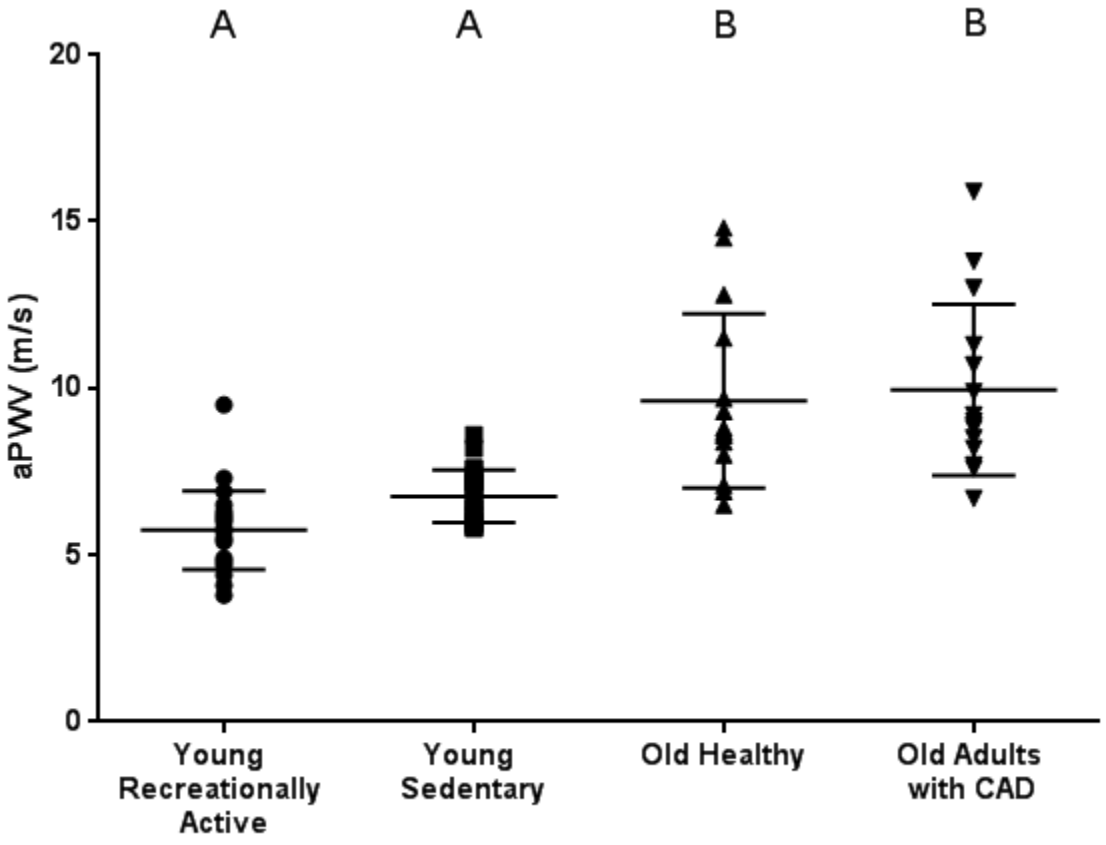
Table 12: Block Entry Multiple Linear Regression Model for Carotid Distensibility

Model	Predictors	Beta	t	P	F	ANOVA sig.	R^2	R^2 Change
1	Age	-.618	-3.077	.003	8.536	.000	.313	.313
	Sex	-.010	-.094	.925				
	BMI	-.069	-.648	.519				
	IMT	.100	.506	.614				
2	Age	-.553	-2.617	.011	7.024	.000	.322	.009
	Sex	-.011	-.114	.910				
	BMI	-.061	-.572	.569				
	IMT	.084	.427	.671				
	EMT	-.110	-.992	.324				

Table 13: Block Entry Multiple Linear Regression Model for Carotid IMT

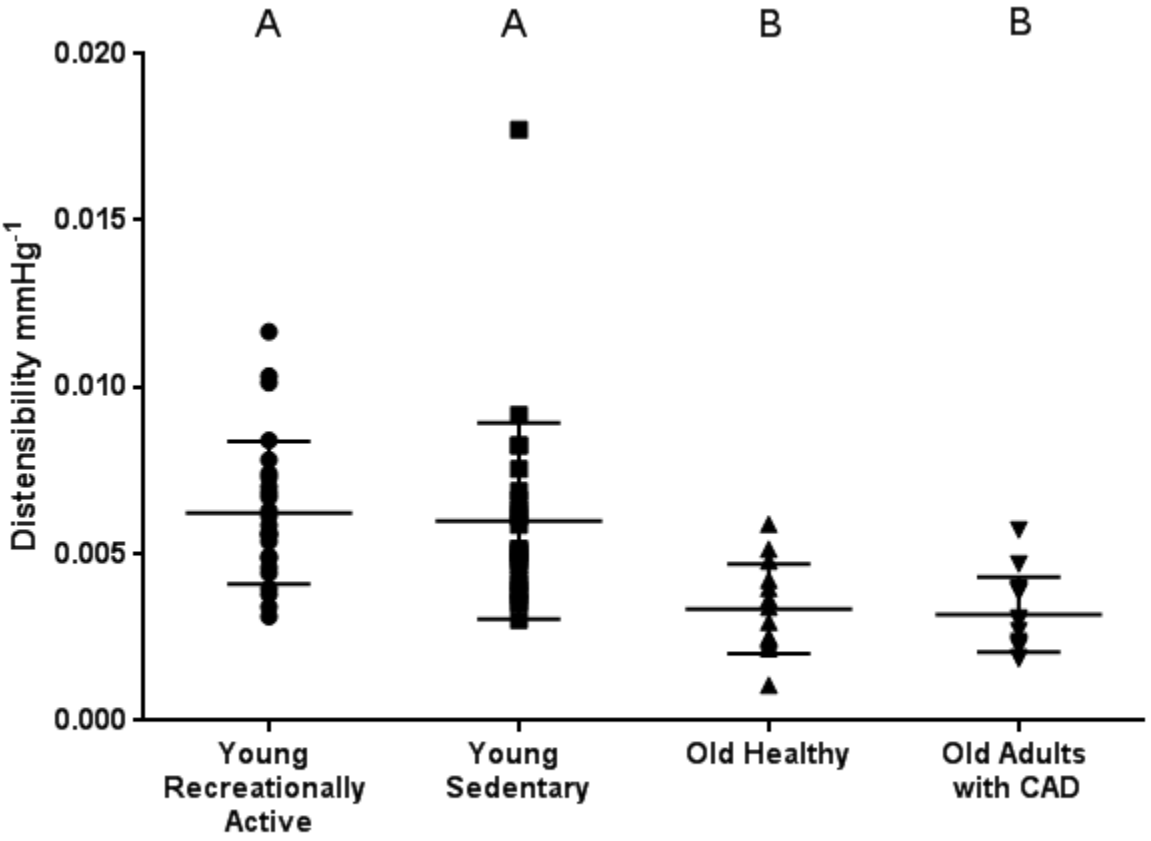
Model	Predictors	Beta	t	P	F	ANOVA sig.	R^2	R^2 Change
1	Age	.823	12.356	.000	41.981	.000	.775	.775
	Sex	-.026	-.448	.655				
	BMI	-.033	-.514	.609				
	SBP	.071	.796	.429				
	DBP	-.150	-1.419	.160				
	MAP	.166	1.333	.187				
2	Age	.841	11.451	.000	35.718	.000	.776	.001
	Sex	-.026	-.446	.657				
	BMI	-.031	-.480	.633				
	SBP	.065	.732	.466				
	DBP	-.144	-1.357	.179				
	MAP	.168	1.340	.184				
	EMT	-.039	-.598	.552				

Figure 2. Aortic Pulse Wave Velocity (aPWV) in Cohorts of Expected Varying Vascular Health



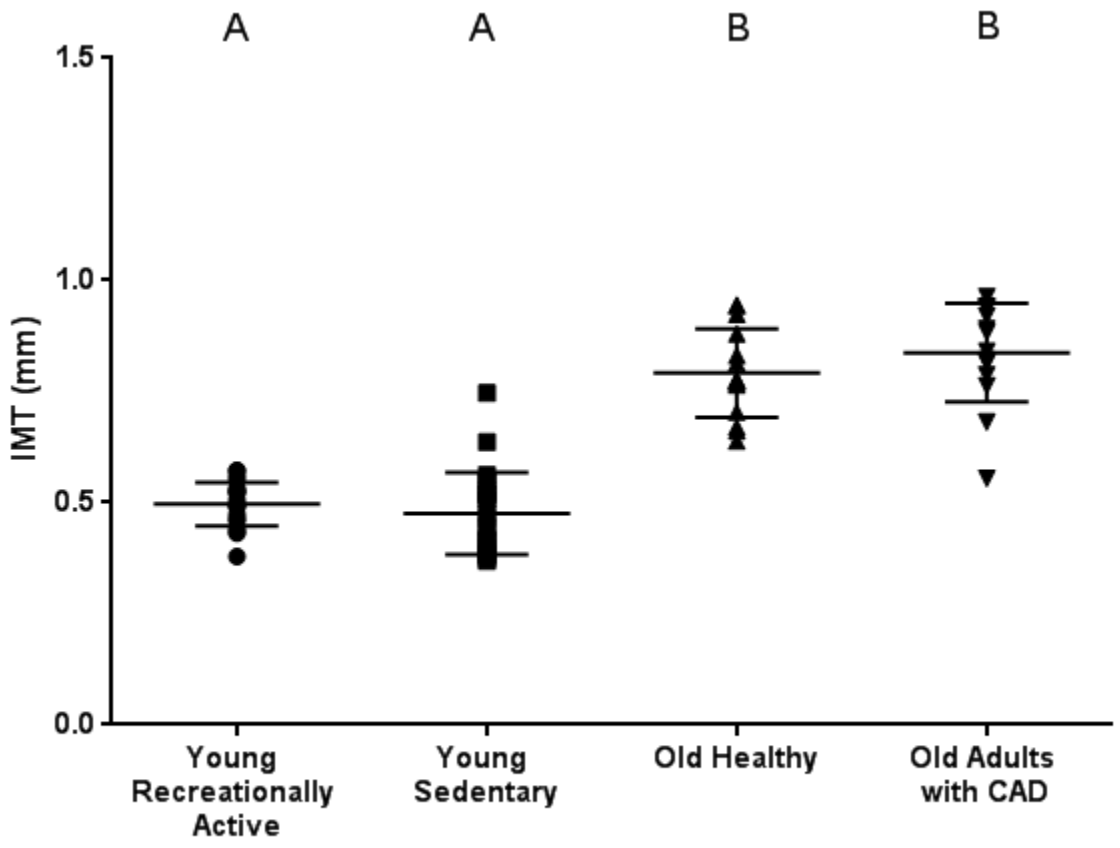
A) Significantly different than Old Healthy and Old Adults with CAD
B) Significantly different than Young Recreationally and Sedentary Adults

Figure 3. Carotid Distensibility in Cohorts of Expected Varying Vascular Health



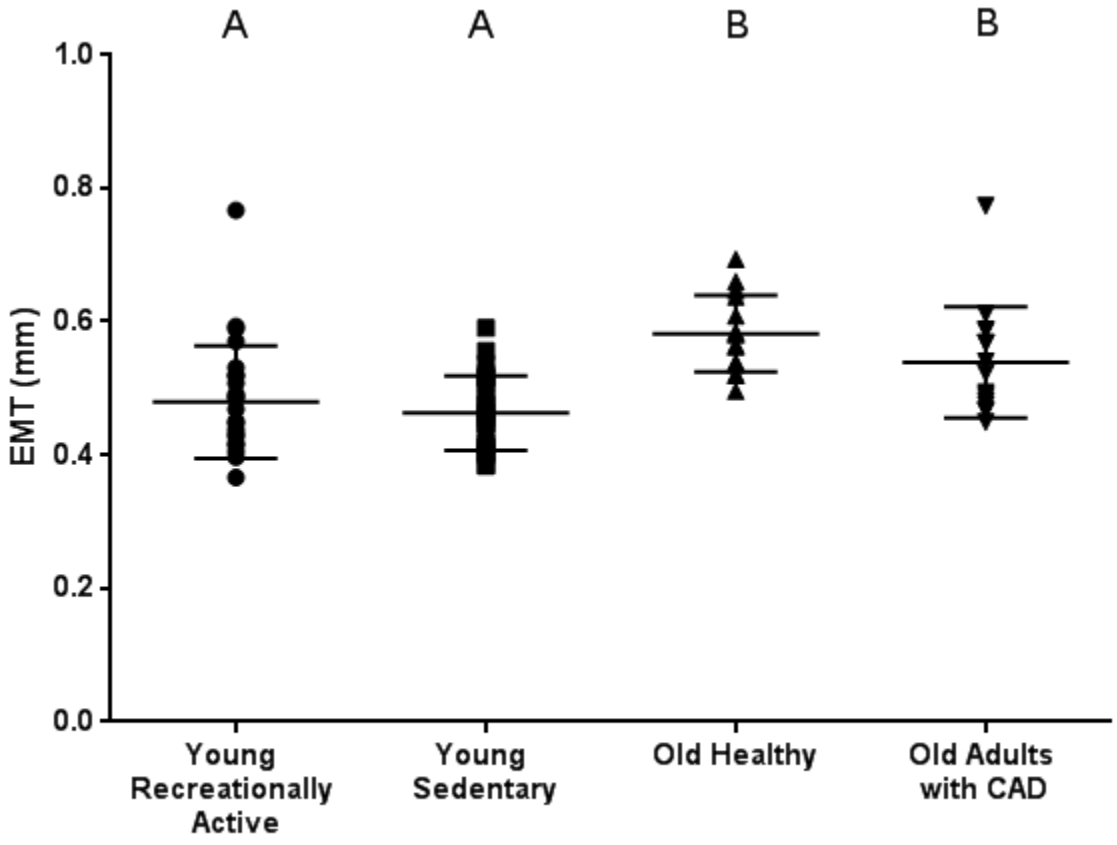
A) Significantly different than Old Healthy and Old Adults with CAD
B) Significantly different than Young Recreationally and Sedentary Adults

Figure 4. Carotid IMT in Cohorts of Expected Varying Vascular Health



A) Significantly different than Old Healthy and Old Adults with CAD
B) Significantly different than Young Recreationally and Sedentary Adults

Figure 5. Carotid EMT in Cohorts of Expected Varying Vascular Health



A) Significantly different than Old Healthy and Old Adults with CAD
B) Significantly different than Young Recreationally and Sedentary Adults

2.4 Discussion

Carotid EMT ultrasonography is a novel technique for measuring PVAT of the common carotid artery. Previous literature indicates that PVAT may have an influence on the development and progression of both arterial stiffness and atherosclerosis [3, 19-20]. The current study explored the use of carotid EMT measurement of PVAT and the potential associations with other indices of arterial health in a cohort of individuals with expected ranges in arterial health.

2.4.1 Impact of Age on Indices of Arterial Health

In agreement with our hypothesis we found that age, influenced both established (arterial stiffness and carotid IMT) and novel (carotid EMT) indicators of arterial health such that there were differences in aPWV, carotid distensibility, IMT and EMT between the younger and older groups. In a previous study that included 16,867 participants, they found that aPWV increased with increasing age groups [21]. This study employed the same aPWV methodology as the present study (with 80% of the distance), with values comparable to the present study. The mean aPWV value for ages <30 years was 6.2 m/s (±1.4 m/s), and for age 60-69 years was 10.3 m/s (±5.4) (both stated as ±2 SD). In comparison the average aPWV observed in our younger group in the current study was 6.2 ±1.1 m/s and in our older group, 9.8 ±2.6 m/s, indicating that we are in agreement with previous research in terms of our population representations. Carotid distensibility data from previous research were, for young adults (age 22 ±10 years), $6.7^{-3} \pm 2.6e^{-3}$ mm Hg^{-1} [22], and for older adults (ages 69.3 ±9.3 years), $2.7e^{-3} \pm 1.1e^{-3}$ mm Hg^{-1} [23]. In comparison to the current study, distensibility for younger adults was $6.1e^{-3} \pm 2.5e^{-3}$ mm Hg^{-1} and for older adults was $3.3e^{-3} \pm 1.2e^{-3}$ mm Hg^{-1}. Previous data on carotid IMT [24] for ages 11-27 years (0.46 ±0.08 mm) and for ages over 65 years (1.0 ±0.21 mm) are similar to the data from the current study for the younger adults (0.47±0.07 mm) and older adults (0.56± 0.04 mm). The current study is the first experiment to make the direct comparison of carotid EMT between older and younger

adults, and similar to the other measures of arterial health, we found that carotid EMT was different between the younger and older groups. These findings suggest that to obtain a comprehensive assessment of the arterial structure and function in models of aging, carotid EMT me useful to include in addition to the other established measures of arterial health.

2.4.2 Impact of Physical Activity on Indices of Arterial Health

Contrary to our hypothesis we did not find differences in any index of arterial health in the younger groups with different physical activity levels, such that aPWV, carotid distensibility, IMT and EMT were not different between younger recreationally active and younger sedentary groups. Based on the literature, different levels of physical activity could potentially reflect different profiles of arterial stiffness [8, 25] and carotid IMT [26-28]. In a cross sectional study involving 28 endurance trained (regularly competed in distance running events, with VO_{2max} at least 1 SD above the mean value from the sedentary group) and sedentary older men (ages >54 years), aPWV was higher in the sedentary group [29]. Rakobowchuk et al found no differences in carotid distensibility pre and post cycling ergometer training in an endurance (65% VO_{2peak} for 5 times/week for 6 weeks) and interval training (Wingate tests at <50 rpm, 3 times a week for 6 weeks) group of 20 younger healthy adults (ages 23.3 ±2.8 years) [30]. With carotid IMT, no consistent relationship with physical activity is apparent, as carotid IMT in sedentary individuals has been observed to be lower, not different or greater than physically active individuals [26, 31]. In a cross sectional study of 137 men, there were no differences in carotid IMT between the sedentary and endurance trained group at any age group (younger: 28, middle age: 50, older: 65 years) [25]. This is similar to the current study, where there were no differences in carotid IMT between the sedentary and recreationally active cohorts.

Without differences in aPWV, carotid distensibility, and EMT, we cannot conclude that physical activity had an influence on either traditional or novel markers of arterial health in our cohort. Previous

research suggests that our observation that physical activity levels did not impact on indices of arterial health may be due to the limitation associated with the use of questionnaires to assess physical activity. Direct measurement of maximal or peak oxygen consumption to assess physical fitness or use of objective tools in the assessment of physical activity could reveal an impact of physical activity or fitness on arterial health [32]. Future investigations can potentially combine both physical activity and fitness measures to assess the effects of physical activity and fitness on differences in traditional and novel assessments of arterial health. It is also possible that in our cohort of younger individuals, their arterial health was not impacted by physical activity due to a ceiling effect in which there was little capacity for improvement. Beck et al. trained 28 younger adults (ages 18-35 years) for 8 weeks, demonstrated no change in aPWV before and after endurance training [33]. The aerobic exercise training involved a regimen of 3 times a week of treadmill exercise at 65-85% of maximum heart rate. 15 individuals were time controlled participants with a mean aPWV of 6.86±0.20 m/s, which was similar to the younger sedentary group of the current study (6.76 ± 0.78 m/s). The endurance trained group had a mean aPWV of 6.81±0.18 m/s post intervention, which was not significantly different from the control.

2.4.3 Impact of Coronary Artery Disease on Indices of Arterial Health

Similarly, we did not observe the expected differences in vascular health indices between the older healthy and older adults with CAD. Although the literature is clear on the relationship between CAD and carotid IMT [9] and arterial stiffness [10], the observed group differences could be compounded by the effects of other cardiovascular risk factors on arterial health. One study suggests that arterial stiffness is not a significant predictor of CAD in the presence of other clinical states, including an increased BMI [34]. Both of the cohorts of older healthy and older adults with CAD in the current study had a mean BMI of greater than 25, which could potentially explain the diminished ability to detect differences in arterial stiffness between the groups.

When comparing the aPWV values of the older healthy and CAD group, these values were very similar to those found in the literature. In a study with 1054 older healthy adults (ages 63.4 ±4.5 years), the obtained aPWV was 9.9 ±0.9 m/s, which was similar to the current study (9.6 ±2.6 m/s) [35]. Ikonomidis et al investigated with 59 adults (ages 53 ±9 years) with CAD obtained a group average aPWV of 10.3 ±2 m/s [36]. The CAD cohort from the current study were relatively older (67.9 ±8.7 years), but had a lower group average aPWV (9.95 ±2.57 m/s). Carotid IMT of the older healthy cohort was similar to previous research [37]. However, carotid IMT was reported higher in previous research (1.00 ±0.32 mm) [38] than the present study (0.84 ±0.11 mm), even though the age group was very similar (60.1±10.3 years). This relatively low carotid IMT in the current study older CAD cohort could potentially explain the lack of differences seen between the older healthy and older CAD groups. Although we could not compare EMT with existing data, carotid EMT group effects are in agreement with the other measures of arterial health.

2.4.4 Day to Day Reliability of Carotid EMT

Our novel measure of arterial health, carotid EMT, was found to be feasible, as quality images were obtained in all four cohorts. We assessed reliability in a relatively small cohort of individuals, in which all 6 participants were from the younger sedentary group. Their baseline measures were taken and were asked to come back for another measure after 12 weeks. They were instructed to continue their regular daily activities, with no change to their physical activity. Previous literature includes information on the coefficients of variation for aPWV in a study of 50 participants ranging from 12.3 to 14.5%, as they were testing different devices for aPWV [41]. A review of carotid IMT reliability studies presented with variation coefficients of 2.4% to 10.6% [40]. Although it was a small cohort, we found that carotid EMT had a relatively good day-to-day repeatability in comparison to existing measures of arterial health. The image acquisition of carotid EMT was fairly straight-forward for trained sonographers, as long as the instructions were clearly conveyed on what are the requirements of the scan. These included the

visualization of the jugular vein, carotid artery and near wall carotid IMT. Good reliability and relatively ease of acquisition adds to the feasibility of obtaining carotid EMT, however further investigation with a larger sample size is needed to improve the strength on the reliability of carotid EMT.

2.4.5 Contributions of Carotid EMT to Predictive Models of Indices of Arterial Health

Aortic PWV has been shown to be an independent predictor of cardiovascular diseases [42-44]. Models that include measures that predict arterial stiffness may provide better risk assessment in a research or clinical setting [39]. In the current study Pearson's correlation data demonstrated significant relationships between carotid EMT and both aPWV and carotid distensibility, however the correlations were moderate to weak indicating the relationships were not strong. The current study also demonstrated that carotid EMT is not an independent predictor of either aPWV or carotid distensibility and that carotid EMT cannot explain additional variance in multiple regression models with traditional variables (age, sex, BMI, etc.) to predict aPWV and carotid distensibility. Carotid EMT is therefore not a likely candidate to use as a surrogate for either aPWV or carotid distensibility in the assessment of arterial stiffness. The literature provides evidence that an increased carotid IMT is related to an increased risk of CAD and stroke [45-46]. The Pearson's correlation data demonstrated a significant positive correlation between carotid IMT and EMT, but this was a moderate to weak relationship and in our study, carotid EMT was not an independent predictor of carotid IMT. The addition of carotid EMT to a model that predicts carotid IMT did not explain a significant amount of additional variance.

Although carotid EMT was correlated with aPWV, carotid distensibility and IMT, it did not independently predict these measures of arterial stiffness and carotid IMT. Despite these findings we cannot rule out the potential for carotid EMT to predict CVD as our study was not designed to assess the contribution of carotid EMT to CVD risk assessment. There is evidence that PVAT is highly associated with components of metabolic syndrome and is predictive of atrial fibrillation [48]. Longitudinal studies

including the measurement of carotid EMT with traditional CVD risk variables can provide further understanding of the potential for measures of PVAT to assess CVD risk.

Previous research has provided evidence that carotid IMT and arterial stiffness are potentially two different markers of vascular disease [48]. Oren et al. found that in 524 younger healthy adults, male gender, age and blood pressure were independent predictors of both carotid IMT and PWV, but in addition, body mass index, and LDL-cholesterol predicted carotid IMT only. In a Finnish study of 1754 younger adults, carotid IMT and flow mediated dilation (a measure of endothelial function) were found to not predict PWV [49]. The current study found that carotid EMT did not an independent predictor of measures of arterial stiffness and carotid IMT. If carotid EMT is a separate marker of vascular disease, it would not be surprising that it was not predictive of arterial stiffness and carotid IMT.

One interesting observation from the current study is the reaffirming of previous findings about the strength of aging on cardiovascular outcomes. In every analysis in the current study age was a significant factor. In the between groups analyses, the differences seen in EMT were between two different age groups. Out of all the different measured factors, age had the strongest relationship with carotid EMT. In all of the multiple regression models, age remained as the strongest independent predictor of arterial stiffness. Increases in age affect the structural and physiological components of the cardiovascular system, which ultimately leads to increases in arterial stiffness [50]. Aging also plays a significant role in determining carotid IMT, with evidence of a strong significant positive correlation between these two variables [51]. Because aging contributes to a large number of anatomical and physiological components of the cardiovascular system, it dominates in terms of effects on arterial health.

2.4.6 Limitations and Future Directions

The current study employed a cross-sectional design, which allowed us to examine the relationship at one "snapshot" in time, limiting our understanding of the relationship between existing measures of arterial health and PVAT. For a more complete assessment of traditional cardiovascular risk profile, factors such as diabetes, smoking, hypertension and dyslipidemia should be included. Unfortunately, due to sample size, waist circumference was not included in some of the models to predict arterial stiffness or carotid IMT, which would have provided insight to the effects of obesity.

Some of the limitations that were encountered in the study included the assessments of the vascular health measures. Every methodology presented with different assumptions and technical challenges, namely ensuring the acquisition of high quality pressure signals and ultrasound images. The participant population also presented with challenges, where larger body habitus may have hindered data signal and image quality, which may impacted the outcomes. Although we had good day-to-day reliability with a small cohort, assessing repeatability across the whole cohort would have provided a stronger support for the feasibility of carotid EMT.

Future studies that examine the predictive value of carotid EMT on cardiovascular disease risk would be helpful, especially determining whether carotid EMT can enhance prediction of cardiovascular disease. Longitudinal studies can also explore the effects of age on PVAT, as it appears to be a strong factor in established measures of arterial health and potentially in carotid EMT. Because carotid EMT is a novel measurement, future studies would need to also focus on validating reliability with a larger sample size.

2.5 Conclusion

The novel findings of this study was that there were differences in carotid EMT between younger and older adults, which are in agreement with established measures of arterial health (aPWV, carotid

distensibility and IMT). Carotid EMT was not an independent predictor of measures of arterial stiffness and carotid IMT, which may indicate the potential to be a separate vascular marker on its own. The study was able to establish the feasibility of the carotid EMT measure through good day-to-day reliability in a small cohort. The current study also identifies useful information for future longitudinal studies to investigate carotid EMT as a valuable measure of PVAT and potential cardiovascular risk assessments.

2.6 References

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APPENDIX A: DATA COLLECTION SHEET

Assessors Initials: ____

Carotid Artery Longitudinal Wall Motion

Participant ID ☐☐☐☐☐☐☐

CALM Mechanisms

Date of Assessment ☐☐☐☐ / ☐☐ / ☐☐
Y Y Y Y M M D D

Time of Assessment: ☐☐ ☐☐

File Name: _____

Age (yrs): _____
Height (m): _____
Weight (kg): _____
WC (cm): _____
HC (cm): _____

Population Specific Information (e.g., YPI, JHS)
Smoking History: _____
A ( ): _____
B ( ): _____
C ( ): _____

COLLECT ALL US ON RIGHT SIDE

Resting BP (Dinamap)

	SBP	DBP	MAP	HR
BP#1				
BP#2				
BP#3				
AVG				

Handgrip (Standing – Right side)
MVC #1: ☐☐☐
MVC #2: ☐☐☐
MVC #3: ☐☐☐
MVC AVG: ☐☐☐

Baseline IHG #1

US: _____

4Ch HFR1: ☐☐
MV HFR2: ☐☐
AP HFR3: ☐☐

Clavicle HFR : ☐☐ Distance : _____

30-sec MBV: ☐☐ 30-sec PBV: ☐☐

PULSE WAVE VELOCITY
Tonometer #: ____ AND ____

Carotid-Femoral: _____ cm
Carotid-SN: _____ cm
SN-Umbilicus: _____ cm
Umbilicus-Femoral: _____ cm
Femoral-TP/DP: _____ cm
SN-Radial: _____ cm

US: _____

LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐

4Ch: ☐☐ MV: ☐☐ AP: ☐☐

IMT: ☐☐ EMT: ☐☐
Dist #1: ☐☐ Dist #2: ☐☐

Finometer @ BL
SBP: ___ DBP: ___ MAP: ___ HR: ___

IHG #1
☐☐%

4Ch HFR1: ☐☐
MV HFR2: ☐☐
AP HFR3: ☐☐

MBV: ☐☐ PBV: ☐☐ Clav: ☐☐

Finometer @ 1:30
SBP: ___ DBP: ___ MAP: ___ HR: ___

LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐
4Ch: ☐☐ MV: ☐☐ AP: ☐☐

Assessors Initials: ____

Carotid Artery Longitudinal Wall Motion

Participant ID ☐☐☐☐☐☐☐

CALM Mechanisms

Date of Assessment ☐☐☐☐ / ☐☐ / ☐☐
Y Y Y Y M M D D

Time of Assessment: ☐☐ ☐☐

File Name: _____

Baseline IHG #2	4Ch HFR1: ☐☐ MV HFR2: ☐☐ AP HFR3: ☐☐ MBV: ☐☐ PBV: ☐☐ Clav: ☐☐	**Finometer @ BL** SBP: ___ DBP: ___ MAP: ___ HR: ___ LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐ 4Ch: ☐☐ MV: ☐☐ AP: ☐☐
IHG #2 ☐☐%	4Ch HFR1: ☐☐ MV HFR2: ☐☐ AP HFR3: ☐☐ MBV: ☐☐ PBV: ☐☐ Clav: ☐☐	**Finometer @ 1:30** SBP: ___ DBP: ___ MAP: ___ HR: ___ LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐ 4Ch: ☐☐ MV: ☐☐ AP: ☐☐
Baseline NTG	4Ch HFR1: ☐☐ MV HFR2: ☐☐ AP HFR3: ☐☐ MBV: ☐☐ PBV: ☐☐ Clav: ☐☐	**Finometer @ BL** SBP: ___ DBP: ___ MAP: ___ HR: ___ LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐ 4Ch: ☐☐ MV: ☐☐ AP: ☐☐
NTG 2:30	4Ch HFR1: ☐☐ MV HFR2: ☐☐ AP HFR3: ☐☐	**Finometer @ 2:30** SBP: ___ DBP: ___ MAP: ___ HR: ___ LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐ 4Ch: ☐☐ MV: ☐☐ AP: ☐☐
NTG 5:30	4Ch HFR1: ☐☐ MV HFR2: ☐☐ AP HFR3: ☐☐ MBV: ☐☐ PBV: ☐☐ Clav: ☐☐	**Finometer @ 5:30** SBP: ___ DBP: ___ MAP: ___ HR: ___ LVOT: ☐☐ 5Ch: ☐☐ 2Ch: ☐☐ 4Ch: ☐☐ MV: ☐☐ AP: ☐☐

Assessors Initials: ____

Carotid Artery Longitudinal Wall Motion

Participant ID ☐☐☐☐☐☐☐

CALM Mechanisms

Date of Assessment ☐☐☐☐ / ☐☐ / ☐☐
Y Y Y Y M M D D

Time of Assessment: ☐☐ ☐☐

File Name: _____

Room	MITRAL Settings	APEX Settings	4CH Settings	Vascular Settings
Temp: ____ Humidity: ____	Depth: _____ Width: _____ FPS: _____ Focus: _____	Depth: _____ Width: _____ FPS: _____ Focus: _____	Depth: _____ Width: _____ FPS: _____ Focus: _____	Depth: _____ FPS: _____ Doppler AC: _____ Sample Volume: _____

Notes:

_____

_____

_____

_____

_____

_____

_____

_____

APPENDIX B: CORONARY ARTERY DISEASE CHECKLIST

Coronary Artery Disease Checklist

Inclusion Criteria (Check)

- ☐ A recent CAD event that was defined as the patient having at least one of the following:
  - ☐ angiographically documented stenosis Q50% in at least one major coronary artery
  - ☐ myocardial infarction
  - ☐ percutaneous coronary intervention
  - ☐ coronary artery bypass graft surgery
- ☐ and positive exercise stress test determined by either
  - ☐ a positive nuclear scan
  - ☐ symptoms of chest discomfort accompanied by ECG changes of 91 mm horizontal or down sloping ST-segment depression.

Medical History (Check if any)

- ☐ Hypertension
- ☐ Diabetes
- ☐ Arthritis
- ☐ DVT
- ☐ Hypercholesteremia
- ☐ Other _____

Medication (Check if any)

- ☐ Hydralazine (Hypertension)
- ☐ Aspirin/Coumadin/Warfarin
- ☐ Tylenol/Advil/Motrin
- ☐ Viagra/Cialis
- ☐ ACE Inhibitors
- ☐ Other _____

Exclusion Criteria (Circle if any)

- smoking within 3 months
- noncardiac surgical procedure within 2 months
- myocardial infarction or coronary artery bypass graft within 2 months
- percutaneous coronary intervention within 1 month
- New York Heart Association class II-IV symptoms of heart failure
- documented valve stenosis
- documented severe chronic obstructive pulmonary disease
- symptomatic peripheral arterial disease
- unstable angina
- uncontrolled hypertension
- uncontrolled atrial arrhythmia
- ventricular dysrhythmia
- any musculoskeletal abnormality that would limit exercise participation.

Nitroglycerin Contraindications (Circle if any)

- known hypersensitivity or idiosyncratic reaction to organic nitrates
- severe anemia (because of potential methemoglobin formation and resulting impaired oxygen delivery)
- hypotension or uncorrected hypovolemia, as the use of nitrates in such states could produce severe hypotension or shock
- head trauma or cerebral hemorrhage (to avoid increased intracranial pressure)
- constrictive pericarditis and pericardial tamponade. Long-acting dosage forms should be avoided in the treatment of acute coronary syndromes.
- Phosphodiesterase 5 Inhibitors: Systolic and diastolic blood pressure may be significantly reduced following coadministration of nitrates and sildenafil, tadalafil or vardenafil. Avoid this combination. Separate doses by at least 24 hours.

APPENDIX C: ANTHROPOMETRIC, HR AND BLOOD PRESSURE MEASURES RAW DATA

Patient ID	Age	Sex	Weight	Height	BMI	HR	SBP	DBP	MAP	Waist Circumference
TR01	21	Male	72.8	1.81	22.22154	56	105	67	84	--
TR02	20	Male	69.3	1.725	23.28922	57	99	59	76	--
TR03	34	Male	76.7	1.84	22.65477	62	108	66	84	--
TR04	19	Male	64.9	1.81	19.81014	47	113	69	86	--
TR05	24	Male	66	1.71	22.57105	54	112	67	85	--
TR06	35	Male	126.8	1.85	37.04894	55	112	72	87	--
TR07	20	Male	76	1.64	28.25699	80	122	67	88	--
TR08	38	Male	70.4	1.75	22.98776	67	115	74	88	--
TR09	21	Male	80.6	1.62	30.71178	82	114	74	89	--
TR10	19	Male	64.2	1.72	21.70092	66	108	64	83	--
TR11	22	Male	128.1	1.97	33.00781	52	129	68	91	--
TR12	21	Male	81	1.765	26.00133	72	122	68	89	--
TR13	19	Male	53.3	1.717	18.07951	75	94	59	74	--
TR14	25	Male	68.8	1.685	24.23196	67	113	69	87	--
TR17	39	Male	107.1	1.785	33.61345	55	113	71	88	--
TR18	37	Male	89.3	1.98	22.77829	58	99	62	78	--
TR19	21	Male	69.3	1.76	22.37216	69	117	74	89	--
TR20	24	Male	73.6	1.71	25.17014	81	115	65	85	--
TR21	22	Male	63.8	1.745	20.95221	76	116	64	85	--
TR22	26	Male	75.4	1.71	25.78571	63	113	66	85	--
TR23	34	Male	102.9	1.85	30.06574	59	128	70	91	--
TR24	26	Male	84.7	1.814	25.74002	60	115	63	84	--
TR25	24	Male	85	1.78	26.82742	50	111	65	84	--
TR26	41	Male	55	1.69	19.25703	43	94	66	78	--
TR27	27	Male	119.2	1.78	37.62151	67	126	75	94	--
VVC01	24	Female	60	1.67	21.51386	51	108	58	81	--
VVC02	28	Male	87.5	1.82	26.41589	67	126	78	94	--
VVC03	26	Male	85	1.84	25.10633	65	125	67	86	--
VVC04	20	Female	56.7	1.66	20.57628	--	--	--	--	--
VVC05	22	Male	75.5	1.83	22.54472	55	122	65	87	--
VVC06	23	Male	88.9	1.79	27.7457	62	127	66	89	--
VVC07	25	Female	53.6	1.68	18.99093	76	114	67	87	--
VVC08	28	Male	103	1.87	29.45466	57	117	67	87	--
VVC09	24	Female	54.6	1.68	19.34524	62	107	69	85	--
VVC10	23	Female	58.9	1.68	20.86876	62	111	64	83	--
LMY01	26	Female	57	1.62	21.71925	56	105	69	84	70
LMY02	23	Male	67.5	1.77	21.54553	51	110	63	83	72
LMY03	23	Female	63	1.68	22.32143	81	125	76	94	73
LMY04	23	Male	78.5	1.81	23.96142	42	108	60	82	86

LMY05	23	Female	64	1.62	24.38653	78	129	79	96	74.5
LMY06	29	Female	61.1	1.61	23.57162	50	110	67	85	75
LMY07	22	Female	61.5	1.63	23.14728	65	106	63	82	71
LMY08	23	Male	72.6	1.73	24.25741	70	127	69	91	74.5
LMY09	21	Male	68.4	1.81	20.87848	61	113	64	84	69
LMY10	21	Female	58.9	1.7	20.38062	67	121	68	89	65.5
LMY11	21	Male	67.6	1.67	24.23895	57	104	69	84	76.5
LMY12	23	Male	86.5	1.78	27.30085	56	135	69	92	87.5
LMY13	21	Male	63.2	1.635	23.64186	57	103	59	79	66
LMY14	20	Male	73.8	1.79	23.03299	66	123	68	90	80
LMY15	19	Female	56.8	1.59	22.46747	70	107	74	86	71
LMY16	22	Female	54.6	1.65	20.0551	60	106	62	82	65
LMO01	70	Male	104.8	1.74	34.61488	42	150	77	104	--
LMO02	83	Male	87.5	1.7	30.27682	61	145	77	102	104.5
LMO03	70	Male	92.9	1.825	27.89266	78	126	70	90	106.5
LMO04	72	Male	78.3	1.63	29.47044	59	147	87	109	97.5
LMO05	70	Female	81.2	1.61	31.32595	55	117	66	86	95
LMO06	68	Female	68.4	1.58	27.39946	52	121	67	88	83.5
LMO07	68	Male	87.6	1.79	27.33997	58	163	102	125	94.5
LMO08	67	Male	68.6	1.79	21.41007	54	103	70	84	77.5
LMO09	65	Female	111	1.66	40.28161	65	126	67	90	--
LMO10	67	Male	90.8	1.7	31.41869	49	143	92	110	109
LMO11	70	Female	76	1.66	27.5802	62	107	59	81	95
LMO12	68	Female	66.2	1.64	24.61333	57	96	59	75	82
LMO13	76	Female	51.5	1.52	22.29051	75	131	68	91	77
LMO14	79	Male	81.7	1.85	23.87144	58	139	67	93	92.5
LMO16	62	Female	66	1.61	25.46198	60	112	69	87	83
LMC01	63	Male	102.2	1.82	30.85376	44	94	76	127	102.5
LMC02	70	Male	81.7	1.7	28.2699	59	172	81	117	95
LMC03	52	Male	80.3	1.83	23.97802	48	106	70	85	86.5
LMC04	59	Male	97.6	1.79	30.46097	45	116	76	89	101
LMC05	71	Male	82.7	1.81	25.24343	49	128	73	92	88
LMC06	68	Male	58.8	1.66	21.33837	52	126	69	90	80.5
LMC07	70	Male	84.8	1.69	29.69084	62	136	76	97	97.5
LMC08	53	Male	117.9	1.75	38.49796	58	137	98	110	113
LMC09	66	Male	80.6	1.65	29.60514	48	102	68	83	101.5
LMC10	63	Male	89.3	1.8	27.56173	72	149	99	116	97
LMC11	81	Male	67.4	1.73	22.51996	46	149	70	98	83
LMC12	74	Male	99.8	1.69	34.94275	49	150	78	104	116
LMC13	74	Male	54.6	1.74	18.03409	64	114	66	85	69
LMC14	74	Male	83.5	1.8	25.7716	53	121	69	89	98
LMC15	80	Male	87.5	1.76	28.24768	44	164	73	108	95.5

APPENDIX D: VASCULAR MEASURES RAW DATA

Patient ID	aPWV	Distensibility	IMT	EMT
TR01	5.8	0.008257	0.4451	0.5903794
TR02	7.2	0.004875	0.7461	0.4125092
TR03	7.2	0.004763	0.6358	0.4787541
TR04	5.9	0.005877	0.3813	0.454673
TR05	7.2	0.00611	0.373	0.5556052
TR06	7.6	0.004659	0.4158	0.4283315
TR07	7.2	0.005154	0.507	0.4022508
TR08	8.6	0.003537	0.5506	0.447892
TR09	6.2	0.004214	0.5176	0.4716254
TR10	5.9	0.005152	0.5179	0.4116399
TR11	6.6	0.007548	0.5494	0.5048348
TR12	6.6	0.003557	0.4324	0.4538906
TR13	6.0	0.006886	0.418	0.4638882
TR14	5.8	0.01772	0.562	0.3835597
TR17	8.2	0.003025	0.3839	0.5160495
TR18	6.4	0.008253	0.505889	0.4180731
TR19	7.6	0.003752	0.3676	0.4457186
TR20	7.4	0.005045	0.3792	0.384516
TR21	7.1	0.006171	0.410625	0.4751898
TR22	7.1	0.005011	0.391778	0.4962282
TR23	5.9	0.004093	0.5194	0.3995558
TR24	6.4	0.006622	0.3982	0.5218742
TR25	6.0	0.009173	0.527667	0.5368271
TR26	6.1	0.003911	0.465444	0.5155279
TR27	7.0	0.006334	0.473	0.4051197
VVC01	3.8	0.010334	0.490333	0.4275491
VVC02	9.5	0.003414	0.521333	0.7667717
VVC03	6.1	0.007014	0.495333	0.3663465
VVC04	6.5	0.006834	0.431	0.4376336
VVC05	4.1	0.008405	0.531	0.396687
VVC06	6.0	0.005865	0.506	0.5894231
VVC07	6.0	0.003121	0.434	0.4473704
VVC08	6.5	0.011668	0.526667	0.4508478
VVC09	6.5	0.00742	0.441333	0.4876216
VVC10	4.8	0.00783	0.378667	0.3984257
LMY01	5.7	0.004938	0.492	0.5927267
LMY02	5.4	0.005641	0.56	0.5078776
LMY03	5.8	0.004594	0.464	0.4146826
LMY04	4.7	0.005567	0.494	0.4913598

LMY05	7.3	0.00443	0.551	0.4312004
LMY06	6.2	0.003962	0.49	0.4916206
LMY07	4.8	0.010138	0.571	0.5308285
LMY08	5.5	0.004873	0.487	0.4685827
LMY09	6.9	0.005371	0.551	0.5188314
LMY10	6.1	0.005587	0.459	0.4851004
LMY11	4.4	0.007291	0.534	0.4179862
LMY12	6.3	0.004958	0.429	0.430418
LMY13	4.9	0.006144	0.491	0.5703842
LMY14	5.4	0.006305	0.473	0.5190922
LMY15	5.7	0.003793	0.572	0.4159867
LMY16	4.4	0.006705	0.523	0.4054675
LMO01	8.8	0.004797	0.923	0.5201355
LMO02	12.8	0.004221	0.943	0.5840331
LMO03	9.7	0.002162	0.764	0.659667
LMO04	9.3	0.001068	0.775	0.53561
LMO05	6.5	0.002423	0.943	0.5192661
LMO06	8.0	0.003974	0.661	0.5619515
LMO07	14.8	0.00246	0.813	0.5807296
LMO08	8.7	0.00515	0.775	0.5807296
LMO09	11.5	--	0.831	0.5640379
LMO10	8.6	0.002258	0.703	0.6933111
LMO11	8.6	0.002957	0.768	0.4956196
LMO12	8.4	0.003412	0.638	0.6370638
LMO13	7.1	0.002502	0.773	0.5390005
LMO14	14.5	0.003651	0.879	0.6088097
LMO16	6.9	0.005891	0.669	0.6466267
LMC01	8.9	0.00222	0.814	0.5681239
LMC02	11.3	0.00187	0.939	0.5883799
LMC03	6.7	0.003869	0.89	0.568037
LMC04	7.7	0.003996	0.762	0.4936201
LMC05	8.5	0.003916	0.84	0.4671048
LMC06	9.9	0.005721	0.818	0.4757114
LMC07	9.2	0.002373	0.882	0.6114178
LMC08	9.0	0.002687	0.786	0.5228305
LMC09	8.2	0.004701	0.554	0.4887517
LMC10	8.8	0.002267	0.681	0.5403045
LMC11	15.9	0.003083	0.941	0.4818838
LMC12	13.0	0.003942	0.821	0.7733788
LMC13	10.7	0.002987	0.963	0.4665832
LMC14	7.6	0.002307	0.919	0.5866412
LMC15	13.8	0.001891	0.937	0.4491091