# THE PREVALENCE OF PRECLINICAL ATHEROSCLEROSIS IN A HEALTHY ADULT POPULATION

**A THESIS SUBMITTED TO THE GRADUATE SCHOOL**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS**

**FOR THE DEGREE**

**MASTER OF SCIENCE**

**BY**

**GARETT J. GRIFFITH**

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**BALL STATE UNIVERSITY**

**MUNCIE, IN**

**MAY 2014**

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BALL STATE UNIVERSITY
MUNCIE, IN
MAY 2014

## ACKNOWLEDGEMENTS

To my family: Thank you for your continued support throughout my academic career. I appreciated every phone call and each time you visited Muncie, and your constant encouragement kept me going during the countless long days in the Human Performance Laboratory. I don't know if I could have made it through graduate school without all of your support and the occasional trip home for good food and company.

Dr. Kaminsky: Thank you first and foremost for granting me the opportunity to learn and grow as a member of the Clinical Exercise Physiology program. My time in the Human Performance Laboratory during these past two years has been a challenging and rewarding graduate school experience and I cannot express my gratitude for all you have contributed to my academic and professional paths.

Dr. Whaley: Thank you for your guidance as a member of my thesis committee and for the knowledge I gained from you as a previous instructor. I have appreciated your insight and critique during this process.

Dr. Kotecki: Thank you for serving as a member of my thesis committee. I truly appreciate your perspective and advice relating to this project.

To my fellow colleagues: For me, interacting with and learning from each of you has been just as important as all of the classes and work experiences during these past two years. Thank you for the encouragement, laughter, and willingness to lend a helping hand when things got stressful, and for allowing me to be a part of each of your lives. I will truly miss "Atypical Item Wednesdays" and I wish you all the best.

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# CHAPTER I

## INTRODUCTION

Cardiovascular disease (CVD) remains America's leading cause of death,[1,2] and the mortality statistics are staggering. According to 2008 national data, the American Heart Association reported 811,940 deaths attributable to this disease.[2] An updated report from the same organization showed that in 2009, an American died as a result of CVD approximately every 40 seconds.[1] The vast majority of deaths attributable to CVD can be classified as resulting from either myocardial infarction (MI) or hemorrhagic stroke. It has been estimated that roughly 785,000 Americans will suffer a first-time MI and around 610,000 Americans will experience a first time stroke each year.[2] These numbers are likely underestimations of the actual prevalence of these clinical occurrences, as it is probable that hundreds of thousands of individuals experience these events without seeking medical assistance every year.[1] CVD is of particular concern for premature mortality, as the Centers for Disease Control and Prevention report that diseases of the heart and blood vessels serve as the second leading cause of death for individuals aged 25-64 years.[3] Total deaths due to CVD in this cohort are likely to increase as the U.S. Census Bureau estimates that the number of adults in this age range will have increased by 3.38% in America alone from 2010 to the year 2015.[4] CVD and its associated comorbidities are also a primary reason that healthy life expectancy, or the amount of years spent free of major

chronic diseases, is shorter than actual life expectancy by 8 and 9 years for males and females, respectively.[5] As dire as the CVD death rates appear to be, advancements in treatment technologies have actually blunted the progressive increase in mortality rates and allowed for a slight reversal in the national trend since 1970.[6] These intervention options include both medication therapy and revascularization procedures.

Unfortunately, the increasing number of surgeries associated with the improved survival rate for CVD patients comes with the cost of an unsustainable economic burden. CVD inpatient operations and procedures have increased from 5.9 million in the year 2000 to 7.6 million in 2010.[2] Consequently, healthcare costs directly associated with these procedures have exceeded $312 billion in 2009, with a financial toll that is projected to increase yearly.[1] An independent analysis determined that with inclusion of indirect costs such as loss of work productivity due to CVD, this total surpassed $400 billion as early as 2006.[7] Therefore, it is readily apparent that there is a strong need in the healthcare community to be able to successfully identify individuals at risk for the development of CVD in order to initiate early treatment strategies and help alleviate the intolerable stress of CVD on the healthcare system.[8,9]

Medical professionals have the ability to use several non-invasive methods for CVD screening in an asymptomatic population. However, each commonly used approach has inadequacies which prevent definitive identification of those at risk for CVD. One available procedure able to be used in preventive health or fitness centers is a graded exercise test (GXT) with electrocardiographic monitoring. The inclusion of a patient's age and either the presence or absence of angina, in addition to the potential degree of ST segment depression, can allow clinicians to make inferences about the status of the coronary arteries. Results from this test are

indicative of myocardial perfusion status during exercise. These tests, through the identification of significant ST segment changes, can serve as diagnostic tools for the presence of myocardial ischemia. ST segment depression criteria, however, may vary by location and limit the predictive capability of GXT electrocardiography.[10] The GXT is also typically reserved for symptomatic patients when performed in a hospital setting, limiting its detective capabilities in a population earlier in the disease process. Additionally, this test's sensitivity has been to have a range of 23% to 100%,[11,12] suggesting there is a need for alternative tools to help identify individuals at risk for CVD prior to its manifestation during a clinical event.

A medical screening service capable of this is a coronary calcium scan (CCS). This test utilizes either electron beam computed tomography or multi-detector computed tomography, which both rely on X-ray technology to create detailed three-dimensional images of the heart. These images reveal either the presence or absence of calcification of the coronary arteries, which can potentially identify early stage CVD in an asymptomatic population. This test is often reserved for individuals currently considered to be at least at moderate risk (10%-20%) for coronary heart disease (CHD) according to the Framingham Risk Score (FRS). The FRS was developed using data from the Framingham Heart Study,[13] and its algorithm incorporates established CVD risk factors to predict an individual's chance of developing coronary heart disease within the next 10 years.[14] The FRS includes the non-modifiable risk factors of age and gender, as well as the modifiable risk factors such as cholesterol, HDL cholesterol, smoking status, and blood pressure.[15] Other well-known CVD risk factors[16] such as body composition and habitual physical activity level, however, are not included in the prediction equation. Using the FRS to identify candidates for a CCS therefore has inherent limitations since multiple risk factors that have been shown to have strong associations[17] with this disease are absent from its

algorithm. Additionally, a medical center's ability to provide a CCS service relies on highly trained technicians, and the associated cost may limit its availability to the entire eligible population.

Fatal cardiovascular events are often precipitated by the progressive atherosclerotic process for several years or decades in an asymptomatic, apparently healthy population.[18-21] This process may present itself early in the form of signs of arterial dysfunction, which is thought to contribute to the inflammatory process. More widely accessible methods are required to successfully identify a greater percentage of individuals in this early stage of CVD, referred to as preclinical atherosclerosis.[22] Such methods include carotid intima media thickness (CIMT)[23,24] and pulse wave velocity (PWV)[25,26] measurements. These measurements serve as indicators of arterial thickness and stiffness, respectively, and abnormally high measurements have been validated as markers of preclinical atherosclerosis. Signs of potential preclinical atherosclerosis include high CIMT and PWV values, which can be operationally defined as having a mean CIMT value $\geq 75^{th}$ percentile for age-, gender-, and race-matched peers or a mean PWV value $\geq 75^{th}$ percentile for age- and blood pressure category-matched peers according to previously established data sets.[27,28] Early detection of these signs of arterial dysfunction may serve to more accurately guide treatment options that may include lifestyle modification or medical therapy.

CIMT measurements rely on the use of ultrasonography and are comprised of the thickness of the tunica intima and tunica media, the two innermost layers of the artery, which are believed to be responsible for potential inflammatory responses that may initiate the atherosclerotic process.[21] Independent relationships have been shown to exist between excess thickness of the carotid arterial wall and an increased likelihood for both MI and stroke.[29] This

measurement technique is capable of detecting plaque deposits, which can increase an individual's risk for ischemic stroke, as well as tracking the progression of atherosclerosis.[22] Routine assessment of CIMT, however, is not widely performed in a clinical setting for individuals considered to be at low or moderate risk according to classic risk stratification schemes. The American Heart Association and the American College of Cardiology advocated for its use for patients at intermediate risk in 2010, and CIMT may have the potential to more accurately sub-stratify patients in need of alternative intervention options.[30] CIMT measurement offers several advantages to alternative CVD detection methodologies, including lower cost and time commitment by the patient, its non-invasive nature, and lack of intravenous contrast or X-ray radiation exposure.[31]

An alternative method for assessment of arterial dysfunction is the use of PWV measurement, an indicator of arterial stiffness. The assessment of PWV using the carotid and femoral palpation sites gives insight into the elastic quality of the aorta, which has been shown to correlate with risk for CVD development and overall mortality.[32,33] This measure has also been shown to be important in both the diagnosis and treatment of hypertension.[34] PWV is a non-invasive and time efficient method to assess arterial function and health, and is highly reproducible.[35] PWV, like CIMT, is not routinely assessed in clinical settings, and therefore quantification of arterial stiffness is not included on common health profiles obtained from screenings performed by health professionals. The comparison of these values against normative data sets can allow individuals at low or moderate risk for CVD to gain a better understanding of their arterial health and work with health professionals to avoid or delay the onset of CVD development.

## Statement of the Problem

It is of paramount importance for health professionals to be able to successfully identify individuals at risk for the development of CVD before they present with signs or symptoms characteristic of an MI or stroke. Since CIMT and PWV measurements have been validated as markers of preclinical atherosclerosis, these screening methods serve as a potential means to detect this condition in its early stages. These tests, however, are not readily available in clinical settings and therefore are under-utilized as screening options for asymptomatic adult populations. The value of these techniques to health professionals as a community screening tool depends on both the prevalence of preclinical atherosclerosis as well as the ability to accurately identify and target those at the highest risk for this condition. Current research and practice fail to determine the prevalence of preclinical atherosclerosis as detected by CIMT and PWV measurements in an adult population, and, as a direct result, risk factors most strongly associated with an elevated chance of having abnormal arterial thickness or stiffness have yet to be fully identified. The identification of certain previously established CVD risk factors or other commonly assessed health parameters in individuals with abnormal arterial assessment results can help to form a more complete risk factor profile for those most at risk for preclinical atherosclerosis. This has the potential to allow health professionals to more effectively utilize these assessment tools as screening services for the community. Additionally, it is critical to identify those in this early stage of CVD to initiate lifestyle modification strategies such as exercise training or more intensive medical or pharmacologic therapies.[8]

## Purpose

The primary purpose of this study was to determine the prevalence of high CIMT and PWV values as an indicator for potential preclinical atherosclerosis in a healthy adult population aged 40-70 years using a cross-sectional, descriptive design. The secondary purpose was to compare established CVD risk factors and other subject health characteristics between those found to have abnormal results and those below the 75$^{th}$ percentile for both CIMT and PWV.[27,28]

**Delimitations**

CIMT, PWV, anthropometric measurements, body composition, blood lipid profile, and physical activity were assessed on members of the Ball State University Adult Physical Fitness Program and surrounding Muncie, IN community in the Human Performance Laboratory. CIMT was measured using ultrasonography of the posterior carotid arterial wall. PWV was determined by analyzing the time delay from the contraction of the ventricles (as detected by electrocardiographic monitoring) to the arrival of the pulse wave form at both the carotid and femoral palpation sites. Anthropometric variables included height, weight, and waist and hip circumferences. Body composition was assessed using dual-energy X-ray absorptiometry. Blood lipid profile was analyzed from an antecubital venous blood draw. Physical activity was monitored for one week using tri-axial accelerometry.

**Limitations**

The study population was comprised primarily of Caucasian subjects, limiting the generalizability and comparison of these results to other ethnicities. Similarly, recruitment of subjects aged 40-70 years is not representative of the entire population and therefore comparisons to individuals outside of this age range may be inappropriate. Last, the APFP population from which the study sample was recruited represents a cohort of habitual aerobic

exercisers, potentially masking the effects of a less physically active lifestyle reported in most individuals in the target age range. Study subjects from the surrounding Muncie community displayed varied physical activity profiles which may be more representative of a typical adult population.

**Definitions**

1. **Preclinical atherosclerosis:** The early or "silent" stage of atherosclerosis, often present for years or decades before the clinical manifestation of CVD
2. **Prevalence:** The widespread presence of a condition or disease state in the general population, expressed as a percentage.
3. **Carotid intima media thickness (CIMT):** A measurement obtained using ultrasound imaging which determines the thickness of the tunica intima and tunica media, the innermost two layers of the arterial wall.
4. **High CIMT:** A mean CIMT value ≥$75^{th}$ percentile for age-, gender-, and race-matched peers.
5. **Pulse wave velocity (PWV):** A measurement obtained using a tonometer, or pressure sensor, which determines the speed of blood flow through the arterial system and allows for the indirect assessment of arterial stiffness.
6. **High PWV:** A mean PWV value ≥$75^{th}$ percentile for age- and blood pressure category-matched peers.

# CHAPTER II

## REVIEW OF LITERATURE

Cardiovascular disease (CVD) continues to be the number one killer of Americans,[1] and the number of heart disease patients continues to rise annually. This is evidenced by an increasing amount of revascularization procedures performed each year.[2] The economic burden placed on the healthcare system due to direct and indirect consequences of CVD necessitates improvements in healthcare professionals' abilities to identify individuals and initiate therapies earlier in the disease process. There is a new potential to identify patients during the long asymptomatic phase of atherosclerotic development known as preclinical atherosclerosis.[21] Carotid intima media thickness (CIMT) and pulse wave velocity (PWV) measurements have been validated as screening tools for the detection of preclinical atherosclerosis; however, its prevalence in a healthy adult population has yet to be fully explored. These assessment strategies have several advantages over more commonly used modalities such as a graded exercise test (GXT), coronary calcium scan (CCS), or coronary catheterization, and their non-invasive nature may allow for their more routine use in a preventive health setting. A more thorough understanding of the relationship between these signs of preclinical atherosclerosis and established CVD risk factors may allow for the more accurate identification of individuals living

with preclinical atherosclerosis, and could improve the ability to target those most in need of these services.

The primary purpose of this study was to investigate the prevalence of preclinical atherosclerosis, as determined by high CIMT and PWV measurements, in a healthy adult population aged 40-70 years. The secondary purpose was to compare established CVD risk factors and other health characteristics between those found to have this condition and those determined to be free of preclinical atherosclerosis in order to identify potential differences in these health parameters.

**Commonly Utilized CVD Screening Methods**

Clinicians are able to utilize several screening or diagnostic tools in order to search for the presence of atherosclerosis in an apparently healthy adult population. However, each technique has inherent inadequacies which may limit the accurate identification of CVD on a large scale in asymptomatic individuals.

*Graded Exercise Test*

One such method involves the use of a GXT along with electrocardiographic (ECG) monitoring. Such tests involve either an incremental or ramp protocol on either a treadmill or cycle ergometer,[36] and are designed to increase the amount of stress placed on the cardiovascular system in order to potentially elicit an ischemic response past a patient-specific work threshold. This widely accessible diagnostic tool has been shown to be a relatively inexpensive modality to evaluate suspected coronary artery disease as well as assess its severity.[37-39] It has been demonstrated that the stable, symptomatic coronary lesions for which healthcare professionals

typically search with a GXT and ECG imaging may be less likely to result in infarction and sudden death than less stable, asymptomatic plaque deposits. This has resulted in an evolution of the use of a GXT with ECG monitoring, placing more focus on the secondary treatment of global atherosclerosis as opposed to a direct, short term intervention.[40] However, the use of GXTs as a potential community-wide screening tool is limited as this procedure is often reserved for physician-referred or symptomatic individuals.

The GXT with ECG monitoring most commonly relies on the detection of significant ST segment changes in order to identify potential myocardial ischemia. These changes represent alterations in intraventricular conductance during each cardiac cycle with increases in workload, which alert test technicians to the potential presence of CVD.[41,42] GXTs are often determined to be "positive" or "negative" in reference to these segment changes; however the diagnostic accuracy of these tests are limited when these dichotomous terms are used in place of stating the conditional probability of CVD based on the Bayesian theory.[38] The post-test classification of patients into low, moderate, or high likelihood for the presence of CVD based on symptom history and ECG findings can strengthen the predictive capability of this test. However, more definitive tests are required to verify these findings. Studies that have reported on the sensitivity or specificity of the diagnostic GXT without the incorporation of the gold standard for the detection of CVD, coronary angiography, have the potential to inflate estimated sensitivity and deflate estimated specificity.[43]

It has been postulated that alternative ECG changes may be more appropriate than ST changes for the detection of CVD during a GXT, and one such theory suggests the direct measurement of QRS complex duration.[44] During exercise, Michaelides and colleagues

demonstrated that normal subjects experienced a 3ms shortening compared to a 6-8ms lengthening of the QRS complex seen in individuals with known CVD.[45] This lengthening has been shown to reach approximately 15ms in patients at an increased risk for ischemia-related life threatening arrhythmias.[46] Computer-based programs capable of detecting these subtle changes were first implemented by Cantor et al.,[47] yet the widespread use of this method of ischemia detection remains limited. An additional ECG change that has been suggested to represent myocardial ischemia is QRS amplitude. Bonoris and colleagues demonstrated that failure of the R wave amplitude to decrease during exercise was an indicator of ischemia, and that the incorporation of R wave amplitude changes could effectively improve the sensitivity and specificity of ST changes alone from 48% to 63% and from 59% to 79%, respectively.[48] These findings have been disputed and opposite directional changes in R wave amplitude during exercise were shown by Talwar et al.,[49] leading to decreased confidence in this measure as an indicator of ischemia. Overall, normalization of ST depression for R wave amplitude appears to have positive clinical applications, however this appears to be true primarily in patients with extreme amplitudes.[50] The lack of consistent ECG interpretation strategies makes comparisons among studies difficult, and the failure to incorporate these additional ECG changes into their analyses inherently limits the diagnostic capabilities of these assessments.

The prognostic value of the GXT can be obtained from markers of cardiorespiratory fitness, primarily time until exhaustion or maximal oxygen uptake. Strong, independent associations exist between aerobic capacity and all-cause mortality in men[51,52] as well as women,[53,54] demonstrating the importance of assessing physical fitness in addition to using a GXT as a diagnostic tool in a clinical setting. Similar relationships have been observed in both healthy individuals and adults known to have CVD, with a landmark analysis in 2002

demonstrating that having an exercise capacity in the lowest quintile among peers results in a relative risk of death of roughly 4.5 and 4.0 when compared to the highest quintile for normal subjects and those with CVD, respectively.[55] Decreased aerobic capacity may exist as a result of myocardial ischemia, and therefore assessment of this marker of physical fitness has the potential to alert health and fitness professionals to occult ischemia. These indicators of cardiorespiratory fitness can serve as important tools for sub-stratification of overall patient risk, adding to the diagnostic yield of a GXT and potentially serving to expand the use of this tool to a greater percentage of the asymptomatic community. Ultimately, lack of uniform criteria for GXT interpretation and its potential inaccessibility among certain populations inhibits it from successfully and consistently detecting asymptomatic individuals with preclinical atherosclerosis.

*Coronary Calcium Scan*

An alternative, yet still non-invasive test for the identification of preclinical atherosclerosis is the CCS. Atherosclerotic plaque masses are estimated to be made up of approximately 20% calcium, and strong associations have been demonstrated between the presence of peripheral plaque deposits and atherosclerosis of the coronary arteries.[56] The development of ultrafast computed tomography (CT) scans has allowed clinicians to quantify this coronary calcification in vivo, which was previously an impossible task. Coronary calcification has been shown to be more prevalent in men when compared to women in a sample of 10,377 asymptomatic individuals ($p<0.0001$), and relative risk seemingly increases in both genders with associated increases in coronary calcification scores.[57] Although this imaging modality has important long-term epidemiological implications, its short-term coronary artery

disease detection capabilities have several limitations that prevent its use as a gold standard for CVD evaluation.

First, the distribution of calcium in coronary lesions is not uniform. It has been shown that some smaller, active plaques as well as some higher-grade stenoses may completely lack identifiable levels of calcium.[58] Additionally, the sensitivity for detecting obstructive coronary artery disease has been demonstrated to be as low as 80%,[59] with an even lower estimated specificity when compared to angiography.[60] The presence of coronary artery calcium deposits has been demonstrated to lack predictive capabilities for adverse cardiovascular events by Detrano et al. with 1,196 high-risk individuals, calling into question its value as an immediate screening tool for preclinical atherosclerosis.[61] Lastly, radiation exposure for patients and potential radiation exposure for technicians, as well as cost, serve as important limitations for the more widespread use of this atherosclerosis detection modality.[62]

*American College of Sports Medicine Risk Classification*

A screening tool designed to aid in the identification of community members who are at an elevated risk for CVD and should therefore seek physician clearance before participating in a new exercise or physical activity routine was designed by the American College of Sports Medicine (ACSM). The ACSM's risk classification scheme was specifically designed to alert health and fitness professionals to an individual's risk of experiencing an immediate ischemic event during exercise testing or training. This system categorizes individuals as either "Low" (asymptomatic men and women who have ≤1 CVD risk factor), "Moderate" (asymptomatic men and women who have ≥2 CVD risk factors), or "High Risk" (individuals who have known cardiovascular, pulmonary, or metabolic disease or one or more signs and symptoms of these

conditions).[36] This questionnaire-based system results in an individual being placed into one of three mutually exclusive "tiers" of risk stratification, ultimately failing to sub-stratify individuals in the "Moderate Risk" category. These individuals may remain asymptomatic while in later stages of atherosclerotic development, warranting additional CVD screening services. Additionally, this system has no long-term predictive implications. This system, therefore, successfully identifies those for whom additional and more sensitive screening services are warranted but offers no further assessment of current cardiovascular health.

*Framingham Risk Score and the 2013 ACC/AHA 10 Year ASCVD Risk Pooled Cohort Equations*

A system that identifies individuals at a future risk for the development of CVD is the Framingham Risk Score (FRS). This method was developed using data from the Framingham Heart Study,[13] and its primary algorithm incorporates CVD risk factors to predict an individual's chance of developing coronary heart disease within the next 10 years.[14] The Framingham Heart Study was initiated in 1948 as an attempt by the United States of America Public Health Service to study characteristics that predisposed American citizens to CVD,[14] and these characteristics were termed "risk factors" for CVD as a result of this study. The original cohort in 1948 included 5,209 reportedly healthy subjects aged 30-60 years from the town of Framingham, located 32 kilometers west of Boston, Massachusetts. Offspring of this initial cohort, as well as third generation family members have been involved in follow-up studies in 1971 and 2002, respectively.[63] This system classifies individuals with an estimated risk of less than 10% as "low risk," individuals with an estimated risk of 20% or higher as "high risk," and individuals between these values as "intermediate risk."[64]

The FRS includes the non-modifiable risk factors of age and gender, as well as the modifiable risk factors of total cholesterol, HDL cholesterol, smoking status, and blood pressure.[15] Other well-known CVD risk factors[16] such as body composition and habitual physical activity level, however, are not included in the prediction equation. This method therefore has inherent limitations in its ability to definitively identify individuals at risk for CVD, since multiple risk factors that have been shown to have associations[17] with this disease are absent from its algorithm. Studies associated with the FRS helped transform the perception of the causes of CVD and encouraged health professionals to place a greater emphasis on prevention and early detection strategies in order to help prevent the rise in incidence of CVD related morbidity and mortality.[65]

In 2013, as part of an updated set of national guidelines on the treatment of blood cholesterol in an attempt to reduce the risk for future atherosclerotic cardiovascular disease (ASCVD) in adults, the American College of Cardiology and the American Heart Association released a risk calculator.[66] This new tool is also designed to estimate an adult's 10 year risk for the development of ASCVD; however the variables of interest slightly differ from those utilized by the FRS and consequently an individual's risk level can vary depending upon which risk assessment method is employed. Updated variables that are new to this algorithm include race (differentiating between African Americans or "whites or others"), as well as whether or not blood pressure is being medically managed. New features of this ACC/AHA tool is an additional calculator for an individual's lifetime ASCVD risk as well as a comparison between an individual's values for CVD risk factors and a theoretical adult of the same age with an optimal risk factor profile. Validation studies are currently lacking as this assessment tool has

just recently been released, however investigative studies are warranted to better understand its relation to the FRS and practicality in a primary prevention setting.

**Assessment of Preclinical Atherosclerosis**

*Carotid Intima Media Thickness*

An abnormally high CIMT has been shown to be an intermediate phenotype for early atherosclerosis, and its assessment's non-invasive nature makes it well suited for large population-based studies.[67,68] Measurements of arterial thickness can be made by using B-mode ultrasonography of the carotid posterior wall. This method has been demonstrated to be a valid marker of atherosclerotic lesions[69] and significantly correlates with results of gross pathologic evaluations in human subjects. An investigation in 1986 by Pignoli and colleagues determined that accurate images are most consistently obtained from the posterior carotid wall, and that a percent error of <20% existed in this measurement with normal and pathologic subjects[70] when compared to measurements made post-mortem. The use of CIMT assessment has been shown to be a reliable and reproducible method for examining atherosclerotic presence and development in population-based studies, as demonstrated as part of the Cardiovascular Health Study. Intrasonographer variability was determined to be less for the common carotid artery ($p<0.0001$, $r=0.65$) than for the internal carotid artery ($p<0.03$, $r=0.14$).[71]

Measurements of CIMT are routinely performed at several anatomical landmarks, with the most common being the common carotid artery (CCA), the internal carotid artery (ICA), and bifurcation (Bif). As a part of the British Regional Heart Study, a sample of 425 men and 375 women free of known CVD (mean age 66 years, range 56-77 years) underwent ultrasound examination of different arterial segments. Longitudinal relationships between measurements

made at these sites and cardiovascular health varied depending on location of abnormally thick values. CIMT measured in the CCA was determined to be most strongly associated with risk factors for and prevalent stroke, whereas CIMT measured in the Bif was more directly associated with an increased risk of ischemic heart disease.[29] CIMT at the CCA measurement site was found to be positively associated with systolic blood pressure in both men and women (age-adjusted odds ratios of 1.0, 1.18, 1.48 and 1.0, 1.51, 1.83 across tertiles of CCA IMT for men and women, respectively). Individuals that experienced an ischemic stroke during follow up analyses had a mean CCA IMT of 0.96mm compared to 0.83mm for those free of these events ($p<0.01$) at baseline, which corresponded to an age-adjusted odds ratio of 1.55 (0.6, 3.7). Mean CIMT taken at the Bif was demonstrated to be significantly higher upon initial assessment for individuals that developed ischemic heart disease than those free of this condition (1.97mm and 1.64mm, respectively, $p<0.01$), resulting in an age-adjusted odds ratio of 3.11 (1.7, 5.8).

The relationship between CIMT assessed at these different measurement locations and the relative risk for an MI was supported by Iglesias del Sol and colleagues using data from the Rotterdam Study. These researchers found evidence that ICA CIMT measurements could be used in addition to the more commonly assessed CCA and Bif sites for CVD screening services. In a sub-cohort consisting of 194 (mean age 72 years, 39% female, mean BMI 26.3kg/m$^2$) former MI patients and 2,073 age- and gender-matched controls, CIMT from all three sites were used for analysis and incidence of MI was determined over a mean follow up of 4.6 years. Risk ratio for MI for the ICA, Bif, and CCA were determined to be 5.31, 4.11, and 3.18, respectively for the highest compared to the lowest quintile.[72] Using a combined CIMT score from all three measurement locations, it was determined that there was a risk ratio of 1.38 (confidence interval of 1.21, 1.58) per standard deviation increase independent of age, gender, BMI, systolic and

diastolic blood pressure, total and HDL cholesterol, smoking, and diabetic status. This investigation provided evidence that risk of future ischemic events can be predicted from thickness measurements made at various carotid locations, affirming this technique's validity in CVD prediction.

Increasing CIMT thickness has been demonstrated to have an independent relationship with an increased hazard ratio for CVD, as has been demonstrated by several large-scale population-based investigations. The Atherosclerosis Risk in Communities (ARIC) Study demonstrated hazard ratios of 5.07 and 1.85 for women and men, respectively, for adults with a mean CIMT ≥1mm when compared to individuals with a mean CIMT of <1mm.[73] Using a sample size of 7,289 women and 5,552 men aged 54 years, this study supported the use of CIMT as a screening tool for future coronary heart disease. Study participants were normotensive, and mean HDL values were considered to be a risk factor for both women and men (27mg/dL and 21mg/dL, respectively). Additionally, the use of CIMT has also been suggested for the prediction of an individual's risk for stroke. The Rotterdam Study incorporated 3,996 subjects who underwent several measurements of markers of atherosclerosis. This investigation longitudinally determined the role of arterial thickness with risk for ischemic stroke. Hazard ratios of 2.23 and 1.89 were seen for individuals in the highest and lowest tertile for mean CIMT, respectively. Increased mean CIMT was also found to strongly correlate with aortic calcifications, which both served as better predictors of incident stroke than classic risk factors. This association has been shown to exist across an age range of 19 to 90 years, and the predictive value has been demonstrated to be as high in younger as in older subjects.[74]

The Carotid Atherosclerosis Progression Study (CAPS) consisted of 5,056 subjects (mean age 50 ± 13 years, 49% male) and also investigated mean CIMT at several anatomical landmarks. During a mean follow-up of 4.2 years, hazard rate ratios per 1 standard deviation of common CIMT were 1.43 for MI, 1.47 for stroke, and 1.45 for MI, stroke, or death ($p<0.0001$)[75] for all subjects, confirming the findings of the Rotterdam Study. The CAPS further assessed overall CVD risk according to age, investigating younger and older adults' CIMT values selecting the age of 50 years as the boundary between each group. A study sample of 2,436 young adults (mean age 39 ± 7 years) revealed a mean left CCA CIMT value of 0.66 ± 0.12mm compared to 2,620 older adults' (mean age 60 ± 7 years) value of 0.82 ± 0.23mm at the same location. Interestingly, longitudinal analyses revealed a variance in hazard risk ratios (HRRs) between the two age categories, with individuals aged <50 years and those ≥50 years old having HRRs of 1.26 (1.06 to 1.49) and 1.08 (1.03 to 1.14) per 0.1mm CCA-IMT increase, respectively. These data signify an increased risk of CVD mortality for younger adults when compared to older adults after an identical increase in CIMT. These results suggest a non-linear risk progression, which relates to ARIC findings that HRRs for CVD mortality increase significantly faster for CIMT values >1.0mm than for those below this threshold. The increased HRRs for those <50 years old revealed in this investigation suggest the preventive potential of CIMT measurement may be strongest in younger adults, as smaller increases over time result in larger relative increases of overall risk.

Interpretation of CIMT values relies upon percentiles that have been previously established as a result of large, well-designed population-based investigations. The ARIC study serves as one of the largest cohorts of universally examined subjects, allowing for the comparison of measurements to be made against 15,800 subjects aged 45-64 years. Howard and

colleagues analyzed these data and developed percentiles that were representative of the general adult population.[27] These percentiles have been established at the CCA, Bif, and ICA for both the left and right anatomical side, and are categorized by age, gender, and race. These nomograms can help determine if individuals have "at risk" CIMT values and identify those in the early stages of CVD progression. Abnormally thick values (i.e. those at or above the 75$^{th}$ percentile among the appropriate peer category) range from 0.64mm to 0.85mm in black women aged 45-65 years, from 0.72mm to 0.99mm in black men aged 45-65 years, from 0.61mm to 0.81mm in white women aged 45-65 years, and from 0.70mm to 0.93mm in white men aged 45-65 years according to these percentile values.

*Pulse Wave Velocity*

PWV measurement allows for the objective assessment of arterial stiffness, for which abnormally high values serve as a sign of overall arterial dysfunction. Arterial stiffness describes a condition in which a blood vessel has a reduced capacity to expand and contract in response to a pulse wave form moving through the local vasculature.[76] Arterial stiffness can be measured by either compliance or distensibility, parameters that describe an artery's ability to respond to pressure changes. Stiff blood vessels experience a decrease in compliance, or volume change for any given pressure, compared to healthier counterparts.[76] This phenomenon results in a measurable difference in the speed of blood propagation through the arterial tree, detected as a time delay from ventricular contraction to the recognition of the arrival of the pulse at two distinct and previously measured arterial locations. Carotid-to-femoral PWV is calculated by $d \bullet (t2-t1)^{-1}$, where d is the distance between the two arterial sites and (t2-t1) represents the difference in time of pulse waveform arrival between the two palpation sites referenced to the R

wave of an electrocardiogram. The carotid and femoral arteries are most commonly used because measurements taken there allow for inferences to be made in regards to the age-related stiffening observed throughout the aorta.[77]

Carotid-femoral PWV has been widely accepted as a marker of the extent of the progression of atherosclerosis in the aorta.[78] The extent to which abnormally high PWV can be measured depends on the heterogeneous composition of advanced atherosclerotic formation, consisting of lipids, fibrous connective tissue deposits, and calcification.[79] Further advancement of atherosclerosis may lead to the detection of plaque buildup, which is associated with additional increase in PWV. These changes are associated with an increased collagen content in conjunction with a decreased elastin content of the arterial wall.[80] Additional progression of the atherosclerotic process may lead to calcification in the media or intima of the aortic wall,[81] visible by chest X-ray and often a marker of coronary artery calcification and potentially blood-limiting lesions. Therefore, PWV has the potential to serve as a valuable screening tool for the early stages of CVD in a seemingly healthy population.[78]

Arterial stiffness, as assessed via PWV, has been demonstrated to increase with age by as much as 0.1 meter•second$^{-1}$•year$^{-1}$ in populations with low a prevalence of atherosclerosis and documented CVD ($r=0.673$, $p<0.001$).[82] These findings support what had been first documented in 1922 by Bramwell and Hill, who calculated an increase in PWV from 520cm/s to 855cm/s (which represents a 176% increase in arterial stiffness) from age 5 to 84 years, with the assumption of a linear increase over time.[32] These results have led to the development of multiple sets of normative and reference standards for PWV across the lifespan, most notably those of Elias et al. and Boutouyrie et al. With a sample of 502 subjects (aged 40 to 93 years),

Elias et al. demonstrated a similar age-related increase in PWV that was more pronounced in hypertensive individuals when compared to their normotensive counterparts in 2011 (8.1 to 11.4m/s and 8.6 to 13.1m/s from age 40-93 years for normotensive and hypertensive adults, respectively).[83] Boutouyrie and colleagues developed another set of reference values for PWV with 1,455 subjects free of CVD and diabetes, and showed that mean PWV values increased from 6.2m/s to 10.9m/s from age 30 to age 70 years.[28]

The importance of the degeneration of aortic distensibility as a result of atherosclerosis in carotid-femoral PWV has been demonstrated in both humans and experimental animals.[84-87] This association has been demonstrated in German subjects by Schimmler et al.,[88] and has been verified in a Western cohort.[89] This age-related increase of arterial stiffness has been repeatedly reported to exist in a linear relationship throughout a lifetime,[82] however other studies have found evidence for a progressively increasing rate of PWV acceleration between 50 and 60 years of age.[90] A sample of 998 Caribbean Hispanic subjects with a mean age of 46 ± 17 years (40.6% hypertensive and 14.6% with diagnosed diabetes) displayed a relationship between arterial stiffness and age that was better explained by a quadratic equation rather than linear regression. This may be explained by an increase in the calcification of the aortic media commonly seen in the fifth decade.[91] Additionally, other molecular and genetic changes accumulate over time to attribute to increases in arterial stiffness.[92-94] Support for the role of these molecular markers in the stiffening of arteries has been shown by a reduction of PWV compared to placebo with the introduction of a collagen cross-link breaker in hypertensive subjects, who experienced no significant change in mean arterial pressure.[95]

Arterial stiffness has also been shown to have a close relationship with hypertension, as functional stiffness depends on the pressure exerted by the blood on the vessel wall. Independent of structural change, higher systolic blood pressures result in an increase in measured arterial stiffness.[96] This relationship is further exemplified by an increased arterial stiffness measured after an acute elevation in blood pressure.[97] Additionally, arterial stiffness and PWV are higher in hypertensive individuals when compared to their pre-hypertensive or normal age-matched controls.[98,99]

Lastly, the role played by genetics in determining an individual's aortic PWV cannot be overlooked, as it has been demonstrated that various genetic syndromes have a significant impact over arterial stiffness. Genetic conditions in which fibrillin-1 is suppressed, such as Marfan's syndrome, lead to insufficient production of elastin fibers, effectively increasing arterial stiffness.[100] Additionally, William's syndrome disrupts the expression of the elastin gene and has been strongly correlated with an increase in PWV throughout the arterial system.[101] Although instances of these syndromes are relatively rare, it has been demonstrated that genetic factors result in similar global responses and impact arterial stiffness independent of other potentially modifiable risk factors. The estimates of the role of genetics on arterial stiffness range from 19%-54%, even after adjustment for significant confounding variables such as age, gender, etc.[102-105]

*The Association Between Arterial Thickness and Stiffness*

Investigations in regards to potential associations between arterial thickness and stiffness have been conflicting, leading to an unclear understanding of the potential relationship between these two markers of arterial dysfunction. This relationship was studied in a subset of the

Atherosclerosis Risk in Communities (ARIC) study including 10,920 black and white, men and women subjects aged 45-64 years. Using diameter change as a measure of arterial stiffness and ultrasonography for assessment of mean CIMT, results showed that stiffness of the arteries remained nearly unchanged for individuals with CIMT $\leq 90^{th}$ percentile. Above the $90^{th}$ percentile for CIMT in this cohort at approximately 0.80mm, diameter change decreased suggesting that elasticity remains unchanged with differences in wall thickness, except in extreme phenotypes.[106] This suggests a chronological development of these two indicators of arterial dysfunction, with thickening preceding stiffening. This phenomenon may provide more detailed insight into the development of preclinical atherosclerosis; however the current literature fails to thoroughly investigate the relationship between CIMT and PWV measurements in a variety of subpopulations.

Aortic stiffness, as measured by PWV, has shown a similar weak association with CIMT after adjustment for age and blood pressure. Zureik and colleagues studied 564 hyper- and normotensive subjects without a documented history of CVD, and determined that gender-adjusted PWV values were significantly higher for individuals with carotid plaques than those without these deposits ($12.7 \pm 0.2$ vs. $11.1 \pm 0.1$ m/s, respectively, $p<0.001$). Mean CIMT, however, was not significantly associated with PWV.[107] In the Northern Manhattan Family Study, arterial strain, stiffness, distensibility, and elastic modulus, which are all accepted markers of arterial stiffness, were found to have no correlation with CIMT in 693 Caribbean Hispanic subjects after adjustment for age and gender.[108] It is possible that mean CIMT increases in response to increased arterial blood pressures as a protective mechanism. This theory is supported by research that has shown an increase in radial IMT in individuals with essential hypertension when compared to age-matched controls without a corresponding increase

in circumferential stress in the arterial system. In 1994, Laurent and colleagues evaluated circumferential stress, distensibility, and the elastic modulus (all markers of arterial stiffness) as well as CIMT in 22 normotensive control subjects (mean age 44 ± 11 years) and 25 age- and gender-matched patients with non-treated essential hypertension (mean age 48 ± 12 years).[109] Circumferential stress is a force applied to the luminal wall tangential to the axis. Arterial distensibility is compliance relative to volume, which therefore relates to stiffness of the arterial wall.[76] Lastly, the elastic modulus is an indicator of the artery's tendency to deform elastically during each cardiac cycle. Mean blood pressure was 90 ± 15mmHg and 121 ± 24mmHg ($p<0.0001$) for the control and hypertensive groups, respectively. Ultrasound evaluation of arterial walls revealed a significantly increased thickness of the radial IMT (0.28 ± 0.05mm and 0.40 ± 0.06mm for control and hypertensive subjects, respectively [$p<0.001$]), however the indicators of arterial stiffness showed no significant variation between each test group. The radial artery elasticity of the hypertensive patients included in this investigation was preserved, maintaining normal compliance despite a marked increase in intravascular pressure. These data suggest that hypertrophy of the arterial wall may take place in response to an increase in shear stress caused by a chronic elevation in blood pressure as seen in individuals with diagnosed hypertension, and that measurement of either arterial thickness or stiffness alone may result in dissimilar diagnostic findings.

## CVD Risk Factors and Arterial Health

### *Physical Activity and Exercise*

CIMT has been shown to have a strong inverse relationship with regular participation in physical activity and exercise. In 2013, Galetta and colleagues demonstrated that in adult males

free of documented CVD and its associated risk factors, athletes had significantly lower CIMT values as measured in both the left and right carotid arteries by ultrasound imaging than their age- and sex-matched sedentary peers.[110] This investigation utilized 32 male athletes (mean age 65 ± 3 years), recruited from local regional running clubs, as well as 32 sedentary subjects (mean age 64 ± 4 years) who were not participating in any form of regular aerobic exercise training as assessed by the Baecke questionnaire. Subjects were asymptomatic and were considered for the study after a normal basal and stress ECG. Physical activity scores determined by the subjective Baecke questionnaire were 10.25 ± 1.50 and 6.75 ± 1.25 for the athletes and sedentary subjects, respectively ($p<0.0001$). Mean CIMT values were shown to be significantly lower in the athletes (0.74 ± 0.11mm) than in the sedentary counterparts (0.92 ± 0.13mm), suggesting that participation in regular physical activity and exercise may attenuate an age-related increase in vascular remodeling.

The effects of habitual physical activity on CIMT have also been investigated in pre-hypertensive adults, free of known cardiovascular and metabolic diseases.[111] Palatini et al. prospectively studied this relationship with a mean follow-up time of six years in 87 young sedentary and active adults (32 ± 8 years and 29 ± 8 years, respectively) who were untreated after being screened for stage 1 hypertension. Baseline physical activity was assessed with a standardized questionnaire, and subjects were considered to be either sedentary if they did not engage in any physical activity, mild exercisers if they participated regularly in leisure-time physical activity, or exercisers if they regularly jogged, cycled, or performed other aerobic exercise. Mean blood pressure at baseline for sedentary subjects was 141 ± 13 / 91 ± 7mmHg compared to 143 ± 12 / 89 ± 8mmHg for their active counterparts. Both groups saw age-related increases in mean CIMT (increase of 22% and 28% for active and sedentary adults,

respectively), with the physically active adults experiencing an attenuated elevation compared to their habitually sedentary peers (p=0.01). These results indicate that engaging in a physically active lifestyle may have beneficial effects on arterial health in those with a pre-hypertensive history.

This relationship has been further supported with the application of single axis accelerometry by Kozàkovà and colleagues in 2010.[112] By limiting the potential recall bias or subjective misclassification of physical activity intensities, this research helped elucidate the effects of physical activity intensity on the progression of CIMT. Ambulatory movements were monitored in a study population of 614 men and women (mean age 44 ± 8 years) for a one week period by a Computer Science Applications Model AM7164, and follow-up International Physical Activity Questionnaires (IPAQ) were administered for additional analyses. For the baseline analysis, mean common carotid artery CIMT was directly related to time spent in sedentary behaviors independent of age and other established atherosclerotic risk factors such as smoking status and elevated blood pressure ($r^2$=0.33, p<0.0001 and $r^2$=0.37, p<0.0001) for men and women, respectively. The 3 year increase in CIMT was assessed in a subgroup of 495 subjects, and was found to be significantly higher in those with periods of only light or moderate intensity activity compared to those with periods of vigorous intensity activity (.022 ± .051mm, .019 ± .046mm, and .007 ± .040mm for light, moderate, and vigorous, respectively). These data provide important support that objectively quantified periods of vigorous intensity physical activity may help minimize the age-related increase in CIMT.

The effect of different physical activity intensity levels on carotid arterial health throughout a lifespan was further examined as part of The Amsterdam Growth and Health

Longitudinal Study. In 2009, van de Laar et al. examined longitudinal data on habitual physical activity and cardiovascular risk in 373 healthy adult subjects.[113] Habitual physical activity was measured by a structured, detailed face to face interview on 8 separate occasions from age 13 to 36. The intensity, frequency, and duration of all physical activities lasting at least 5 minutes and at an intensity level >4 METs were recorded. Light-to-moderate, hard, and very hard physical activities were defined as 4-7 METs, 7-10 METs, and >10 METs, respectively, with time spent in hard and very hard intensities being termed "vigorous" for analyses. When grouped into tertiles of arterial stiffness as measured with noninvasive ultrasonography and determined by calculating the beta-stiffness index, the distensibility coefficient, the compliance coefficient, and Young's elastic modulus, it was determined that individuals with the stiffest arteries spent, on average, less time in vigorous activities compared to those with the least stiffness (-26.5 min/week [95% CI: -45.9 to -7.1]). Mean beta-stiffness index ($\ln(SP/DP)/(\Delta D/D)$) was $6.0 \pm 0.5$ and $9.0 \pm 1.1$ for the least stiff and most stiff arteries, respectively. This difference was attenuated but still significant after adjustment for known CVD risk factors such as blood lipids, cardiorespiratory fitness, fat distribution, resting heart rate, and mean arterial pressure (-11.2 min/week [95% CI: -29.4 to -7.0]), showing an independent relationship between physical activity and arterial health parameters. This study demonstrates support for the promotion of vigorous intensity physical activity for a favorable impact on arterial function in young, asymptomatic adults.

The relationship between physical activity and arterial stiffness has also been explored in a healthy older adult population (aged 70-79 years). As part of The Health, Aging, and Body Composition Study, Havlik and colleagues investigated self-reported physical activity and its relationship to aortic PWV.[114] A sample size of 2,488 subjects from Pittsburgh, PA and

Memphis, TN completed the Leisure Time Physical Activity Questionnaire, designed to capture a range of activities commonly performed by older adults. Researchers assessed speed of blood flow through the arterial system by measuring the distance from the carotid to the femoral artery locations, and using Doppler flow probes in order to detect the onset of flow during each cardiac cycle. Aortic PWV and therefore arterial stiffness was inversely related with both kilocalorie expenditure and minutes of vigorous physical activity performed per week, as estimated by a conversion compendium and the subject's body weight and consideration of the subject's perception of effort. Individuals with a physical activity kilocalorie expenditure of <200kcal/week showed a mean aortic PWV of 8.71m/s, which was significantly higher than the value of 8.03m/s for those spending ≥1,500 kcal/week ($p<0.001$). Similarly, ≥180 minutes of weekly vigorous physical activity resulted in a significantly lower aortic PWV when compared to those accumulating no vigorous activity ($p<0.0005$). These findings support others and agree that vigorous intensity activities have important arterial health benefits, even for elderly adults.

The importance of vigorous physical activity for arterial health as measured by PWV was further confirmed as part of The Northern Ireland Young Hearts Project. As part of this investigation, Boreham et al. demonstrated an inverse relationship between participation in vigorous physical activity and PWV that was found to exist independent of cardiorespiratory fitness level.[115] A study sample of 405 men and women (mean age 22 ± 1 and 22 ± 1 years, respectively) completed a modified Baecke questionnaire in order to subjectively quantify time spent in work, sports, and non-sports leisure physical activity. Only sports-related physical activities were inversely related with arterial stiffness, and this relationship appeared to be strongly mediated by cardiorespiratory fitness. These findings suggest that physical activity has

the potential to help favorably modify arterial health if the intensity is adequate to lead to improvements in aerobic capacity.

In 2008, Duren and colleagues demonstrated that participation in lower intensity physical activities, such as yoga, may be sufficient to minimize PWV and accrue benefits for overall arterial function in healthy adults.[116] Study subjects were free of established CVD risk factors and a total of 10 regular aerobic exercisers, 8 regular yoga participants, and 8 sedentary controls had arterial stiffness assessed via PWV (mean age 51 ± 7, 48 ± 5, 51 ± 8 years, respectively). Yoga subjects participated in yoga that included inversion positions at least 2 days/week for at least one year and aerobic exercisers engaged in at least 30 minutes/day of aerobic training at least 3 days/week for at least one year. Mean PWV for the sedentary group was significantly higher than the aerobic and yoga exercisers ($p<0.001$), and there was no difference between the yoga and aerobic groups ($p=0.21$, effect size of 0.08). It is important to note that both exercising groups reported similar physical activity levels on the Baecke questionnaire (Physical Activity Score of 8.04 ± 0.82 and 8.56 ± 1.37 for aerobic and yoga groups, respectively), so conclusions based solely on habitual participation in different exercise intensities require further investigation.

*Body Composition*

Several parameters of body composition have also been shown to significantly correlate with arterial function and, therefore, the overall health of these vessels. In 2011, Hacihamdioglu et al. demonstrated that increased abdominal obesity, as measured by waist circumference, contributes to the risk for a pediatric population to have at-risk CIMT values.[117] A study population of 104 obese children, defined as having a BMI over the 95$^{th}$ percentile for age-

matched peers, and 30 age- and gender-matched controls underwent physical examinations involving anthropometric measurements and other health information. Waist circumference proved to be significantly different between obese children and the control group (84.7 ± 10.1cm and 73.6 ± 7.8cm, respectively, $p<0.001$), as did CIMT (0.49 ± 0.05mm and 0.40 ± 0.02mm, respectively, $p<0.001$). The relationship between these two variables ($r=0.58$) demonstrates that increased abdominal obesity elevates an individual's likelihood of increasing the rate of progression of atherosclerosis. Additionally, this effect can occur early in one's lifespan in the presence of increased abdominal adiposity.

This finding was supported and extended into healthy, diabetic, and hypertensive adult populations by Recio-Rodriguez and colleagues in 2012.[118] This cross-sectional, descriptive study consisted of 305 individuals considered to be diabetic, hypertensive, or healthy (33%, 37%, and 30% of the study sample, respectively). The study sample included 163 men and 142 women, and mean age was 53 ± 12 years. Additionally, percentage of prescription medication treatment in the sample for hypertension, diabetes, and dyslipidemia was 24%, 29%, and 23%, respectively. Traditional quantifiers of obesity such as BMI, waist circumference, body fat percentage (measured via OMRON bioelectrical impedance analysis body fat monitor), and waist/height ratio were analyzed in order to assess which marker most strongly correlated with signs of arterial dysfunction including elevated CIMT and PWV values. After adjusting for age, gender, and CVD risk factors such as blood pressure and inflammatory blood markers, multiple regression analyses determined that an increase in waist circumference of 1cm resulted in an associated increase in PWV of 0.029m/s and in CIMT of 0.001mm. Furthermore, waist circumference's relationship with these indicators of early stage CVD was stronger than the more traditionally utilized BMI value ($r^2=0.178$ vs. 0.115 and $r^2=0.144$ vs. 0.068 for PWV and

CIMT, respectively). These findings show support for the routine assessment of measures of abdominal obesity in clinical practice, as they demonstrate a stronger correlation with measures of arterial stiffness and thickness independent of clinical comorbidities such as hypertension or diabetes.

Data from the British Regional Heart Study were analyzed in a 2004 report by Lawlor and colleagues in an effort to determine the effects of gender-based differences in body fat distribution on CIMT and therefore arterial health.[119] Data from 800 men and women (aged 56-75 years, mean age 65 ± 6 and 65 ± 5 years for males and females, respectively) were analyzed for potential correlations between waist to hip ratio and CIMT in an effort to better understand the early process of atherosclerotic development. Mean waist to hip ratio for females and males was 0.80 and 0.93, respectively, and mean CIMT was 1.36 vs. 1.54 mm for males and females, respectively. The correlation coefficients were 0.23 (0.14 to 0.32) and 0.19 (0.10 to 0.28) for women and men, respectively ($p<0.001$), showing that both genders showed a relationship between body fat distribution and arterial thickness. Findings from this study indicate that both genders experience a body composition-related effect on CIMT, supporting that this risk factor for cardiovascular and metabolic disease universally impacts arterial health.

Gender differences in body composition and the associated impact on arterial stiffness have also been investigated in a healthy, non-obese adult population (mean age 38 ± 13 and 37 ± 9 years for men and women, respectively) by Budimir et al. in 2012.[120] PWV was assessed in a group of 352 subjects free of documented CVD, in addition to measuring BMI, waist to hip ratio, waist to height ratio, and percent body fat by skinfold caliper. After adjusting for potentially confounding variables such as blood pressure, smoking status, age, and blood lipid profile

values, BMI, waist circumference, and waist to height ratio remained significant but inverse predictors of arterial stiffness (β coefficient from -0.06 to -0.16, $p<0.05$) in females. Interestingly, the only body composition-related variable determined to remain significant in males after adjustment for identical confounders was BMI (β coefficient from -0.09 to -0.13, $p<0.05$). These associations may be strongly confounded by established CVD risk factors and therefore these variables must be addressed when investigating the effects of one or more of them on overall arterial health.

Support for the close relationship between body composition and arterial stiffness was further gathered by Dengo et al. in 2010 through an intervention study investigating the effects of weight loss on arterial health in 36 overweight and obese but otherwise healthy adults.[121] A 12-week hypocaloric (1200-1500kcal/day) diet was employed for 25 subjects, compared to 11 controls that had no change in lifestyle habits during this same time frame. The intervention resulted in significant reductions in body fat as measured via dual energy x-ray absorptiometry (-4.6 ± 0.6kg vs. -0.1 ± 0.4kg, $p<0.05$) as well as total abdominal fat as assessed via computed tomography (-101 ± 14$cm^3$ vs. -8 ± 13$cm^3$, $p<0.05$). These changes significantly correlated with arterial stiffness as measured by carotid-femoral PWV, which decreased by 187 ± 29cm/s for the test group and increased 15 ± 42cm/s in controls ($r=0.482$ and $r=0.461$ for total fat mass and total abdominal fat, respectively, $p<0.05$). The influence of weight loss on arterial stiffness appeared to exist independently of alterations in subjects' blood pressure, indicating that body composition interventions may serve as effective ways to improve overall arterial health in moderate risk adults.

The effects of body composition on common carotid artery function were explored in a study by Kozàkovà and colleagues in 2008[122] in which percentage of total body fat was measured with bio-electrical impedance analysis using a Body Composition Analyzer Model TB-300. The 627 subjects (252 men and 375 women) were determined to be at low, below average, or average risk (77%, 18%, and 5% of the sample, respectively) for the development of coronary heart disease based on the Framingham Heart Study prediction score. Findings of this study support that waist girth significantly impacts arterial health by influencing CIMT in healthy adults, in addition to demonstrating the importance of fat free mass (FFM) on this indicator of arterial function. Male subjects had a mean FFM of 52.4 ± 11.1kg and females averaged 44.5 ± 4.2kg. Researchers investigated the previously demonstrated relationships between increased FFM, systemic blood pressure, total blood volume, and these factors' resultant effect on posterior wall thickness.[123] An increase in metabolically active FFM has been shown to relate to an increase in total blood volume and therefore cardiac output, potentially leading to elevated levels of shear stress between blood and the luminal wall within the arterial system.[124-126] Kilograms of fat free mass remained a significant correlate of posterior wall CIMT when adjusted for age in a subgroup of 75 subjects (standardized regression coefficient=0.43 ± 0.08). Data from this analysis suggest that CIMT may be remodeled partly in response to body composition characteristics of healthy adults, particularly lean tissue mass.

*Blood Lipid Profile*

The independent associations between several commonly assessed elements of a standard blood lipid profile and arterial health parameters such as thickness and stiffness have not been studied in as much depth as other established CVD risk factors, however significant relationships

have been shown to exist in several studies. The presence of diabetes mellitus, clinically defined as having a fasting blood glucose level ≥126mg/dL.[127] has been demonstrated to significantly correlate with CIMT.[128] As a part of a cross-sectional investigation by Alizadeh et al., 40 adults with diabetes and 40 age- and gender-matched healthy controls (aged 51 ± 9 and 51 ± 10 years, respectively) underwent CIMT measurements. Subjects with diagnosed diabetes had been clinically diagnosed as such for an average of 10 ± 6 years, and had a mean CIMT value of 0.98 ± 0.4mm vs. 0.63 ± 0.23mm in controls ($p<0.05$). Further analysis revealed that in individuals without a history of documented coronary artery disease (i.e. low- to moderate-risk adults), individuals with diabetes had a mean CIMT of 0.92 ± 0.38mm compared to 0.62 ± 0.16mm seen in controls ($p=0.019$). Multivariate regression analysis revealed that diabetes mellitus was the strongest correlate with CIMT (coefficient=0.344, 95% CI = 0.209-0.479). This study showed that diabetes mellitus is an independent risk factor for CVD, even after controlling for potential confounders such as age, blood pressure, and smoking. The relationship between coronary artery disease and diabetes mellitus shows support for the use of CIMT assessment in this population as a screening method for early detection of atherosclerosis development.

The relationship between fasting blood glucose level and arterial health was further strengthened in the Tanno and Sobetsu Study, in which Ohnishi and colleagues investigated the link between diabetic status and PWV.[129] In a sample of 232 men (mean age 65 ± 10 years), PWV was assessed and groups were partitioned based on fasting blood glucose. Mean fasting blood glucose levels for the normal (n=185), impaired fasting glucose (n=24), and diabetic (n=23) groups were 95 ± 7mg/dl, 116 ± 5mg/dl, and 143 ± 41mg/dl, respectively. Mean HbA1c values for these groups were 5.0 ± 0.3%, 5.6 ± 0.3%, and 6.6 ± 1.2%, respectively. Results suggested that the association between arterial health and both fasting blood glucose levels and

HbA1c existed in a linear relationship, as mean PWV for the normal, impaired fasting glucose, and diabetic groups were 1,518cm/sec, 1,673cm/sec, and 1,771cm/sec, respectively. The association between PWV and both diabetic parameters was significant ($r=0.329$, $p<0.0001$ and $r=0.292$, $p<0.001$ for fasting blood glucose and HbA1c, respectively), suggesting that arterial health may be compromised before an individual is clinically diagnosed as having diabetes. These results emphasize the importance of early detection of the atherosclerotic process through assessment of markers of arterial health, especially in populations at an elevated risk for the development of diabetes.

Other elements that are routinely assessed during blood lipid profiles appear to have important interactions with the health of arteries as well. In 2013, Shah et al. demonstrated that ratios of sub-fractions of blood lipids have significant correlations with CIMT.[130] In a sample of 244 young adults with clinically diagnosed type II diabetes mellitus (mean age 18 years), laboratory blood values were collected in addition to CIMT measurements. Results suggested that LDL/HDL ratio was the strongest predictor of CIMT ($p<0.02$), indicating that specific sub-components of the whole blood lipid variables may have important implications for arterial health. Additionally, the protective effect of HDL resulted in an independent and inverse correlation with mean CIMT ($p<0.05$). Altogether, these elements of a routinely assessed blood lipid profile as well as other traditional CVD risk factors like age, gender, and body composition helped to explain <20% of CIMT variance in this population, suggesting other risk factors need to be analyzed when determining an individual's risk for atherosclerotic development.

An alternative blood lipid ratio that has been shown to significantly relate to arterial stiffness in a sample of 893 adolescents and young adults (mean age 18 years) is triglyceride to

HDL ratio.[131] Subjects were stratified into groups of triglyceride to HDL ratio, with the low, mild, and high groups being made up of 227, 288, and 379 subjects, respectively. Across triglyceride to HDL groups, there was a progressive rise in CVD risk factors such as body composition and blood pressure in addition to arterial stiffness as assessed via carotid-femoral PWV. The high triglyceride to HDL ratio group had the stiffest arteries among the groups ($p<0.03$). These findings suggest that the use of this ratio may improve the identification of young, asymptomatic adults that are at an increased likelihood of showing early signs of atherosclerosis as detected via PWV assessment.

**Summary**

The early and accurate detection of atherosclerosis in an asymptomatic, seemingly healthy adult population depends on the ability of healthcare professionals to utilize valid and effective screening tools to identify individuals in the early stages of CVD. CIMT and PWV measurements serve as viable options for this assessment, and the practicality of these methods in a primary prevention setting depends on determining the prevalence of at-risk values in an adult cohort free of documented CVD. Additionally, a continual development of the understanding of the risk factors that may most strongly predispose an individual to develop this condition is required to help more effectively identify individuals in need of these services. While the complex relationships among CVD risk factors and signs of preclinical atherosclerosis, primarily arterial thickness and stiffness, have been elucidated with well-designed analyses, the utilization of more advanced assessment tools has the potential to improve overall understanding of the process of preclinical atherosclerosis. Mainly, physical activity assessment with tri-axial accelerometers may more accurately quantify time spent in sedentary

behavior or moderate-to-vigorous intensities. Additionally, body composition as measured via DXA scan will improve upon previous investigations that utilized less accurate means to examine relationships between total body fat percentage, total lean mass, regional adiposity, and arterial health. Successful determination of preclinical atherosclerosis prevalence and identification of objectively assessed CVD risk factors or other health characteristics that may predispose an individual to early development of atherosclerosis may help prevent the annual increase of CVD related morbidity. Ultimately, CIMT and PWV have the potential to serve as practical and effective screening tools and aid in preventive health strategies for the general public.

# CHAPTER III

# METHODOLOGY

The primary purpose of this study was to determine the prevalence of high carotid intima media thickness (CIMT) and pulse wave velocity (PWV) values, which both represent an elevated risk for preclinical atherosclerosis, in a healthy adult population aged 40-70 years. Secondarily, this study aimed to compare established CVD risk factors and other health-related characteristics between those found to have elevated arterial assessment values and those whose CIMT and PWV values are considered to be within normal limits.

## Subjects

Members of the Adult Physical Fitness Program (APFP) and surrounding Muncie, Indiana community were recruited for participation in this study by word of mouth, email communication, and recruitment flyers posted in the APFP fitness center. Subjects consisted of regular aerobic exercisers that have been following exercise prescriptions for ≥6 months, new fitness center members with a variety of former physical activity profiles, and Ball State University faculty and staff. Prior to enrollment in the study, subjects were informed of procedures as well as risks and benefits of participation. Subjects provided a written informed consent prior to involvement in the study. Inclusion criteria consisted of adults aged 40-70 years. Exclusion criteria included any history of documented cardiovascular disease (CVD),

including past myocardial infarction, stroke, peripheral artery disease, heart failure, or revascularization procedure.

### Study Overview

The study protocol was approved by the Ball State University Institutional Review Board. All assessments were completed at the Ball State University Human Performance Laboratory (HPL). Participation in the study involved two separate visits to the HPL, spaced approximately one week apart. Visit one included the measurement of anthropometric variables, CIMT and PWV assessment, and the issuance of an accelerometer. Visit two consisted of a blood draw and body composition assessment.

## Procedures

### Visit One

*Health History Questionnaire (HHQ)*

Subjects completed an HHQ involving information on medication status, family and personal history, symptoms, recent hospitalizations, as well as lifestyle and physical activity habits. The HHQ included smoking information to allow for the quantification of pack-years, as well as information on frequency and type of both alcoholic and caffeine consumption per week. Additionally, subjects were asked to describe any participation in current or former weight reduction diets. Physical activity was subjectively assessed by having subjects describe occupational physical activity levels and detail their vigorous and recreational participation in physical activity.

*Anthropometric Measurements*

Subjects were instructed to remove footwear and excess clothing as well as empty pockets before measurements were taken. Weight was assessed using a Health-O-Meter Model 349 klx digital scale (Bridgeview, IL) to the nearest 0.1 pound. Height was measured using a wall-mounted SECA (Germany) stadiometer to the nearest 0.25 inch, and values were obtained after exhalation. Body mass index was calculated as $kg/m^2$. Waist circumference was measured on bare skin at the narrowest part of the torso above the umbilicus and below the xiphoid process. Hip circumference was measured over the subject's shorts at the widest portion of the buttocks. Both waist and hip circumferences were measured by a single technician using a tape measure with a spring-loaded tension gauge. Two measurements were taken, and if these values differed by more than 2cm a third measurement was obtained to allow for the average of the two values within the 2cm tolerable limit to be used in analysis.

*Carotid Intima Media Thickness*

CIMT was measured by a single trained technician. Measurements were taken from the left common carotid artery (LCCA) from the longitudinal plane. Two-dimensional B-mode ultrasonography images were obtained using the General Electric LOGIQe InSite ExC system (Milwaukee, WI) 12 L linear-array transducer. With the subject in the supine position, the head was turned away from the technician at 45 degrees. After locating the carotid bulb, a 5-10 second cinematic loop was taken. A still image was then saved at the point of end-diastole, identified as the period of smallest luminal diameter. A series of 7 images were obtained and subsequently used for measurement. Measurements of the LCCA were taken at a point 1cm from the carotid bulb on the posterior carotid wall. The highest and lowest values were excluded, and the mean of the 5 remaining measurements was used for statistical analyses.

Images were saved as a "JPEG image (*.jpg)". Data were classified according to percentiles for age-, gender-, and race-matched peers originally established by Howard et al. in 1993.[27] Percentiles did not exist for individuals <45 and >65 years old; therefore, subjects aged 40-44 were classified according to CIMT values for 45 year old adults and subjects aged 66-70 were classified according to CIMT values for 65 year old adults.

*Pulse Wave Velocity*

PWV was measured by gender-matched technicians, with one male technician obtaining the measurements for all male subjects and two female technicians assisting with data acquisition for female subjects. Measurements were taken using an AtCor Medical SphygmoCor (Itasca, IL) on the right side of the body, using the carotid and femoral palpation sites. All PWV measurements were taken in the supine position. The carotid palpation site, generally located between the sternocleidomastoid and the trachea roughly at the level of the cricoid cartilage, was identified by having the subject's head turned at 45 degrees away from the technician. The femoral palpation site, generally located below the inguinal ligament and about midway between the symphysis pubis and anterior superior iliac spine, was identified by having the subject externally rotate the hip and flex the knee. Both sites were marked on the subject's skin, allowing for consistent measurement at each location. Supine resting blood pressure was measured using auscultation by an aneroid sphygmomanometer calibrated against a mercury column after at least 5 minutes of rest. The SphygmoCor detects the time delay between the ventricles contracting and the pulse arriving at predetermined locations, allowing velocity of blood flow to be calculated since velocity equals distance•time$^{-1}$. Distance was measured from the suprasternal notch to both the carotid (proximal) and femoral (distal) palpation sites and

recorded in the SphygmoCor unit. Electrocardiogram electrodes were placed on the right arm, left arm, and left leg in order to identify a clear R wave for each cardiac cycle and determine precisely when contraction of the ventricles occurred during assessment. The tonometer was placed on the previously located carotid and femoral artery palpation sites for 10 seconds of consistent pulse wave form recognition in order to assess the proximal and distal sites, respectively. The SphygmoCor calculated the PWV based off of these measurements. A series of two measurements were obtained and the average values were used in statistical analyses. Quality control required the standard deviations of the mean timing data from the ECG to the proximal and distal sites to be ≤6%, the heart rate to be within 5 beats per minute at each site, and the pulse-transit time to be <10%. Data were classified according to reference values for age- and blood pressure category-matched peers originally established by Boutouyrie et al. in 2010.[28]

*Physical Activity (PA) Assessment*

Subjects wore a GT3X+ accelerometer (ActiGraph, Fort Walton Beach, FL) for a one week PA assessment period. The accelerometer was worn on the subjects' waist band at the midline of the thigh on the right hip. Subjects were instructed to wear the accelerometer during all waking hours, except for during water-based activities. A log sheet containing usage instructions was given to each subject and was to be returned along with the accelerometer upon arriving for the second visit. Subjects were instructed to record any periods during which they did not wear the accelerometer, performed a bout of exercise, or engaged in any atypical activities. The epoch was set at 60 seconds. Validation criteria required that the accelerometer be worn for ≥600 minutes per day on ≥4 days per week, and any data not meeting these criteria

were excluded from analysis. Data were analyzed for steps per day, time spent in sedentary bouts per day, vigorous intensity minutes per day, and moderate to vigorous physical activity (MVPA) per day. Non-ambulatory activities included on subjects' accelerometer log sheet that were classified as vigorous physical activity (≥6 METs) according to the Compendium of Physical Activities were manually added to subjects' objectively quantified vigorous physical activity totals.[132]

**Visit Two**

*Blood Lipid Profile*

For subjects with a change in cholesterol medication status, weight, or self-reported change in physical activity level in the past 12 months, subjects arrived to the HPL after having completed a 10-12 hour fast. The HHQ detailed the subjects' current medications, dosage, frequency, and the date on which medications were started. Subjects' weight history was determined by self-report and analyzing previous body composition assessments taken as part of the Ball State University Adult Physical Fitness Program annual renewal process. The HHQ specified current physical activity participation and the length in which subjects have participated in such activities. A trained phlebotomist took an 8mL blood sample from an antecubital vein using a 22G needle. After the blood sample was drawn, the sample was inverted 5 times to prevent clotting. The sample was allowed at least 30 minutes to settle, at which point the sample was centrifuged in a Unico® PowerSpin™ VX Centrifuge for 15 minutes at 3,400rpm. The serum was analyzed by LabCorp (Muncie, IN) for total cholesterol, HDL cholesterol, estimated LDL cholesterol, triglycerides, and glucose concentration.

For subjects with no change in cholesterol medication status, weight, or physical activity level in the past 12 months, blood lipid profile results from within the past 12 months were used for analysis. These values were obtained, when possible, from either the APFP database or from the subject's physician. Subjects that did not have access to blood lipid profile results had their blood drawn in the Human Performance Laboratory by the procedures outlined above.

*Body Composition*

For subjects with a change in weight, self-reported physical activity status, or participation in resistance training in the past twelve months, body composition was assessed using dual energy X-ray absorptiometry (DXA) scan (GE Lunar iDXA, enCORE™ 2010, GE Healthcare, Madison, WI). Subjects' weight history was determined by self-report and analyzing previous body composition assessments taken as part of the Ball State University Adult Physical Fitness Program annual renewal process. The HHQ specified current physical activity participation and the length in which subjects have participated in such activities. Resistance training status was assessed by self-report. A total body scan was used to distinguish fat mass from lean and bone mineral tissue. Additionally, the DXA scan assessed android and gynoid fat distributions. Subjects were instructed to lay supine on the DXA scanning bed within the designated scanning field. Subjects who exceeded the scanning field width were instructed to align the right side of their bodies with the scanning boundary, and the left side was subsequently estimated with the assumption that the subject's left side of the body was symmetrical and therefore had an identical body composition as the right side of the body.

For subjects with no change in weight, self-reported physical activity status, or participation in resistance training in the past 12 months, body composition results from within

the past 12 months were used for analysis. These values were obtained, when possible, from the APFP database. Subjects that did not have access to DXA results had their body composition assessed in the Human Performance laboratory by the procedures outlined above.

*Accelerometer Analysis*

ActiGraph GT3X+ (ActiGraph, Pensacola, FL) accelerometers were initialized in ActiLife v6.8.0 using 60Hz sampling rates and processed using a 60 second epoch. Raw data collected from the accelerometers were downloaded using ActiLife v6.8.0 and exported into a Microsoft Excel file. Data used for analyses were calculated using cutpoints previously established by Santos-Lozano for young adults.[133] Sedentary behavior was defined as <100 counts•min$^{-1}$.

**Statistical Analysis**

Statistical analyses were performed with SPSS (SPSS Inc., Chicago, Illinois; version 20.0). Prevalence of preclinical atherosclerosis, defined as having high CIMT or PWV values, was calculated as a percentage from total sample size for all subjects and independently for both male and female subjects. Groups were divided according to the presence or absence of high arterial assessment values as previously described. Chi-squared analyses were performed in order to identify potential significant differences in prevalence of high CIMT or PWV between men and women, as well as in significant differences in the distribution of high CIMT or PWV values between those above or below commonly utilized health profile thresholds. Mean and standard deviation were calculated for each objectively measured CVD risk factor and screening variable (including body composition, blood lipid profile, and physical activity level) for each group. Independent samples t-tests were performed in order to compare relevant health

parameters between each study group. Gender-specific analyses were performed in order to investigate differences in both blood lipid profile and body composition results, as these values are expected to significantly differ between genders. The correlations between CIMT and PWV as well as the respective percentile values were investigated with the Pearson product-moment correlation coefficient.

# CHAPTER IV

# RESEARCH MANUSCRIPT

Journal Format: American Journal of Preventive Medicine

# TITLE PAGE

## Title

The prevalence of preclinical atherosclerosis in a healthy adult population

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## Word Count

5,602

## Conflict of interest statement

The authors of this manuscript certify that there is no conflict of interest with any financial organization regarding the material discussed in this research manuscript.

## Financial disclosure

No financial disclosures were reported by the authors of this paper.

## ABSTRACT

**Title:** The prevalence of preclinical atherosclerosis in a healthy adult population

**Background:** Cardiovascular disease (CVD) is a progressive disease that presents signs, such as abnormal thickening or stiffening of arteries, early in its preclinical stage, and screening tools such as carotid intima media thickness (CIMT) measurement and pulse wave velocity (PWV) assessment have the potential to identify individuals prior to the clinical manifestation of CVD.

**Purpose:** The purpose of this study was to determine the prevalence of preclinical atherosclerosis, as indicated by high CIMT and PWV values, in an adult population aged 40-70 years and free of diagnosed CVD using these screening tools. Secondarily, this study aimed to compare established CVD risk factors and other health parameters between those with elevated or normal arterial health values.

**Methods:** Sixty subjects made 2 visits to the Ball State Human Performance Laboratory. The first visit included basic anthropometric measurements as well as assessment of CIMT via ultrasonography and carotid-femoral PWV via applanation tonometry. After a one week objective physical activity assessment, subjects returned to the laboratory for assessment of blood lipids and body composition. Prevalence of preclinical atherosclerosis was calculated from the total sample as well as within both genders, and an independent samples t-test was conducted in order to identify significant differences in health characteristics between those in the normal and high groups.

**Results:** Abnormal CIMT or PWV values were present in 43% of study subjects; 30% and 18% of the test sample met the criteria for elevated CIMT and PWV, respectively. Significant

differences existed between normal and high CIMT and PWV study groups for physical activity, body composition, and blood lipid profile variables. Comparisons within each gender revealed differences in health profile elements.

**Conclusions:** Both the CIMT and PWV measurement techniques may be valuable options for community CVD screenings, as certain health profile abnormalities may impact each marker of arterial health differently. Additional research is needed in order to determine the cost-effectiveness of these screening tools in a preventive health setting.

**Key Words:** preclinical atherosclerosis, prevalence, carotid intima media thickness, pulse wave velocity, cardiovascular disease

# TEXT

## Introduction

Cardiovascular disease (CVD) remains America's leading cause of death, and the mortality statistics are staggering. According to 2008 national data, the American Heart Association reported 811,940 deaths attributable to this disease.[2] The vast majority of deaths attributable to CVD result from either myocardial infarction (MI) or hemorrhagic stroke. Nationally reported statistics such as these are likely underestimations of the actual prevalence of these clinical occurrences, as it is probable that hundreds of thousands of individuals experience these events without seeking medical assistance.[1] Risk of CVD development is of particular concern for young adults, as the Centers for Disease Control and Prevention report that diseases of the heart and blood vessels serve as the leading cause of death for individuals aged 25-64 years.[3] Total number of deaths due to CVD in this cohort are likely to increase as the U.S. Census Bureau estimates that the number of adults in this age range will have increased by 3.38% in America alone from 2010 to the year 2015.[4]

In order to decrease the reliance on acute revascularization procedures and ease the unsustainable economic burden placed on the healthcare system as a result of CVD, allied health professionals must focus additional resources towards primary prevention and detection of individuals most likely to be at risk for the development of CVD. Traditional screening methods that may be employed in an asymptomatic population such as a graded exercise test or coronary calcium scan have inherent limitations including cost and potential radiation exposure and therefore limit the feasibility of their routine usage in the general public. These tests, therefore, may not be the best options in trying to identify those who may be showing signs of preclinical

atherosclerosis, or the buildup of fatty deposits inside of arteries that precedes CVD's manifestation during a clinical event.[21,22]

A more thorough understanding of the health profile associated with early CVD development is required in order for those at the highest risk for this chronic disease to be consistently identified. Signs of arterial dysfunction such as abnormal thickening and stiffening of arteries have been recognized as valid markers of preclinical atherosclerosis.[23-26] The CVD-related risk of each sign of preclinical atherosclerosis, as measured with carotid intima media thickness (CIMT) and pulse wave velocity (PWV), have been delineated through well-designed studies.[29,33] The efficacy of these two potential community screening modalities relies on both the prevalence of abnormal values in a seemingly healthy adult population as well as an in-depth understanding of a risk factor profile that may predispose an individual to display this arterial phenotype. Therefore, the purpose of this investigation was to determine the prevalence of preclinical atherosclerosis, as determined by the presence of high CIMT or PWV values, in a healthy adult population aged 40-70 years. The secondary purpose was to compare established CVD risk factors and other health parameters between those found to have high arterial assessment scores and those determined to be within normal limits.

## Methods

### *Subjects*

The study population consisted of 60 subjects (30 male) from Muncie, Indiana and its surrounding communities recruited via word of mouth as well as recruitment flyers and emails sent through on-campus communication systems. Subjects provided a written informed consent prior to involvement in the study. Inclusion criteria consisted of adults aged 40-70 years, and

exclusion criteria included any history of documented CVD (including prior MI, stroke, peripheral artery disease, heart failure, or revascularization procedure). The original sample size consisted of sixty-three subjects; however, 3 subjects were excluded from all analyses due to incomplete physical activity data. Among the sixty subjects, thirty-one were current BSU Adult Physical Fitness Program members.

*Study Overview*

The study protocol was approved by the BSU Institutional Review Board. All assessments were completed at the BSU Human Performance Laboratory (HPL). Participation in the study involved two separate visits to the HPL, spaced approximately one week apart. Visit one included anthropometric measurements, CIMT and PWV assessments, and the initialization of an accelerometer. Visit two included a blood lipid profile and body composition assessment.

*Visit One*

Subjects completed a health history questionnaire involving information on medication status, family and personal history, symptoms, recent hospitalizations, as well as lifestyle and physical activity habits.

*Anthropometric Measurements*. Weight was measured with subjects in minimal athletic attire using a Health-O-Meter Model 349 klx digital scale (Bridgeview, IL) to the nearest 0.1 pound after subjects had removed shoes, heavy items from their pockets, and any additional layers of heavy clothing. Height was measured using a wall-mounted SECA stadiometer (Germany) to the nearest 0.25 inch after subjects had fully exhaled. Body mass index (BMI) was calculated as $kg/m^2$. Waist circumference was measured at the narrowest portion of the torso

above the umbilicus and below the xiphoid process. Hip circumference was measured at the widest part of the buttocks. Circumference measurements were taken in duplicate by a single technician using a tape measure with a tension gauge, and the average of these two measurements were used in analyses provided measurements were within 2cm. A measurement >2cm apart from a previous circumference measurement resulted in a third measurement, with the mean of the 2 values within 2cm being used for analysis.

*Carotid Intima Media Thickness*. CIMT was measured in all subjects by a single trained sonographer. CIMT was defined as the mean distance from the media-adventitia interface to the lumen-intima interface. Two-dimensional B-mode ultrasonographic images were obtained from the subject's left common carotid artery (LCCA) using the General Electric LOGIQe InSite ExC system (Milwaukee, WI) 12 L linear-array transducer. The subject's head was turned at 45 degrees away from the technician after subjects assumed the supine position. A 5-10 second cinematic loop was taken at a position adjacent but inferior to the carotid bulb, and a still image was captured then saved at the point of end-diastole, identified as the period of smallest luminal diameter. A total of 7 images of the LCCA were obtained and used for measurement of CIMT. Measurements of the LCCA CIMT were taken 1cm from the origin of the carotid bulb on the posterior wall. The highest and lowest values were excluded from analyses, and the mean of the 5 remaining measurements were used in statistical analyses. Images were saved as a "JPEG image (*jpg)" file, and data were classified according to percentiles for age-, gender-, and race-matched peers originally established by Howard et al. in 1993.[27] Subjects aged 40-44 years were classified according to CIMT percentiles for adults aged 45 years and those aged 66-70 years were classified according to CIMT percentiles for adults aged 65 years. This study defined

elevated arterial thickness as having a mean CIMT value ≥75$^{th}$ percentile for age-, gender-, and race-matched peers.

*Pulse Wave Velocity*. PWV was measured by trained gender-matched technicians, with two female technicians obtaining measurements for all female subjects and one male technician obtaining measurements for all male subjects. Measurements used an AtCor Medical (Itasca, IL) SphygmoCor PWV unit and were taken on the subject's right side using the carotid and femoral artery locations. With the subject in the supine position, the distances between the suprasternal notch and both the carotid artery palpation (proximal) and femoral artery palpation (distal) sites were recorded. Supine resting blood pressure was also recorded. Electrocardiogram electrodes were placed on the subject's right arm, left arm, and left leg. Data collection consisted of placing the tonometer on the previously located arterial palpation sites for a minimum of 10 seconds of consistent wave form analysis. A series of 2 measurements were taken and mean values were used in statistical analyses. Data were classified according to previously established age- and blood pressure category-matched peers, created by Boutouyrie et al. in 2010.[28] This study defined elevated arterial stiffness as having a mean PWV value ≥75$^{th}$ percentile for age- and blood pressure category-matched peers. This value was calculated by multiplying the difference of the 90$^{th}$ percentile and median values, multiplying the difference by 0.625, and subsequently adding this product to the median.

*Physical Activity (PA) Assessment*. Subjects were issued a GT3X+ accelerometer (ActiGraph, Fort Walton Beach, FL) for a 7 day wear period. Subjects were instructed to wear the accelerometer on the waistband of the right hip during all waking hours, except for during water-based activities. A log sheet was given to each subject in order to record periods of

exercise, atypical physical activities, or times during which the accelerometer was not worn. Vigorous physical activities (≥6 METs) included on the subjects' log sheet were included in objective assessment of vigorous physical activity. The epoch was set at 60 seconds. Validation criteria included ≥600 minutes per day and ≥4 days per week. In the event that subjects wore the accelerometer for more than 7 valid days, the first 7 days of valid wear time were used in all analyses.

*Visit Two*

*Blood Lipid Profile*. Blood lipid profile variables for individuals who have had a blood draw performed within the past 12 months and have not had a change in cholesterol medication status, weight, or self-reported change in physical activity level within this time frame were obtained from the previous blood tests (n=46). For individuals that have had a change in one or more of these factors or were unable to obtain recent blood lipid profile results, subjects had blood drawn from an antecubital vein by a trained phlebotomist after a 10-12 hour fast (n=14). Blood samples were inverted 5 times and allowed 30 minutes to settle, and were subsequently centrifuged in a Unico® PowerSpin™ VX Centrifuge for 15 minutes at 3,400rpm. Samples were analyzed by LabCorp in Muncie, Indiana.

*Body Composition*. Body composition variables for individuals (n=9) who have had a dual energy x-ray absorptiometry (DXA) scan within the past 12 months and have not had a significant change in weight (±5 lbs.), self-reported physical activity status, or participation in resistance training within this time frame were obtained from the BSU APFP database. For individuals that have had a change in one or more of these factors, subjects (n=51) underwent a total body DXA scan (GE Lunar iDXA, enCORE™ 2010, GE Healthcare, Madison, WI).

Subjects were instructed to lay supine on the scanning bed within the scanning field after having removed all metallic objects from their person. Subjects (n=4) who exceeded the scanning field were instructed to align themselves with the right side of the scanning boundary, allowing the DXA to estimate the composition of the subjects' left sides.

*Accelerometer Analysis*. ActiGraph GT3X+ (ActiGraph, Pensacola, FL) accelerometers were initialized in ActiLife v6.8.0 using a sampling rate of 60Hz and processed using a 60 second epoch. Data were analyzed using triaxial cutpoints previously established for young adults (aged 40-55 years) by Santos-Lozano in 2013.[133]

*Statistical Analysis.* Total prevalence was calculated as the percentage of individuals with elevated CIMT and PWV values divided by the total sample size. Independent samples t-tests were performed between the elevated CIMT and PWV groups and those with normal values for anthropometric, blood lipid profile, body composition, and physical activity variables. Further analyses investigated unique differences within each gender between those with elevated CIMT and PWV values and those within normal limits. Correlational relationships between CIMT and PWV in addition to these markers of arterial health and established risk factors were investigated using Pearson product-moment correlation coefficients. Significance for all analyses was set at $p<0.05$.

## Results

### *Prevalence of Elevated CIMT and PWV*

Descriptive data of all study subjects as well as results of gender-specific comparisons are displayed in Table 1. In this study sample, prevalence of abnormally high arterial assessment

values was 43% (n=26). Eighteen study subjects (30% of the total sample) displayed high CIMT values and eleven subjects (18% of the total sample) showed elevated PWV results. Both CIMT and PWV measurements were determined to be high in 3 subjects, two of whom were male. In men, the prevalence of high CIMT and PWV values was 17% and 33% (n=5 and n=10), respectively. Female subjects, however, had a higher prevalence of elevated CIMT values than high PWV values, which were 43% and 3% (n=13 and n=1), respectively. As displayed in Table 2a and 2b, chi squared analyses revealed that the distributions of high CIMT and PWV values were significantly greater in female and male subjects, respectively.

*The Relationship Between CIMT and PWV*

The correlations between CIMT and PWV were investigated for all subjects as well as specifically within males and females, and all analyses failed to reach statistical significance ($r^2$ values of 0.0287, 0.0061, and 0.0776 for all subjects, males, and females, respectively). Correlations were even weaker when explored by CIMT and PWV percentiles in all subjects as well as within each gender subset, once again failing to reach statistical significance ($r^2$ values of 0.0009, 0.0069, and 0.0425 for all subjects, males, and females, respectively).

*Comparisons of CVD Risk Factors and other Health Characteristics*

Comparisons between those with normal and high CIMT and PWV among the entire sample are presented in Table 3. Gender-specific comparisons of health characteristics found to be different among male and female subjects for those with both normal and high CIMT and PWV values are presented in Tables 4 and 5, respectively. As shown in Table 6a and 6b, analyses suggested that obese individuals have a higher prevalence of elevated CIMT but not PWV. As shown in Table 7a and 7b, chi squared analyses revealed that subjects with abdominal

obesity, defined as a waist circumference >102cm for males and >88cm for females, had a significantly higher prevalence of high CIMT values but not PWV values. As shown in Table 8a and 8b, additional analysis of body composition and fat distribution revealed that those with an A/G Ratio ≥1.0 were more likely to display abnormally high PWV values, but not CIMT values. Table 9a and 9b showed HDL's role in these arterial health assessments was investigated with chi squared analyses, which revealed that HDL may moderate PWV values but not CIMT. Correlations between both CIMT and PWV and subject health characteristics are shown in Appendix A.

## Discussion

### *The Prevalence of Preclinical Atherosclerosis*

To the author's knowledge, this is the first prevalence-based study utilizing both CIMT and PWV values to detect those at risk for the development of preclinical atherosclerosis. While normative data is available for these assessment tools and it can be assumed that 25% of the population is above the 75$^{th}$ percentile threshold chosen as "high" for the purposes of this study, no information is available on the prevalence of at-risk, asymptomatic adults as determined by graded exercise testing or coronary calcium scan. The current study found that 43% of men and women aged 40-70 years display abnormally high arterial assessment values, and perhaps more interestingly that men and women are more likely to exhibit high PWV and CIMT results, respectively. This significant difference in arterial dysfunction phenotypes provides important evidence for primary prevention or community screening professionals, as it appears that gender should play a strong role in helping inform decisions about detection of early stage atherosclerosis.

*The Association between Arterial Thickness and Stiffness*

In the current investigation, CIMT and PWV measurements were not significantly correlated with one another. This relationship was further diminished when investigating the correlation between the age-, gender-, and race-matched CIMT percentile and the age- and blood pressure category-matched PWV percentile. These findings support previous research that shows an inconclusive association between these two arterial health parameters. Riley et al. determined that diameter change, a marker of arterial stiffness, remained relatively unchanged in adults aged 45-64 years below the gender-matched $90^{th}$ percentile for CIMT, which was determined to be generally 0.8mm in their cohort. Above this threshold, it was shown that elasticity decreased in a linear relationship with increasing arterial thickness.[106] The current findings support Riley's previous research and suggest that there is no correlation between these assessments in adults aged 40-70 years with CIMT values that reach above the 0.8mm mark set by Riley and colleagues. Further evidence for the lack of association between CIMT and PWV was provided by Zureik et al., who established a positive linear relationship between PWV and carotid plaques and refuted any relationship relating to arterial stiffness and CIMT.[107] Using carotid-femoral PWV in a population of middle-aged adults similar to those utilized in the current study, Zureik's non-significant findings ($r^2$=0.15, p>0.05) match those of the current investigation and support that CIMT and PWV share no direct association. Juo and colleagues reported a significant correlation between these two assessments in a population of normal weight Caribbean Hispanic subjects (mean age 47 years), which to date provides the strongest evidence for such an association.[108] The current investigation's sample consisted of 97% Caucasian subjects, allowing for the possibility of race playing a role in adults' development of abnormal arterial characteristics. However, more extensive research is required before this

potential relationship can be further suggested as the current literature fails to adequately explore these assessments' relationship in minority populations.

*Physical Activity*

It has been demonstrated that both subjectively and objectively quantified time spent in physical activity can influence both CIMT and PWV assessment results in adult populations comparable in age to the sample used in the present analysis. As subjective questionnaires have repeatedly been shown to over-estimate time spent in different physical intensity categories,[134] in particularly moderate physical activity intensity and walking-based activities,[135] objective physical activity assessment may better serve to elucidate differences in activity levels between those with normal or high arterial assessment results. The current investigation found significant differences in objectively quantified time spent in vigorous physical activity between males with normal and high CIMT, which may suggest a protective effect of vigorous intensity physical activity. Interestingly, these findings were not shared by the total study group or females when analyzed separately. These results must be interpreted with caution as the small gender subcohorts may not be a true representation of the entire population. These results for males support previous research by Kozàkovà and colleagues, who showed that periods of vigorous intensity exercise as quantified by uniaxial accelerometry can help minimize the progression of CIMT in a population of men and women aged 44 years.[112] Subjects in the present study accumulated an average of 2 minutes per day of vigorous intensity physical activity, which is notably less than the 10 minutes of vigorous activity per day achieved by subjects in Kozàkovà's study. However, additional adjustment of vigorous activity according to self-report exercise failed to reveal significant differences in activity between study groups. These objective

vigorous physical activity results provide preliminary evidence that protective effects of vigorous intensity physical activity on arterial health may be conferred with less vigorous activity accumulation than suggested previously, although additional research is required in order to establish a true dose-response relationship. Evidence for the protective effects of habitual vigorous intensity physical activity for arterial health has also been demonstrated by Van de Laar and colleagues in a population of healthy adults under the age of 36 years.[113] This 2010 investigation included detailed face to face interviews in order to assess the effects on arterial stiffness of regular participation in vigorous physical activity, and determined that vigorous physical activity provided protection against stiffening, as assessed via ultrasonography, of the arterial system. These findings were supported during the Northern Ireland Young Hearts Project, which demonstrated a similar protective effect of vigorous intensity physical activity participation for arterial stiffness in young adults aged 22 years as assessed by carotid-femoral PWV.[115] The current study found no difference in vigorous intensity exercise participation between those with normal and high PWV values, which may suggest that the relationship previously shown in young adults may not be as strong in older adults. Van de Laar's investigation utilized ultrasonography and measurement of distensibility for assessment of arterial stiffness rather than the applanation tonometry method used to assess aortic stiffness in the current study, potentially masking similar trends and making direct comparisons difficult. It must be noted that the physical activity intensity accelerometry cutpoints that were chosen for the current analyses were originally established for adults aged 40-55 years,[133] which may result in an underestimation of time spent in MVPA for study subjects above this age cutoff.

Objective physical activity monitors are also capable of assessing sedentary behavior, which in this study was a significant 27% higher in women with high CIMT compared to their

normal CIMT counterparts. Although this behavior is expected to be equal between both genders in adults aged 30-59 years,[136] this difference was not apparent in analyses involving the total sample or those specific to male subjects. The relationship between objectively quantified sedentary behavior and either CIMT or PWV has yet to be elucidated and studies targeting this association are currently lacking. The current investigation utilized time spent in sedentary bouts as an indicator of daily participation in sedentary behavior, which in no instance was significantly different for groups based on PWV category. As no guidelines are currently established for sedentary behavior and its importance as a potential independent risk factor for CVD is still being recognized by the medical community,[137,138] additional research into its moderation of arterial health is warranted.

*Body Composition*

BMI, or the direct mathematical relationship between an individual's weight and height, may be the most commonly assessed body composition metric in a clinical setting. A BMI at or above 30kg/m$^2$ is an established risk factor for CVD,[36] and the link between obesity and other health concerns is widely recognized. The current investigation provides additional evidence for this relationship, and its focus on detecting markers of both arterial thickness and stiffness abnormalities offers unique insight into BMI's modification of arterial health. These data suggest that obese individuals are significantly more likely to have high CIMT values, yet not PWV (Tables 6a and 6b). Longitudinal analyses investigating the link between BMI and these markers of arterial health are needed; however these data provide evidence that it is likely that having an elevated BMI may predispose an individual to abnormal thickening rather than stiffening of the arterial walls.

Body composition can easily be assessed in terms of body fat distribution, and waist circumference has been repeatedly demonstrated to differ between individuals with normal and abnormal arterial health characteristics. Waist circumference can be dichotomized into the presence or absence of abdominal obesity, defined as having a waist circumference >88cm for adult women and >102cm for adult men. Hacihamdioglu and colleagues demonstrated that those with abdominal obesity are at an increased risk for high CIMT values.[117] The current findings support this previous research and suggest this relationship is present in adult females, as those with a high CIMT showed a significantly higher waist circumference than peers with normal CIMT values. Similar findings were not present in male subjects, perhaps due in part to the small subset of males determined to have high CIMT results. Analysis of the total sample also revealed that those with abdominal obesity were significantly more likely to have high CIMT levels, providing evidence that excess adipose tissue around the waist plays a role in increasing one's likelihood of exhibiting high CIMT values in adults of this age range. Body fat distribution can also be analyzed by relating an individual's waist and hip circumferences, allowing for relative comparisons to be drawn between individuals of different sizes. Waist/hip ratio was once again significantly higher in the high CIMT women when compared to their normal CIMT peers in the current study, which supports findings of the British Regional Heart Study that suggested a direct correlation between waist/hip ratio and mean CIMT measured at the bifurcation in adults aged 56-75 years.[119] Mean bifurcation CIMT values in the British regional Heart Study analysis were 1.36mm and 1.54mm for women and men, respectively, which as expected were notably higher than the 0.68mm and 0.71mm values when taken at the common carotid artery seen in women and men in this study, respectively.[29] The current data support the previous findings and suggest that waist to hip ratio may help modify arterial

thickness measurements which can be recognized at multiple anatomical landmarks. These findings reinforce the importance of routine assessment of waist to hip ratio in a community screening setting.

A complimentary assessment to waist and hip circumference measurements is the DXA scan, which is able to determine body fat distributions and identify those with elevated body fat percentages who may not show increased abdominal girth. The current investigation utilized the DXA scan in order to compare regional fat distributions as well as total lean and fat mass between those with normal and high CIMT and PWV. An intervention study by Dengo et al. illustrated the beneficial effects of a decrease in total fat mass, as measured via DXA scan, on PWV in obese male and female adults aged 61-65 years, demonstrating the link between adipose tissue and arterial health.[121] The current study identified no significant differences in total fat mass between normal and high PWV groups in all subjects or independent gender analyses. Interestingly, those with high CIMT had significantly greater total fat mass than their normal CIMT counterparts both in the total sample and among female subjects separately. This indicates that Dengo et al.'s claims of the importance of reduced fat mass for arterial function may apply to arterial thickness as well as stiffness. To the author's knowledge, the current study is the first to compare total fat mass between CIMT independently for men and women, offering insight into the potential for gender differences in the body's modification of carotid thickening based on body fat.

The link between total body fat percentage and CIMT has also been investigated in order to better understand body composition's effect on arterial health markers. Kozàkovà and colleagues utilized bioelectrical impedance analysis in order to study this relationship in a

population of normal weight adults aged 44 ± 8 years, and ultimately suggested that this health characteristic was a weaker predictor of arterial thickness than other body composition measurements.[122] The current investigation's use of the DXA scan showed older adults with high CIMT showed a mean body fat percentage 6.5% higher than their normal CIMT peers. The current study sample was older than the former investigation and had a mean BMI in the overweight classification, making direct comparisons with Kozàkovà's findings difficult. However, these data support that total body fat percentage indeed modifies the thickness of the carotid wall and that analysis via DXA scan offers promising research opportunities. Android/Gynoid ratio as measured via DXA scan offers similar information to waist/hip circumference, however this precise assessment's relationships with both CIMT and PWV have yet to be fully explored. The current research identified a significantly higher android/gynoid ratio in high PWV subjects compared to their normal PWV peers. This finding agrees with the significantly increased waist/hip ratio seen in the same sub-cohort, supporting the importance of routinely assessing regional fat distributions. Interestingly, android fat percentage was significantly higher in the high CIMT group when compared to the normal CIMT adults, yet this relationship was absent between PWV categories. As the DXA assessment tool is rarely utilized in arterial health investigations, long-term implications of these differences have yet to be identified and further research into these relationships are warranted.

*Blood Lipid Profile*

Groups for all analyses had an even distribution of individuals being treated with lipid-lowering medications ($p>0.05$), therefore no adjustments were made for this potentially confounding variable. Specific elements of a standard blood lipid profile have been shown to

significantly impact arterial health parameters including CIMT and PWV. The presence of diabetes mellitus, specifically, has been demonstrated to influence these markers of arterial function. Research by Alizadeh shows that adults aged 40-60 years with clinically diagnosed diabetes mellitus (i.e. having a fasting blood glucose level of ≥126mg/dL) have significantly increased CIMT values compared to their non-diabetic peers (0.98 ± 0.4mm vs. 0.63 ± 0.23mm, $p<0.05$).[128] The current results show a non-significant increase in fasting blood glucose between adults with high PWV and their normal PWV peers; however this difference is non-existent when subjects are grouped by CIMT category. It must be noted that only 3 subjects reported a history of diagnosed diabetes mellitus in the current study, making comparisons among groups based on this clinical condition difficult. These findings do not support those Alizadeh and colleagues; however the small sample size on which the current analyses are based may be masking more significant differences in fasting blood glucose between these groups.

Sub-fractions of a basic blood lipid profile, such as HDL levels, have also been shown to significantly correlate with CIMT, as was demonstrated by Shah and colleagues in a population of adolescents and young adults.[130] In Shah et al.'s study, HDL was demonstrated to be the only lipid to be independently and significantly associated with CIMT. The current investigation supports these results and extends them into an older, non-diabetic adult population, suggesting that HDL's protective effects are exerted further across the lifespan and may impact both CIMT, as previously suggested, as well as PWV. When data from all subjects in the current study were analyzed, HDL levels were significantly higher in the normal PWV group compared to those with high PWV. A chi-squared analysis supported that individuals with an HDL level above 60mg/dL, which has been established as a cardio-protective health characteristic, are significantly more likely to exhibit healthy arterial stiffness properties (Table 9b). HDL was also

significantly higher in a female-specific analysis of CIMT, with women showing a normal CIMT having an HDL level on average 13mg/dL higher than their high CIMT peers. Neither LDL cholesterol nor triglycerides were significantly different between any normal or high CIMT or PWV groups, suggesting HDL may be the strongest moderator of arterial health characteristics among the blood lipid profile.

**Strengths**

A strength of this study is the incorporation of both CIMT and PWV assessment modalities, as potential differences in risk factor profiles between those with normal and high results for each measurement may exist due to their non-existent correlation. This study therefore had the ability to analyze both aspects of arterial health and compare previously established health characteristic differences between those with normal and elevated values in regards to both arterial thickness and stiffness. Additional strengths included the use of tri-axial accelerometry to more accurately and objectively quantify time spent in different physical activity categories as well as sedentary behavior, an area of research which has yet to be explored in relation to both CIMT and PWV. This advancement may offer a more complete and accurate physical activity profile compared to uniaxial accelerometers and questionnaires which have commonly been employed in this area of research. Last, the utilization of the DXA scan in order to more fully understand regional body fat distributions and their potential effects on both CIMT and PWV values offers improvements over skinfold calipers or bioelectrical impedance analysis, perhaps more accurately demonstrating body composition's role in the remodeling and function of arteries.

**Limitations**

The limitations of this study include three primary areas. First, the recruitment of a primarily homogeneous study sample limits the generalizability to other populations. Only two of the final sample subjects were not Caucasian, and the study parameters dictated that all subjects be between the ages of 40 and 70 years. This decision was made based both on the currently available range of CIMT percentile rankings, which inhibited comparisons among younger or older study subjects, and the potential benefit of improved early CVD detection for this age group. This may limit the findings' ability to extend to younger populations, who may be at risk for the development of preclinical atherosclerosis as well. Second, the logistics of the study required that some data for analyses be acquired from a retrospective database, limiting the true cross-sectional nature of the study. Although criteria were established in order to standardize data collection, potential relationships may not be as clearly demonstrated due to this. Additionally, medical management of comorbidities including hypertension, dyslipidemia, and diabetes mellitus was monitored via the health history questionnaire in order to determine whether or not significant distributions of these medications existed between study groups. Third, the total sample size and gender subsets had a relatively small number of subjects, once again limiting the overall strength of statistical analyses and the potential to further detect differences in health among the different study groups.

**Conclusion**

Prevalence of preclinical atherosclerosis as detected by CIMT and PWV measurement techniques may be as high as 43% in an adult population aged 40-70 years and free of documented cardiovascular disease. Male subjects may be more susceptible to exhibiting abnormal arterial stiffness values, while women may be more likely to show abnormally thick

arterial walls. This is a novel finding in the study of arterial health parameters. When evaluating an individual's likelihood of showing either abnormal CIMT or PWV, asymptomatic adults' health characteristics should help guide screening decisions and possible lifestyle modifications by identifying those that may be in need of more aggressive therapy options. The true interconnectedness of each of these health parameters makes identifying isolated differences in CVD risk factors between study groups difficult, however elements of body composition were repeatedly and significantly different between CIMT study groups. Health professionals should be aware of these results and track patients' body composition trends in more depth than simple BMI, as abdominal obesity was shown to significantly increase the likelihood of adults displaying abnormal CIMT results. Additionally, HDL's relationship with PWV values illustrates its importance for overall arterial health. Clinical professionals should consider focusing on management of patients' HDL cholesterol levels, as values above the protective threshold of 60mg/dL may result in lower prevalence of elevated PWV in this population. Lastly, physical activity may play a role in arterial health modification, although additional research is needed in this area in order to make stronger recommendations for this element of patient care plans.

## ACKNOWLEDGEMENTS

The author thanks the Ball State University's ASPiRE Graduate Research Award Program for helping fund this study.

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**Table 1. Subject Characteristics**

| | Total (n=60) | Males (n=30) | Females (n=30) |
|---|---|---|---|
| Age (y) | 59 ± 8 | 59 ± 8 | 59 ± 8 |
| Supine SBP (mmHg) | 122 ± 15 | 127 ± 15 | 118 ± 14* |
| Supine DBP (mmHg) | 73 ± 8 | 75 ± 8 | 72 ± 8 |
| BMI ($kg/m^2$) | 27.2 ± 4.7 | 27.7 ± 3.8 | 26.7 ± 5.4 |
| Waist Circumference (cm) | 88.9 ± 12.8 | 94.6 ± 10.0 | 83.3 ± 12.9* |
| Hip Circumference (cm) | 104.1 ± 10.6 | 103.4 ± 6.4 | 104.7 ± 13.6 |
| Waist/Hip Ratio | 0.85 ± 0.08 | 0.91 ± 0.06 | 0.79 ± 0.05* |
| Total Cholesterol (mg/dL) | 191 ± 32 | 186 ± 27 | 195 ± 36 |
| LDL (mg/dL) | 111 ± 27 | 112 ± 25 | 111 ± 30 |
| HDL (mg/dL) | 58 ± 15 | 54 ± 15 | 62 ± 15 |
| Triglycerides (mg/dL) | 112 ± 56 | 103 ± 30 | 122 ± 73 |
| Glucose (mg/dL) | 96 ± 11 | 100 ± 10 | 92 ± 11* |
| Total Fat Mass (kg) | 26.1 ± 10.5 | 24.9 ± 9.1 | 27.2 ± 11.8 |
| Total Lean Mass (kg) | 50.8 ± 10.8 | 59.7 ± 5.4 | 41.8 ± 6.5* |
| Total Body Fat (%) | 33.3 ± 8.9 | 28.7 ± 6.9 | 37.9 ± 8.5* |
| Android Fat (%) | 37.6 ± 11.3 | 35.6 ± 10.6 | 39.6 ± 11.9 |
| Gynoid Fat (%) | 34.6 ± 9.8 | 28.1 ± 5.9 | 41.1 ± 8.6* |
| A/G Ratio | 1.1 ± 0.3 | 1.3 ± 0.2 | 1.0 ± 0.2* |
| Steps/Day | 7205 ± 2618 | 7223 ± 2415 | 7188 ± 2849 |
| MVPA (minutes/day) | 31 ± 19 | 31 ± 16 | 30 ± 22 |
| Vigorous Activity (minutes/day) | 2 ± 4 | 2 ± 3 | 3 ± 5 |
| Log-Adjusted Vigorous Activity (minutes/day) | 4.7 ± 6.1 | 6 ± 7 | 4 ± 5 |
| Time in Sedentary Bouts (minutes/day) | 388 ± 94 | 396 ± 92 | 380 ± 98 |

Data are presented as means ± SD. SBP= systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; A/G=android body fat percent/gynoid body fat percent; MVPA=moderate to vigorous physical activity. *$p<0.05$ between Male and Female subjects.

**Table 2a. Cross Tabulations for CIMT and Gender**

| | Males | Females | Totals |
|---|---|---|---|
| CIMT < 75$^{th}$ Percentile | 25 | 17 | 42 |
| CIMT ≥ 75$^{th}$ Percentile | 5 | 13 | 18 |
| Totals | 30 | 30 | 60 |

$p<0.05$

**Table 2b. Cross Tabulations for PWV and Gender**

| | Males | Females | Totals |
|---|---|---|---|
| PWV < 75$^{th}$ Percentile | 20 | 29 | 49 |
| PWV ≥ 75$^{th}$ Percentile | 10 | 1 | 11 |
| Totals | 30 | 30 | 60 |

$p<0.05$

**Table 3. Comparison of Health Characteristics in all Subjects Between Normal and High CIMT and PWV**

| | CIMT < 75th Percentile (n=42) | CIMT ≥ 75th Percentile (n=18) | PWV < 75th Percentile (n=49) | PWV ≥ 75th Percentile (n=11) |
|---|---|---|---|---|
| Age (y) | 59 ± 8 | 60 ± 7 | 60 ± 7 | 54 ± 9# |
| Supine SBP (mmHg) | 122 ± 14 | 124 ± 17 | 122 ± 15 | 123 ± 16 |
| Supine DBP (mmHg) | 72 ± 8 | 76 ± 7 | 73 ± 8 | 74 ± 6 |
| BMI (kg/m$^2$) | 26 ± 4 | 29 ± 5 | 27 ± 5 | 28 ± 3 |
| Waist Circumference (cm) | 87.3 ± 12.9 | 92.6 ± 12.1 | 87.9 ± 13.3 | 93.6 ± 9.3 |
| Hip Circumference (cm) | 102.3 ± 8.9 | 108.1 ± 13.1* | 104.2 ± 11.4 | 103.3 ± 5.9 |
| Waist/Hip Ratio | 0.85 ± 0.09 | 0.86 ± 0.07 | 0.84 ± 0.08 | 0.91 ± 0.06# |
| Total Cholesterol (mg/dL) | 188 ± 41 | 188 ± 39 | 189 ± 43 | 184 ± 31 |
| LDL (mg/dL) | 112 ± 25 | 111 ± 33 | 111 ± 28 | 113 ± 26 |
| HDL (mg/dL) | 60 ± 16 | 56 ± 15 | 61 ± 15 | 48 ± 11# |
| Triglycerides (mg/dL) | 103 ± 43 | 128 ± 80 | 110 ± 63 | 113 ± 23 |
| Glucose (mg/dL) | 96 ± 11 | 96 ± 11 | 95 ± 11 | 99 ± 12 |
| Total Fat Mass (kg) | 24.0 ± 9.3 | 30.8 ± 11.9* | 26.1 ± 11.2 | 25.9 ± 7.3 |
| Total Lean Mass (kg) | 51.5 ± 11.8 | 49.1 ± 7.9 | 49.0 ± 10.5 | 59.0 ± 8.3# |
| Total Body Fat (%) | 31.5 ± 8.5 | 37.6 ± 8.7* | 34.0 ± 9.4 | 30.3 ± 6.1 |
| Android Fat (%) | 35.6 ± 10.9 | 42.3 ± 11.2* | 37.5 ± 11.9 | 38.2 ± 9.0 |
| Gynoid Fat (%) | 32.9 ± 9.7 | 38.7 ± 9.1* | 35.9 ± 10.2 | 29.2 ± 5.9# |
| A/G Ratio | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.3 ± 0.2# |
| Steps/Day | 7508 ± 2682 | 6499 ± 2386 | 7184 ± 2523 | 7297 ± 3143 |
| MVPA (minutes/day) | 32 ± 19 | 28 ± 18 | 31 ± 20 | 32 ± 14 |
| Vigorous Activity (minutes/day) | 2 ± 4 | 2 ± 4 | 2 ± 4 | 2 ± 4 |
| Log-Adjusted Vigorous Activity (minutes/day) | 4 ± 6 | 5 ± 7 | 5 ± 6 | 6 ± 6 |
| Time in Sedentary Bouts (minutes/day) | 377 ± 97 | 412 ± 86 | 388 ± 98 | 387 ±81 |

Data are presented as means ± SD. SBP= systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; CIMT=carotid intima media thickness; PWV=pulse wave velocity; A/G=android body fat percent/gynoid body fat percent; MVPA=moderate to vigorous physical activity. *$p<0.05$ between Normal and High CIMT for all subjects. #$p<0.05$ between Normal and High PWV for all subjects.

**Table 4. Comparison of Health Characteristics Between Normal and High CIMT**

| | Males (n=30) | | Females (n=30) | |
|---|---|---|---|---|
| | CIMT < 75$^{th}$ Percentile (n=25) | CIMT ≥ 75$^{th}$ Percentile (n=5) | CIMT < 75$^{th}$ Percentile (n=17) | CIMT ≥ 75$^{th}$ Percentile (n=13) |
| Age (y) | 58 ± 8 | 63 ± 5 | 60 ± 8 | 58 ± 7 |
| Supine SBP (mmHg) | 125 ± 14 | 135 ± 20 | 117 ± 13 | 120 ± 13 |
| Supine DBP (mmHg) | 74 ± 8 | 79 ± 8 | 70 ± 9 | 74 ± 6 |
| BMI (kg/m$^2$) | 27.6 ± 3.9 | 28.0 ± 3.5 | 24.8 ± 4.0 | 29.3 ± 6.1$^{\#}$ |
| Waist Circumference (cm) | 93.9 ± 10.1 | 98.0 ± 9.5 | 77.7 ± 10.4 | 90.6 ± 12.6$^{\#}$ |
| Hip Circumference (cm) | 103.4 ± 6.7 | 103.3 ± 4.8 | 100.7 ± 11.4 | 110.0 ± 14.9 |
| Waist/Hip Ratio | 0.91 ± 0.06 | 0.95 ± 0.06 | 0.77 ± 0.05 | 0.82 ± 0.04$^{\#}$ |
| Total Cholesterol (mg/dL) | 176 ± 44 | 204 ± 40 | 205 ± 32 | 182 ± 39 |
| LDL (mg/dL) | 109 ± 21 | 125 ± 40 | 116 ± 30 | 105 ± 29 |
| HDL (mg/dL) | 54 ± 16 | 58 ± 9 | 68 ± 12 | 55 ± 17$^{\#}$ |
| Triglycerides (mg/dL) | 104 ± 29 | 105 ± 42 | 103 ± 58 | 137 ± 91 |
| Glucose (mg/dL) | 99 ± 11 | 99 ± 8 | 91 ± 10 | 95 ± 13 |
| Total Fat Mass (kg) | 24.6 ± 9.7 | 26.8 ± 5.8 | 23.3 ± 9.0 | 32.3 ± 13.4$^{\#}$ |
| Total Lean Mass (kg) | 60.1 ± 5.6 | 58.0 ± 4.1 | 38.9 ± 5.0 | 45.7 ± 6.2$^{\#}$ |
| Total Body Fat (%) | 28.2 ± 7.2 | 31.4 ± 4.4 | 36.2 ± 8.0 | 40.0 ± 8.9 |
| Android Fat (%) | 34.6 ± 10.8 | 40.7 ± 8.4 | 37.0 ± 11.2 | 42.9 ± 12.4 |
| Gynoid Fat (%) | 27.9 ± 6.3 | 29.3 ± 3.8 | 40.3 ± 9.3 | 42.2 ± 7.9 |
| A/G Ratio | 1.2 ± 0.2 | 1.4 ± 0.3 | 0.9 ± 0.2 | 1.0 ± 0.2 |
| Steps/Day | 7311 ± 2514 | 6781 ± 2017 | 7798 ± 2966 | 6390 ± 2581 |
| MVPA (minutes/day) | 32 ± 16 | 27 ± 19 | 32 ± 25 | 28 ± 19 |
| Vigorous Activity (minutes/day) | 2 ± 3 | 0 ± 0* | 3 ± 6 | 3 ± 4 |
| Log-Adjusted Vigorous Activity (minutes/day) | 3 ± 5 | 3 ± 7 | 7 ± 8 | 5 ± 6 |
| Time in Sedentary Bouts (minutes/day) | 403 ± 90 | 362 ± 100 | 340 ± 95 | 432 ± 75$^{\#}$ |

Data are presented as means ± SD. SBP= systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; CIMT=carotid intima media thickness; A/G=android body fat percent/gynoid body fat percent; MVPA=moderate to vigorous physical activity. *$p<0.05$ between Normal and High CIMT for Males. $^{\#}p<0.05$ between Normal and High CIMT for Females.

**Table 5. Comparison of Health Characteristics Between Normal and High PWV**

| | Males (n=30) | | Females (n=30) | |
|---|---|---|---|---|
| | PWV < 75th Percentile (n=20) | PWV ≥ 75th Percentile (n=10) | PWV < 75th Percentile (n=29) | PWV ≥ 75th Percentile (n=1) |
| Age (y) | 61 ± 6 | 55 ± 9* | 59 ± 7 | 47 |
| Supine SBP (mmHg) | 129 ± 15 | 121 ± 16 | 117 ± 13 | 144 |
| Supine DBP (mmHg) | 75 ± 9 | 73 ± 6 | 71 ± 8 | 82 |
| BMI ($kg/m^2$) | 27.4 ± 4.0 | 28.2 ± 3.5 | 26.7 ± 5.5 | 27.8 |
| Waist Circumference (cm) | 94.8 ± 10.4 | 94.2 ± 9.6 | 83.1 ± 13.1 | 88.0 |
| Hip Circumference (cm) | 103.3 ± 6.7 | 103.6 ± 6.1 | 104.9 ± 13.8 | 100.5 |
| Waist/Hip Ratio | 0.92 ± 0.06 | 0.91 ± 0.06 | 0.79 ± 0.05 | 0.88 |
| Total Cholesterol (mg/dL) | 179 ± 49 | 186 ± 32 | 196 ± 37 | 167 |
| LDL (mg/dL) | 110 ± 24 | 115 ± 27 | 112 ± 30 | 95 |
| HDL (mg/dL) | 58 ± 16 | 47 ± 11 | 63 ± 15 | 56 |
| Triglycerides (mg/dL) | 97 ± 33 | 117 ± 20 | 119 ± 76 | 76 |
| Glucose (mg/dL) | 99 ± 8 | 99 ± 13 | 92 ± 11 | 99 |
| Total Fat Mass (kg) | 24.6 ± 10.0 | 25.6 ± 7.6 | 27.1 ± 12.0 | 29.4 |
| Total Lean Mass (kg) | 59.3 ± 5.1 | 60.7 ± 6.1 | 41.9 ± 6.6 | 41.0 |
| Total Body Fat (%) | 28.5 ± 7.8 | 29.2 ± 5.0 | 37.8 ± 8.6 | 41.7 |
| Android Fat (%) | 34.9 ± 11.6 | 37.1 ± 8.7 | 39.3 ± 12.0 | 48.7 |
| Gynoid Fat (%) | 28.3 ± 6.7 | 27.9 ± 4.1 | 41.1 ± 8.8 | 42.4 |
| A/G Ratio | 1.2 ± 0.2 | 1.3 ± 0.2 | 0.9 ± 0.2 | 1.2 |
| Steps/Day | 7046 ± 2013 | 7575 ± 3167 | 7280 ± 2853 | 4518 |
| MVPA (minutes/day) | 30 ± 17 | 33 ± 13 | 31 ± 22 | 15 |
| Vigorous Activity (minutes/day) | 1 ± 2 | 3 ± 4 | 3 ± 5 | 0 |
| Log-Adjusted Vigorous Activity (minutes/day) | 3 ± 4 | 5 ± 6 | 6 ± 7 | 0 |
| Time in Sedentary Bouts (minutes/day) | 402 ± 96 | 384 ± 85 | 378 ± 99 | 412 |

Data are presented as means ± SD. SBP= systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; PWV=pulse wave velocity; A/G=android body fat percent/gynoid body fat percent; MVPA=moderate to vigorous physical activity. *$p<0.05$ between Normal and High PWV for Males. #$p<0.05$ between Normal and High PWV for Females.

**Table 6a. Cross Tabulations for CIMT and BMI**

| | BMI < 30kg/m$^2$ | BMI ≥ 30kg/m$^2$ | Totals |
|---|---|---|---|
| CIMT < 75$^{th}$ Percentile | 36 | 6 | 42 |
| CIMT ≥ 75$^{th}$ Percentile | 11 | 7 | 18 |
| Totals | 47 | 13 | 60 |

$p<0.05$

**Table 6b. Cross Tabulations for PWV and BMI**

| | BMI < 30kg/m$^2$ | BMI ≥ 30kg/m$^2$ | Totals |
|---|---|---|---|
| PWV < 75$^{th}$ Percentile | 39 | 10 | 49 |
| PWV ≥ 75$^{th}$ Percentile | 8 | 3 | 11 |
| Totals | 47 | 13 | 60 |

$p>0.05$

**Table 7a. Cross Tabulations for CIMT and Abdominal Obesity**

| | No Abdominal Obesity | Abdominal Obesity | Totals |
|---|---|---|---|
| CIMT < $75^{th}$ Percentile | 31 | 11 | 42 |
| CIMT ≥ $75^{th}$ Percentile | 7 | 11 | 18 |
| Totals | 38 | 22 | 60 |

$p<0.05$

**Table 7b. Cross Tabulations for PWV and Abdominal Obesity**

| | No Abdominal Obesity | Abdominal Obesity | Totals |
|---|---|---|---|
| PWV < $75^{th}$ Percentile | 31 | 18 | 49 |
| PWV ≥ $75^{th}$ Percentile | 7 | 4 | 11 |
| Totals | 38 | 22 | 60 |

$p>0.05$

**Table 8a. Cross Tabulations for CIMT and A/G Ratio**

| | A/G Ratio < 1.0 | A/G Ratio ≥ 1.0 | Totals |
|---|---|---|---|
| CIMT < 75$^{th}$ Percentile | 15 | 27 | 42 |
| CIMT ≥ 75$^{th}$ Percentile | 6 | 12 | 18 |
| Totals | 21 | 39 | 60 |

$p>0.05$

**Table 8b. Cross Tabulations for PWV and A/G Ratio**

| | A/G Ratio < 1.0 | A/G Ratio ≥ 1.0 | Totals |
|---|---|---|---|
| PWV < 75$^{th}$ Percentile | 20 | 29 | 49 |
| PWV ≥ 75$^{th}$ Percentile | 1 | 10 | 11 |
| Totals | 21 | 39 | 60 |

$p<0.05$

**Table 9a. Cross Tabulations for CIMT and HDL**

| | HDL < 60mg/dL | HDL ≥ 60mg/dL | Totals |
|---|---|---|---|
| CIMT < 75$^{th}$ Percentile | 18 | 23 | 41 |
| CIMT ≥ 75$^{th}$ Percentile | 11 | 7 | 18 |
| Totals | 29 | 30 | 59 |

$p>0.05$

**Table 9b. Cross Tabulations for PWV and HDL**

| | HDL < 60mg/dL | HDL ≥ 60mg/dL | Totals |
|---|---|---|---|
| PWV < 75$^{th}$ Percentile | 20 | 28 | 48 |
| PWV ≥ 75$^{th}$ Percentile | 9 | 2 | 11 |
| Totals | 29 | 30 | 59 |

$p<0.05$

**APPENDIX A. Correlations Between both CIMT and PWV and Subject Health Characteristics**

| | Mean CIMT (mm) ($r^2$) | Mean PWV (m/s) ($r^2$) |
|---|---|---|
| Age (y) | .134* | .024 |
| Supine SBP (mmHg) | .001 | .230* |
| Supine DBP (mmHg) | .009 | .173* |
| BMI ($kg/m^2$) | .079* | .047 |
| Waist Circumference (cm) | .091* | .118* |
| Hip Circumference (cm) | .081* | .000 |
| Waist/Hip Ratio | .027 | .272* |
| Total Cholesterol (mg/dL) | .019 | .054 |
| LDL (mg/dL) | .016 | .004 |
| HDL (mg/dL) | .025 | .095* |
| Triglycerides (mg/dL) | .017 | .001 |
| Glucose (mg/dL) | .102* | .110* |
| Total Fat Mass (kg) | .058 | .005 |
| Total Lean Mass (kg) | .031 | .091 |
| Total Body Fat (%) | .012 | .003 |
| Android Fat (%) | .024 | .027 |
| Gynoid Fat (%) | .001 | .048 |
| A/G Ratio | .032 | .265* |
| Steps/Day | .010 | .003 |
| MVPA (minutes/day) | .003 | .001 |
| Vigorous Activity (minutes/day) | .027 | .007 |
| Log-Adjusted Vigorous Activity (minutes/day) | .006 | .022 |
| Time in Sedentary Bouts (minutes/day) | .006 | .002 |

SBP= systolic blood pressure; DBP=diastolic blood pressure; BMI=body mass index; CIMT=carotid intima media thickness; PWV=pulse wave velocity; A/G=android body fat percent/gynoid body fat percent; MVPA=moderate to vigorous physical activity. *$p<0.05$.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

Cardiovascular disease (CVD) has remained the primary killer of Americans, and the costs associated with revascularization procedures and other interventions have reached unsustainable levels. Increasing emphasis is being placed on screening for this disease in its occult phase, prior to its manifestation as a myocardial infarction or stroke during a clinical event. However, current preventive screening modalities have inherent limitations and require both equipment and costs that inhibit their large scale use in the community. Newer screening modalities include the measurement of carotid intima media thickness and pulse wave velocity as means to assess arterial thickness and stiffness, respectively. These markers have been validated as signs of preclinical atherosclerosis and may be able to detect those at an increased risk for the development of CVD on a larger scale than other screening modalities. The cost effectiveness of these screening methods can be determined if a thorough understanding of the prevalence of abnormal values and risk factors most strongly associated with them are established. The primary purpose of this study was to determine the prevalence of preclinical atherosclerosis, as determined by high CIMT or PWV values, in an adult population aged 40-70 years and free of documented CVD. Secondarily, this study aimed to compare traditional CVD risk factors and

other health characteristics between individuals found to have normal and high values for both CIMT and PWV.

Of the sixty study subjects, aged 59 ± 8 years and 50% male, eighteen were determined to have high CIMT values and eleven subjects were found to have elevated PWV results and 3 test subjects were found to have abnormally high values for both arterial assessments. High CIMT values were significantly more common in male subjects than elevated PWV values, with high CIMT and PWV results being found in 17% and 33% of male participants, respectively. Comparatively, elevated CIMT values were significantly more common among female subjects when compared to high PWV assessment results, with a prevalence of 43% and 3% for high CIMT and PWV values, respectively.

The correlation between CIMT and PWV values was found to be non-significant for all subjects, as well as within male and female study groups. This lack of a significant relationship was once again demonstrated when the correlation between CIMT and PWV percentiles was explored, revealing even weaker associations between these screening variables in all subjects as well as within gender-specific analyses of both men and women.

Results of independent samples t-tests revealed that with high CIMT had significantly higher hip circumference, total fat mass, and android fat percentage than those with CIMT within normal limits. Similar analyses between normal PWV and high PWV subjects revealed that both age and HDL cholesterol levels were significantly lower in those with high PWV results. A gender-specific independent samples t-test showed that among female subjects, participants with high CIMT had significantly higher waist circumference, waist to hip ratio, and total lean mass than their normal CIMT counterparts. These differences did not exist among male subjects.

Chi-squared analyses investigated the relationships between being above or below commonly utilized health thresholds and the prevalence of high CIMT and PWV values. These analyses suggested that having a body mass index $\geq 30 kg/m^2$ increase adults' prevalence of an elevated CIMT value, but not PWV. The relationship of body composition and markers of arterial health was further supported by chi-squared analysis of abdominal obesity and high CIMT results, which demonstrated that abdominal obesity significantly increased the likelihood of high CIMT values in this cohort. Abdominal obesity's relationship with high PWV was found to be non-significant. Having an A/G Ratio $\geq 1.0$ predisposed adults to elevated PWV prevalence; however, this relationship was not supported for CIMT. Blood lipid profile analysis suggested that having an HDL cholesterol level $\geq 60 mg/dL$ significantly reduces the likelihood of having a high PWV value, yet HDL cholesterol's effect on CIMT was not significant. This relationship was supported by an independent samples t-test between normal and high PWV groups ($p<0.05$).

The results from this study conclude that high CIMT and PWV values, which can serve as surrogate markers of preclinical atherosclerosis, may be present in as much as 43% of adults free of documented CVD. Additionally, this research provides preliminary evidence that men and women manifest signs of arterial dysfunction differently; therefore, clinicians and community health specialists can more effectively screen for these conditions in the general public based on these results. Specifically, it appears as though males at risk for the development of CVD should preliminarily be screened using PWV measurements, whereas females should undergo CIMT assessments in order to most effectively screen for preclinical atherosclerosis. This study provides preliminary evidence that body composition as assessed by dual energy x-ray absorptiometry may play a powerful role in signaling clinicians to an individual's risk of abnormal CIMT or PWV values, suggesting that health professionals should

track these more specific changes in addition to the often-reported body mass index. HDL cholesterol, a commonly reported element of a standard blood lipid profile, was suggested to exhibit a protective effect for PWV, supporting the increased importance of achieving the protective threshold of 60mg/dL either through medication or exercise therapy as opposed to focusing solely on the medical management of LDL. Lastly, vigorous physical activity needs to be included in care plans of those at risk for the development of CVD, as exercise of this intensity may confer a protective effect for arterial health more so than the commonly prescribed moderate to vigorous physical activity alone. Additional research is needed in order to further support these results. These findings should be considered by primary prevention health care professionals in order to more effectively screen for preclinical atherosclerosis and slow the rise in CVD prevalence.

**Recommendations for Future Research**

Further research is needed with larger sample sizes in order to better understand the complex relationships among CIMT, PWV, and established CVD risk factors. Additionally, the recruitment of more sedentary subjects could potentially allow significant differences in physical activity characteristics to emerge between those with normal and abnormal arterial health properties. The targeted recruitment of more adults with diagnosed diabetes mellitus would help to expand the study's findings in regards to blood lipid profile variables, and could help broaden the results' meaningfulness to include this clinical population. Similar health profile studies can be conducted with measurements of CIMT or PWV taken at alternative anatomical locations in order to determine if differences exist. A longitudinal study would be of value in order to follow these subjects for several years and better understand how changes in these health characteristics

may prevent or accelerate changes in both arterial thickness and stiffness. As this study's most important application is its potential to help allied health professionals more accurately identify those in need of lifestyle modifications prior to the development of clinical CVD, intervention studies may help provide important information in regards to which of these health variables can easily be modified to promote increased arterial health. Extending this study design into a younger population would require the development of percentile rankings for CIMT in adults younger than age 40, but this research direction could allow for a more thorough understanding of risk factors that may initiate the earlier stages of CVD progression. Lastly, studies investigating the cost-effectiveness of both CIMT and PWV screenings are required in order to make recommendations on the use of either or both of these screening tools on a community-wide scale.

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