SUBCLINICAL CARDIOVASCULAR DISEASE, VASCULAR HEALTH AND MARKERS OF RISK

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Cardiovascular diseases are a leading cause of morbidity and mortality. To facilitate early treatment and prevention, the relationship between risk markers and measures of subclinical disease should be determined. This dissertation examines how putative markers of risk, including traditional cardiovascular risk factors, rheumatoid arthritis and negative affect, are associated with measures of subclinical cardiovascular disease and vascular health.

First, the relationship of traditional risk factors with carotid artery intima-media thickness (IMT) is evaluated in 453 healthy middle-aged women. It is unknown whether segment-specific associations exist when accounting for the interdependence across IMT locations. Results show unique positive associations between common carotid IMT and weight, bifurcation IMT and smoking and systolic blood pressure, and internal carotid IMT and apoprotein B.

Second, it is postulated that the evaluation of carotid diameters augments knowledge of associations between rheumatoid arthritis and IMT and plaque. In 93 middle-aged patients, diagnosis of rheumatoid arthritis is associated with larger lumen and interadventitial diameters compared to 93 matched healthy women; whereas plaque prevalence is not statistically different and carotid IMT is similar, showing potential influences of vascular adaptation. Positive associations are demonstrated between carotid measures and rheumatoid arthritis medications, hypothyroidism and inflammatory markers.

Third, associations between negative psychosocial indices and brachial artery flow-mediated dilation (FMD) are examined in 332 healthy older men and women. It is not known whether a link exists when considering multiple measures of negative affect in a large sample of both sexes. After controlling for traditional cardiovascular risk factors, FMD is inversely associated with hostility and general anger scores for men, and anger suppression in women.

As demonstrated in this dissertation, associations between cardiovascular health and risk markers are evident early in the disease process. When evaluating cardiovascular disease risk,

including co-morbid conditions and psychosocial symptoms along with traditional risk factors is of public health relevance. Additionally, the implications of appropriate statistical methods, the effects of vascular adaptation, and the importance of including women in epidemiologic research are illustrated.

In conclusion, evaluating associations between markers of risk and subclinical cardiovascular disease and vascular health provides insight into the broader epidemic of cardiovascular disease.

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PREFACE

The motto for this degree could have been, "nothing's going to stop me." Completion of my PhD has been a long and slow moving process, but I've managed to keep it going amidst working full-time, planning a wedding, building & moving into a new house and having two children. There have been lots of starts and stops, with very productive time periods, and weeks where I didn't even think about it; and of course lots of drafts, revisions and resubmissions to various journals. It's almost hard to remember the coursework I started nine years ago and preliminary exam a few years later; or even getting the first paper published and completing the overview/comprehensive exam. Nonetheless, here I am today with a finished product.

I've had help and encouragement along the way, some since the beginning, and from others for segments along the way. Thank you to my co-workers here at the University of Pittsburgh and previously at the National Institutes of Health and those involved with the 'Acute Care for Elders' program in Ohio, who contributed to my affinity to research, broadened my knowledge and skills, and were instrumental in influencing my decision to pursue and ability to complete a doctoral degree.

I'd like to acknowledge my dissertation committee whose knowledge and thoughtful critiques have been very helpful. I've learned from both your participation in my dissertation and through my employment experiences with you. And, a special thank you to the co-authors on each of the individual papers, who have contributed to their success and certainly made each better.

Finally, I'd like to extend a very warm thank you to my family and friends many of whom have been especially supportive and encouraging both in this most recent pursuit and in daily life; with a special acknowledgement to Barb, Deb & Gina and to my immediate family – Mom & Dad, my sister Donna, my brother Mark & his family, my husband Kris, and the two most fantastic kids in the world, Benjamin & Lydia.

1.0 INTRODUCTION AND OVERVIEW

1.1 OVERVIEW

Given that cardiovascular disease continues to be a leading cause of morbidity and mortality,[41,42] it is important to identify individuals with subclinical disease, so that treatment and prevention can be applied early in the disease process. Ultrasound evaluation of peripheral arteries, namely carotid and brachial, provides an easy, noninvasive method for assessing vascular health and aging and thus provides information on subclinical cardiovascular disease. Although subclinical ultrasound measures are not yet part of standard clinical care, they are useful research tools given their strong association with both risk factors and health outcomes. Research measures commonly used to detect early atherosclerosis and evaluate vascular health include carotid intima media thickness (IMT), lumen diameter, interadventitial diameter and plaque prevalence, and brachial artery flow-mediated dilation (FMD). Of particular interest is how putative markers of risk, including traditional cardiovascular risk factors, specific diseases/conditions and psychosocial traits, are associated with these subclinical disease measures.

The ensuing body of work examines three separate but related aspects of subclinical cardiovascular disease and vascular health and aging. First, classic cardiovascular risk factors are examined in conjunction with segment-specific IMT. This is important because unique associations by segment are driven by the underlying biology, and an understanding of how atherosclerosis develops may promote more targeted treatment. The novelty of this paper is the use of a mixed statistical model approach to control for the correlation in IMT between segments, thus allowing potential differences in vessel areas to be compared directly. Second, atherosclerosis in women with rheumatoid arthritis is assessed in comparison to healthy women and in conjunction with measures of rheumatoid arthritis severity. This line of research is imperative given the much higher incidence of cardiovascular disease morbidity and mortality in

patients with rheumatoid arthritis relative to the general population. We make a unique contribution to the existing literature by considering carotid artery diameter along with IMT and plaque, which are the measures of early atherosclerosis typically evaluated. Third, we use FMD to assess the relationship between negative affect and endothelial dysfunction in a sample of healthy older adults. Substantiating evidence of a link between psychosocial constructs and subclinical cardiovascular disease promotes the notion that influences beyond traditional cardiovascular risk factors should be considered when evaluating heart health. By including multiple measures of negative affect in both men and women, this paper broadens the previously published research on FMD and psychosocial measures.

1.2 CARDIOVASCULAR DISEASE

Heart health is an important public health concern and research on subclinical cardiovascular disease and vascular health and aging enables varying aspects of cardiovascular disease to be considered and addressed. Following is a brief synopsis of the global and national impact of heart disease and a review of subclinical cardiovascular disease and vascular health. Awareness of the magnitude and spectrum of causes of cardiovascular disease are the impetus for the individual papers, which examine the associations of traditional cardiovascular risk factors, rheumatoid arthritis and measure of negative affect with atherosclerosis and vascular health.

1.2.1 Facts about Cardiovascular Disease

Reviewing some of the statistics on cardiovascular disease makes the enormity of the problem readily apparent. Cardiovascular disease is a group of disorders involving diseases of the blood vessels supplying the heart, brain, arms and legs, damage or malfunction of the heart muscle, valves and structure, and dislodging of blood clots in the veins.[42] According to the World Health Organization, nearly one-third of all deaths world-wide are due to cardiovascular disease, which is more than any other cause.[42] By 2015, it is estimated that the number of deaths due to cardiovascular disease will rise to 20 million people, a 2.5 million increase from statistics for

2005.[42] For the approximately 20 million people who survive heart attacks and stroke, continuing medical care is needed,[42] which has substantial financial and quality of life implications.

In the United States of America, the prevalence of cardiovascular disease was 37% in 2004; it is the leading cause of death for both sexes and all racial/ethnic groups; and it has been the number one killer for every year but one since 1900.[43-45] In 2006, the estimated cost (direct and indirect) of cardiovascular diseases and stroke was $403.1 billion.[44] For more than past two decades, the number of cardiovascular disease deaths for women has exceeded men, with males representing about 47%.[43] For men, about one-third of cardiovascular disease deaths occur prior to the average life expectancy age of 75 years old.[43,44] One-third of all adult females has some form of cardiovascular disease, and the number of cardiovascular disease deaths for women exceeds the next seven causes combined.[43,46] Of those who die suddenly of coronary heart disease, 50% of men and 64% of women have no previous symptoms.[43,47] Thus, continued research into early identification, including vascular health and markers of risk, is needed.

1.2.2 Subclinical Cardiovascular Disease and Vascular Health

Atherosclerosis and arteriosclerosis together form the foundation for cardiovascular diseases including coronary heart disease, and stroke, congestive heart failure and renal failure, respectively. Atherosclerosis includes subclinical features of stenosis, calcification, plaque and rupture, while arteriosclerosis encompasses changes in collagen, degeneration of elastin, wall thickening and arterial dilation. Clinical and research evaluation of these features include structural (e.g. plaque, wall thickening and calcification) and functional (e.g. arterial dilatation, vascular stiffness, reactive hyperemia) measures.[48,49] Further, while most risk factors (e.g. inflammation, inactivity, smoking, obesity) influence both atherosclerosis and arteriosclerosis, some may be more influential for one or the other (e.g. lipids and atherosclerosis; blood pressure/age/insulin resistance and arteriosclerosis). Here we will consider both atherosclerosis and arteriosclerosis as indicators of subclinical cardiovascular disease and vascular health and aging.

Atherosclerosis, the accumulation of plaques, or fatty deposits on the arterial walls, is the cause of most acute cardiovascular events and accounts for almost 75% of all cardiovascular

disease deaths.[42,44,45,50] As reviewed by Faxon and colleagues from the Atherosclerotic Vascular Disease 2004 Conference,[51] a typical sequence involves[50,52] accumulation in the subendothelium of low density lipoproteins that are oxidized by smooth muscle cells and macrophages. Concurrent with the accumulation of minimally oxidized low density lipoproteins is the development of inflammation, including instigation of adhesion molecules. Also influential is the bioavailability of nitric oxide and other mediators, which provide a balance between vasoconstriction and vasodilatation, anticoagulation and thrombosis, and modulation of the inflammation. These substances and processes result in release of cytokines and growth factors that attract additional monocytes, followed by smooth muscle cell proliferation and foam cell accumulation, which result in plaque growth. Vascular adaptation enables the artery vessel to constrict or enlarge in size during the development of atherosclerosis in an effort to maintain a constant level of blood flow. However, when the plaque encompasses approximately 40% of the vessel area or greater, arterial diameter no longer enlarges and the lumen narrows. The restriction or blockage of blood flow and erosion or rupture in plaque leads to cardiovascular outcomes including heart attack and stroke.

Underlying the initiation and progression of subclinical cardiovascular disease are genetic and lifestyle factors that influence vascular health. A first-degree relative with early-onset atherosclerotic cardiovascular disease (less then age 55 years in men and less than age 65 year in women) or having a condition where increased IMT is seen at a very early stage of atherogenesis (e.g. diabetes mellitus, systemic lupus erythematosus) increases one's risk for heart disease. An unhealthy diet, physical inactivity and smoking all contribute to the formation of traditional cardiovascular risk factors including, obesity, hypertension, poor lipid profile and diabetes.[42,53,54] Additionally, these risk indicators are interacting with and influenced by psychosocial and environmental aspects such as, negative disposition, stress, and socioeconomic status.[55-57] All of the above factors, along with aging itself[58,59] and the fundamental role of inflammation,[52] start in motion the pathophysiological processes that result in the initial insult to the endothelium, accumulation of the fatty streak, atheroma formation and thrombosis promotion. This damage often occurs prior to any overt cardiovascular event and thus often progresses undetected.

1.2.3 Topics of Subclinical Disease and Vascular Health Presented

Endothelial function, atherosclerosis and arterial diameter are useful indicators of subclinical cardiovascular disease and vascular health, and thus evaluation of these conditions provides insight into cardiovascular disease. Not surprisingly, these measures have been linked to disease outcomes, including coronary artery disease, myocardial infarction and stroke.[1,2,60-67] Furthermore, evaluating measures of early atherosclerosis and vascular health, along with related markers of risk may help identify those in jeopardy, lead to new areas of research and treatment, and potentially alleviate some of the burden of cardiovascular disease.

Of particular interest is the assessment of vascular health and aging in women, who are underrepresented in cardiovascular disease research.[68-70] Two of the following papers use female-only samples (Chapters 2 & 3), while the third evaluates both men and women (Chapter 4). Special attention will also be paid to the statistical methodology employed, the effects of aging, and the implications of hemodynamics of the vasculature. The following sections will review individual ultrasound measures and putative markers of risk along with the existing literature on these topics. In evaluating measures of subclinical cardiovascular disease and vascular health in association with putative markers of risk, we plan to address some of the issues underlying the public health significance of heart disease.

1.3 ULTRASOUND MEASURES

Ultrasound measures of subclinical cardiovascular disease are used to evaluate endothelial function, early atherosclerosis and vascular health. Scanning is generally done in a dimly lit testing room with a participant in the supine position after several minutes of rest. Carotid and brachial arteries are examined using an ultrasound scanner equipped with a linear array imaging probe. These measures are sound indicators of subclinical cardiovascular disease and useful tools when examining associations with putative risk markers.[71,72] Carotid ultrasound measures of IMT, plaque, lumen diameter, and interadventitial diameter are useful for evaluating early systemic atherosclerosis and are informative measures of vascular health.[73-77] Ultrasound

imaging of endothelial-dependent FMD evaluates vasodilatation and dysfunction of the brachial artery.[78,79]

1.3.1 Intima-Media Thickness

Carotid arteries can be viewed via ultrasound in transverse and longitudinal projections. For the common carotid artery segment, both near and far walls are normally examined just proximal to the bifurcation. For the bifurcation area, internal carotid, and external carotid artery measurements are generally taken of the far walls only (since near walls cannot be consistently visualized). Digitized images are used to trace the medial-adventitial and intima-lumen interfaces across 1-cm lengths and compute IMT for each segment. IMT can be summarized as the average or as the maximum value of all or some segments.[77] Additionally, the common carotid artery is used as a single indicator of IMT in early atherosclerosis.[8,80] The ultrasound laboratory at the University of Pittsburgh calculates average IMT from the mean of both the right and left carotid arteries of three segments (internal, bifurcation and common; 8 locations total).[30]

Carotid IMT is considered a marker of early atherosclerosis, predicts future risk of cardiovascular disease, and has been found to be high in individuals with coronary heart disease and myocardial infarction (IMT $\geq$ 0.73 mm and $\geq$ 1 mm, respectively).[1,2] Cardiovascular risk factors, such as high blood pressure, body mass index and cholesterol, are associated with average and maximum carotid IMT.[3-7,81-83] To detect the burden of preclinical cardiovascular disease in patients with rheumatoid arthritis, use of noninvasive measures such as carotid IMT has been promoted.[84-99] In the following chapters we will look at average IMT in conjunction with segment-specific IMT in healthy women (Chapter 2), as well as the common carotid artery in rheumatoid arthritis analyses (Chapter 3).

1.3.2 Segment-Specific Intima-Media Thickness

Three segments are typically considered when examining IMT by location; these include the common carotid artery, internal carotid artery and the arterial bifurcation; with some investigators combining the internal and bifurcation areas of the vessel. Body mass index, high

blood pressure, cholesterol and other traditional cardiovascular risk factors have been explored in conjunction with segment-specific locations of IMT.[8-13]

While looking at multiple segment averages provides reliability and precision, examination of individual segments provides worthwhile information. Differences in blood flow, cellar processes and influences of the risk factors themselves suggest it may be beneficial to look at IMT at specific locations along the carotid artery. It has been reported that the association between coronary and carotid atherosclerosis is only marginally increased when IMT in the internal and bifurcation segments is added to the common.[8] We believe unique information can be gained by looking at the three individual carotid IMT segments (Chapter 2).

1.3.3 Carotid Plaque

Carotid arteries can also be evaluated for the presence of eccentric focal plaque in the bifurcation, internal and common areas. Plaque is typically defined as a distinct area protruding into the vessel lumen with ≥ 50% thickness than the surrounding area. For group comparisons, degree of plaque may be dichotomize as presence of plaque versus no plaque, categorized as no/small plaque (< 30% of vessel diameter) versus medium/large/multiple plaques (≥ 30% stenosis), or summed as the total number of plaques. Notably, while degree of stenosis (i.e. plaque size and number of lesions) is important in clinical outcomes, plaque vulnerability and composition (i.e. plaque type) are more likely to influence rupture and erosion.[100-105]

The risk of plaque disruption is a function of rupture triggers (i.e. extrinsic effects) and vulnerability of the plaque (i.e. intrinsic effects).[100-105] Major determinants of vulnerability are thickness of the fibrous cap, size and consistency of the atheromatous core, calcification burden and pattern, endothelial denudation, and plaque inflammation within the cap. Biomechanical and hemodynamic forces acting extrinsically on the plaque include location of the plaque, shear stress, myocardial susceptibility, and blood coagulability. And stability of the plaque includes evaluation of both structural and functional properties.[102] Clinical outcomes can be linked to plaque rupture, which is most common, as well as plaque erosion and lumen occlusion.[100-105]

Like IMT, carotid plaque is a measure of subclinical atherosclerosis. Carotid plaque has been linked with increased age, smoking, higher blood pressure, hypercholesterolemia and ischemic stroke.[39,60,106,107] Plaque is most commonly found in the carotid bifurcation.[39]

1.3.4 Carotid Artery Diameter

Lumen diameter is measured as the distance between the lumen-intima interfaces of the common carotid artery, where the walls are parallel and there is no evidence of plaque. Interadventitial diameter is measured as the distance between the adventitia-media interfaces. Typically, these widths are calculated by averaging the distances of several measurements taken across a pre-specified segment.

Larger lumen and interadventitial diameters are markers of vascular aging. Arterial dilatation (i.e. vascular remodeling) is considered to begin as an adaptive response to changes in wall shear stress concomitant with increased wall thickness.[63,74,75,108-110] Previous cross-sectional analyses show increased carotid diameters are positively associated with hypertension, diabetes, coronary heart disease, myocardial infarction and left ventricular mass.[61-63] Increased diameter has also been found to be independently associated with traditional cardiovascular risk factors including male sex, increased weight, high blood pressure and pack-years of smoking.[9,62,75,111,112] In the general population, carotid arteries free of atherosclerosis and unexposed to risk factors maintain normal size with aging.[75] Here we will take a novel approach to comparing early atherosclerosis in patients with rheumatoid arthritis and healthy women by examining lumen and interadventitial diameters in addition to IMT and plaque (Chapter 3).

1.3.5 Brachial Artery Flow-Mediated Dilation and Endothelial Dysfunction

For the measurement of brachial artery FMD,[78,79] a blood pressure cuff is typically placed distal to the elbow with the arm extended out on a board and pillow perpendicular to the body. Baseline brachial lumen diameter is computed as the distance between the lumen-intima interfaces 1-2 centimeters proximal to the antecubital fossa. An ischemic trigger at the forearm is achieved by inflating a blood pressure cuff approximately 30 mmHg above the participant's systolic pressure or greater than 250 mmHg for 4-5 minutes. After release of the cuff, brachial artery images are obtained over several minutes and usually digitized for later scoring. At pre-specified time intervals, end-diastolic images from separate cardiac cycles are averaged from measurements taken across small arterial segments. Absolute change in diameter is calculated as the peak (widest) of these diameter measures minus the baseline brachial diameter. FMD is

calculated using the absolute difference (i.e. change in diameter) divided by the baseline brachial diameter times one-hundred.[79]

The vascular endothelium is critical in vascular growth, vasoprotection and vasoregulation. Impaired vasodilatation is associated with cardiovascular risk factors, such as increased age, systolic blood pressure, and body mass index and with lipid-lowering medication and smoking;[78,113-117] Endothelial dysfunction is one of the earliest signs of insult in the cardiovascular disease continuum and is significantly related to atherogenesis.[118,119] It is associated with cardiovascular outcomes and has been suggested as a biomarker of disease, similar to IMT and coronary calcification (reviewed by [64,120,121]).

For evaluation of endothelial dysfunction, brachial artery FMD is an easy to assess, non-invasive technique.[78,122,123] A higher FMD value indicates greater vasodilatation capacity, and thus healthier endothelial function. FMD, a measure of impaired vasodilatation, is thought to reflect nitric oxide bioavailability and the effects of shear stress on the endothelium.[124-127] For healthy populations, mean values for FMD range from -3 to 26%,[113,128,129] and although there is great overlap amongst groups, values for coronary artery disease and diabetic patients are generally lower.[78,130] FMD is considered an endothelium-dependent process that manifests the relaxation of a conduit artery when exposed to increase flow and as such, is also referred to as reactive-hyperemia.[131] It is related to traditional cardiovascular risk factors,[117] provides a surrogate marker for health of the coronary arteries,[130,132,133] and is an independent predictor of cardiac risk and events.[65,134,135,135] FMD and has been shown to improve with treatment.[136,137] In Chapter 4, FMD will be used to evaluate the influences of negative affect on endothelial dysfunction.

1.3.6 Inter-relationship, Reproducibility and Application

While each of the above ultrasound measures conveys unique information, they are clearly interrelated. FMD is inversely related to IMT,[118,119] and evidence of endothelial dysfunction has been found prior to the development of measurable atherosclerosis.[119,138] It has been suggested that damage to the endothelium is required before risk factors can "induce" atherosclerotic changes in the arterial wall. Healthy young adults with FMD evidence of endothelial dysfunction and cardiovascular risk factors are at increase risk for thickened carotid arterial

walls.[119] Presence of plaque or stenosis is strongly and positively associated with IMT and occurs subsequent to foam cell accumulation and wall thickening.[39,139] Widening of arterial diameters is a dynamic, early response to risk factors and wall thickening.[75] Carotid diameters are positively related to IMT.[112]

All four measures are influenced by hemodynamics within the blood vessels and are indicators of vascular aging; and as such, convey different aspects of underlying disease. Shear stress is inversely related to carotid diameter, IMT and plaque,[18,19,109,140,141] and positively related to FMD.[127] An individual's overall vascular aging profile is determined by age-associated endothelial dysfunction, intima media thickening, and other evidence of insult to the vasculature, along with risk factor exposure and genetic makeup.[59]

Reproducibility and application varies across these ultrasound indicators of subclinical disease and vascular health. Of these measures, IMT is most often employed with frequent research application and high clinical potential. It is easy to assess, highly reproducible and has standard cut-points suggesting pre-clinical disease. [1,2,77] Plaque is similarly accessible, with good reproducibility. However, categorization of disease by plaque is less straight forward because rupture and erosion are more dependent on composition and vulnerability than on degree of stenosis, which is what is captured by ultrasound.[100-105] And while the degree of obstruction should not be underestimated, it is the stability of the plaque that is most relevant to clinical outcomes.

Diameter measures are harder to compare at the individual level and are likely better suited to research evaluations, which use group or holistic comparisons. Arterial diameters (e.g. carotid lumen and interadventitial, baseline brachial) are highly dependent on body size with narrower diameters (found in shorter individuals) having greater capacity to dilate and adapt relative to larger ones. Carotid diameter measures are not as widely used as IMT and plaque, nonetheless these carotid structures are interdependent-and occur both as part of the natural aging process and in response to insult or injury. Brachial artery diameter reproducibility is excellent, and while not as high, FMD is a stable and reproducible measure.[119,123]

Overall, ultrasound measures of carotid IMT, plaque, lumen diameter and interadventitial diameter and brachial artery FMD represent a spectrum of subclinical cardiovascular disease and vascular health and aging. They can be used as surrogate endpoints of disease and are targeted for primary prevention.[53] They compliment one another by evaluating both the structural (e.g.

IMT, plaque) and functional (e.g. FMD) health of the vasculature.[48,49] Measures of the peripheral arteries are useful research tools for evaluating early systemic atherosclerosis in large populations. Finally, it is worth noting that all of these structures are in constant flux because of vascular adaptation, showing both negative and positive progression over time, and reversibility with treatment.

1.4 PUTATIVE RISK MARKERS

When evaluating cardiovascular risk, consideration of a global risk profile provides better predictive power than any single risk factor.[72,142] While biological factors are typically considered, many other markers have been found to be associated with pre-clinical cardiovascular disease and disease outcomes. Thus, in addition to traditional cardiovascular risk factors (e.g. age, blood pressure), certain disease conditions (e.g. inflammatory autoimmune disorders, metabolic syndrome) and psychosocial aspects (e.g. hostility, depression, life stress) have also been found to be associated with cardiovascular health. By definition, risk factors are neither necessary nor sufficient to cause a disease; however, they are useful indicators for determining who is more likely to develop one.[143] Additionally, associated risk factors can sometimes be targeted for treatment in hopes of helping the overall burden of disease. Therefore, additional research into the associations of putative risk markers and measures of early atherosclerosis is warranted. Here we will specifically consider traditional cardiovascular measures, rheumatoid arthritis and negative affect as potential precursors and consequences of atherosclerosis and vascular health.

1.4.1 Traditional Cardiovascular Risk Factors

Traditional cardiovascular risk factors have been extensively examined in conjunction with subclinical measures of endothelial dysfunction, atherosclerosis and vascular aging.[3-7,9,39,62,75,78,81-83,107,111-117] In general, several categories of risk factors are typically considered.[53,71,142,144] These include blood pressure (diagnosis of hypertension or taking

antihypertensive medications), lipids (total cholesterol, low density lipoproteins, high density lipoproteins, triglycerides, apoprotein B), insulin (glucose, diabetes, anti-diabetic treatment), body size (weight, body mass index, waist circumference), smoking/exposure to passive smoking, physical activity, aging and gender; and more recently, menopausal status, lipoprotein subfractions, homocysteine, metabolic syndrome and inflammatory markers.[42,53,54,142,145,146] For example, the Framingham risk score, a common algorithm for assessing 10-year cardiovascular disease risk, assesses age, lipid levels, blood pressure, and smoking and diabetes status.[147] Familial predisposition, that is a first-degree relative with early-onset atherosclerotic cardiovascular disease, can also be considered as a traditional marker of risk.[142]

Because of their high association with cardiovascular disease and their link to unhealthy living habits, many of these factors are naturally targeted for lifestyle and behavior changes.[53,54,148,149] A task force from the American College of Cardiology[53] suggests consideration of primordial cardiovascular prevention (i.e. the prevention of risk factors). Thus, healthy eating, physical activity, ideal weight and psychosocial factors would be implemented to curtail the development of hypertension, diabetes, obesity and hypercholesterolemia. Primary prevention of these latter factors occurs prior to overt disease, but after inflammation, endothelial dysfunction, carotid stenosis and coronary calcification have begun.[53] Aggressive secondary prevention measures, including medication, surgery and rehabilitation, are critical for patients with established cardiovascular disease who are at high risk for future events.[53,148,150]

1.4.2 Disease Specific Risk – Rheumatoid Arthritis

Rheumatoid arthritis is a chronic, inflammatory autoimmune disorder that affects 1% of the U.S. population, particularly women of childbearing and early menopausal ages.[151-155] Patients with rheumatoid arthritis have a reduced life expectancy, most commonly attributable to their increased incidence of cardiovascular events including, atherosclerosis of the coronary arteries and myocardial infarction.[156-159] In patients with rheumatoid arthritis, cardiovascular disease is a major determinant of morbidity and is the leading cause of death.[156-159]

Inflammatory aspects of the disease are of particular interest, given their link with cardiovascular disease in the general population and the role of inflammation in both atherogenesis and rheumatoid arthritis.[91,160-165] While, the relationship between inflammatory

markers and atherosclerosis in patients with rheumatoid arthritis has been inconsistent across some studies, logical pathways by which systemic inflammation contributes to atherogenesis in patients with rheumatoid arthritis have been proposed.[166-168]

Other factors associated with rheumatoid arthritis, such as pharmacological treatment, disease duration, and swollen joint counts may also be useful for identifying specific aspects of rheumatoid arthritis with potential links to atherosclerosis.[84,85,161,169] Interestingly, traditional cardiovascular risk factors have not been consistently linked with rheumatoid arthritis, [156,158] although components of the metabolic syndrome show positive associations with IMT[170] and combining traditional and nontraditional risk factors increases the accuracy of predicting carotid plaque.[171]

1.4.3 Psychosocial Risk – Negative Affect

It has long been postulated that negative emotions play a role in the etiology of physical illness. Multiple behavioral and physiological pathways have been identified in both human and animal research, particularly in studies examining coronary heart disease morbidity and mortality. Further, certain psychological traits (e.g. hostility and anger, [172] and possibly depression[173]) can be considered chronic or dispositional rather than episodic or acute; therefore their influence could be comparable to the lasting or additive effects of some traditional cardiovascular risk factors such as hypertension or obesity. And just as traditional risk factors play independent and overlapping roles in the progression of cardiovascular disease, measures of negative affect can be considered individually or as a general disposition.

Four types of negative affect including, depression, anxiety, anger (specifically, Anger In, Anger Out and Trait Anger) and hostility, will be considered here (Chapter 4). Depressed mood can be described as feeling sad, irritable, hopeless and discouraged, as a loss of pleasure or interest in usual activities, and as feeling a sense of inadequacy or worthlessness. Anxiety includes physical symptoms such as accelerated heart rate, restlessness, shortness of breath and nausea, as well as feeling tense, nervous, fearful, “on edge” and worrisome. Anxiety and depression share some of the symptoms of chronic diseases such as fatigue/loss of energy, weight loss/gain, feeling shaky, trouble sleeping and diminished ability to concentrate/think.

Anger and hostility are commonly linked to Type A personality with exaggerated response and high stress being typical covariates. Hostility reflects a person's attitudes and beliefs and is characterized by a tendency to view the world in a negative, cynical fashion. Anger is an emotional state that encompasses generalized distress (ranging from mild annoyance to outrage and fury), as well as interpersonal components of anger suppression and expression. Martin and colleagues describe the trait anger domain with a 3-factor "ABC" model of anger (affect), aggression (behavior) and hostility (cognition).[174] Interestingly, it has been suggested that the cognitive response, rather than overt behavior, may be the aspects of anger and hostility that are most influential in cardiovascular disease.

Several pathways by which negative emotions influence cardiovascular health have been considered. These include health related behaviors that are typical of individuals with these characteristics (e.g. smoking),[175-178] poor access to or seeking of medical care,[179-182] and the occurrence of direct acute or chronic pathophysiological changes.[172,183-188] Genetics and environmental factors also influence psychosocial disposition.[189-193] Finally, cardiovascular disease itself is associated with depression and anxiety, particularly after surgical procedures.[194,195]

1.5 RESULTS AND LIMITATIONS OF EXISTING LITERATURE

The specific manner in which each of the above types of risk markers are associated with subclinical disease and cardiovascular events varies. Some markers may be causal, others exacerbate the disease, and still others may occur in conjunction with or as a result of cardiovascular outcomes, or in some combination of these modes. Following is a review of the existing literature on the three topics of interest including, traditional cardiovascular risk factors and segment-specific IMT, rheumatoid arthritis and subclinical outcomes, and measures of negative affect and FMD.

1.5.1 Traditional Cardiovascular Risk Factors and Segment-Specific IMT

Both similar and unique associations have been reported between cardiovascular risk factors and the common, internal and bifurcation segments of the carotid artery. Similarities across segments seem natural given the proximity of the segments along the vessel. However, variations in blood flow and sheer stress experienced by each arterial segment could provide distinct predisposing factors. Likewise, differences in cellular processes and the risk factors themselves suggest unique associations.

Some studies have generally found the three carotid segments similarly associated with cardiovascular risk factors.[10,11,13] Previously published studies have reported positive associations of age, male sex, systolic blood pressure, body composition, and smoking with IMT of the common and bifurcation areas.[10,11,13] Associations with lipids, while not as common, have also been reported such that higher low density lipoproteins and lower high density lipoproteins have been associated with higher IMT in all three segments.[9,11,13] It is possible that examining more diseased populations may have made it harder for these studies to tease out any unique associations that may exist.

The level of IMT in the common carotid artery, which is typically more than the internal carotid artery but less than the bifurcation area, is partly due to the lower sheer wall force[18,19,32] that is consistent with the laminar blood flow[33] in this location. In terms of risk factors, a positive relationship is commonly found between body size and atherosclerosis in the common carotid artery.[34] Diabetes, which is associated with obesity, is thought to decrease wall shear stress and is positively associated with common carotid artery IMT.[35] Aging is also inversely related to shear stress[32] and shows a positive relationship with common carotid artery wall thickness. [3,11,13,32] The common carotid artery segment, which is easily and reliably viewed via ultrasound, is the most frequently evaluated, and studies of general IMT often report results only on this segment.

In the internal carotid artery segment, the smaller wall thickness, relative to the other segments, is likely attributable to the mix of laminar and oscillatory shear stress around the arterial wall.[19] Infiltration of lipids during extended contact of blood with the wall has been linked to areas of low shear stress,[36] which portions of this segment experience. Additionally, shear stress influences cellular processes such as an increase in growth-regulatory factors,[16,22]

expression of inflammatory molecules[24] and response of pro-oxidant processes,[33] which have also been linked to low density lipoproteins and atherosclerosis,[37,38] although not specifically to the internal carotid artery segment.

The bifurcation, which connects the common carotid artery and internal carotid artery segments, is likely influenced by factors related to each of these sites. The bifurcation experiences the most oscillatory stress and turbulence as a result of reverse flow velocity components occurring during pulsatile flow.[19,32,33] Consequentially, the bifurcation may exhibit greater wall thickness and be associated with higher systolic blood pressure. Higher IMT in the bifurcation area may also be a function of plaque, which is commonly found in this location.[39] The influence of shear stress on the extracellular factors of hypertension such as increased pressure,[16] decreases in vasoactive agents[17] and inflammatory effects,[14] would have the largest impact on the bifurcation segment. Research on increased residence time of blood[36] and adherence of platelets and macrophages to the arterial wall,[40] which influence lipid infiltration and plaque formation, is consistent with associations between lipids and bifurcation IMT.[11,13] The increased residence time might also make the bifurcation more susceptible to the toxic components of cigarette smoke.[15]

Variations in methodology, including use of older subjects or heart clinic patients, make others' results of IMT by carotid segment challenging to appraise.[8-13] Further, previous studies have not evaluated the segments in the same statistical model, so associations can not be directly compared and tested. Thus, examining IMT locations in healthy subjects using a repeated measures analytic model should provide more definitive results regarding segment-specific associations with risk factors.

1.5.2 Rheumatoid Arthritis and Subclinical Outcome Measures

Carotid ultrasound findings comparing subjects with rheumatoid arthritis to controls have been somewhat inconsistent. While studies typically find common carotid IMT to be higher in patients with rheumatoid arthritis compared to controls,[84-89,95] several studies have found no difference[90-92,99] or the opposite result.[94] In regards to carotid plaque, several studies have reported no difference in plaque prevalence,[84-86,89,91] while others have found increased carotid plaque in patients with RA.[88,90,92,94,95,97] Further, in examining patients with rheumatoid arthritis,

research on associations between markers of inflammation and other measures of rheumatoid arthritis severity and atherosclerosis are not clear cut.[85-87,89,91,95,160,161,171,196,197] Choice of study population, disease-specific influences and/or vascular adaptation may explain these inconsistencies in the literature.

Several studies have described increased IMT in patients with rheumatoid arthritis when compared to controls,[84-89,95] while others have found no difference,[90-92] or lower IMT in patients with rheumatoid arthritis.[94] Given the high prevalence of cardiovascular morbidity and mortality in patients with rheumatoid arthritis, reports of higher common carotid wall thickness in patients would be expected. However, a large study of rheumatoid arthritis men and women found no difference in carotid IMT compared with controls.[91,92] Similarly, no difference in IMT was reported between women with rheumatoid arthritis, women with systemic lupus erythematosus, and controls.[92] Interestingly, patients with lupus had larger carotid artery diameters than controls. In a small Italian study, internal carotid IMT was higher in patients with rheumatoid arthritis, while common carotid IMT was similar to controls.[90]

Differences in study populations (rheumatoid arthritis and controls) may explain some of the discrepancies in IMT results, given variations in age and gender of subjects. Research indicates that IMT increases with age,[75,198] with menopausal status independent of age,[146] and is greater in men.[4] Average age of subjects was 55 years old in three studies that report greater IMT in patients with rheumatoid arthritis compared to controls.[84-86] In contrast, in studies where the average age was less than 50 or greater than 60 years old, IMT was higher in controls or did not differ between groups.[91,92,94] Taken together, these results suggest a curvilinear relationship, where IMT is similar between patients and controls at younger and older ages. Ethnicity and control characteristics may also be influencing results given that those that found greater IMT in patients with rheumatoid arthritis all occurred outside the United States, while the others were US-based and comprised primarily of Caucasians.

With a closer look at the discrepancies between previously published studies, the apparent contradiction in IMT results may in fact be mediated by differences in stage of arterial remodeling. Changes in vessel hemodynamics (primarily wall shear stress)[63,74,75,108-110] initiates arterial dilatation, which allows lumen cross-sectional area to be kept constant and maintains or decreases IMT by distributing it over a larger area. However, the artery has a limited capacity to dilate,[73,74,199] and further increase in IMT and plaque progression will prevail. In other words,

the lack of association between rheumatoid arthritis and IMT may be a function of the stage of vascular remodeling, whereby younger rheumatoid arthritis samples[92] may be at an earlier stage of atherogenesis (prior to differences in IMT), while older samples[91] may be at the final stages of remodeling (thus showing greater IMT than controls). Then with continued aging, cardiovascular disease morbidity and mortality may be influencing results such that patients with severe rheumatoid arthritis are not able to participate in research. Interestingly, a study of IMT progression[93] in women with rheumatoid arthritis found an accelerated rate of annual wall thickening when compared to healthy controls. Thus, while there is evidence that IMT may differ between patients with rheumatoid arthritis and controls, the course of progression may be distinct from other conditions where increased IMT is seen at a very early stage of atherogenesis.

Alternatively, a unique, rheumatoid arthritis-related modification of atherogenesis may exist. Perhaps the underlying autoimmune disease process, its treatment, or an interaction between the two may account for a change in the evolution of atherosclerotic vascular disease in these patients. In other words, the subclinical atherosclerosis found in several studies may not reflect the typical interplay between diameters and IMT as just described.

The association between prevalence of plaque and rheumatoid arthritis similarly remains unresolved. Patients with rheumatoid arthritis were more likely to have carotid plaque than controls in a recent study of arterial stiffness,[92] and in a study examining the role of insulin resistance in atherosclerosis.[88] Another small preliminary study[90] found patients with rheumatoid arthritis had a higher stenosis/IMT score in the internal carotid artery than controls. Conversely, several previous investigations have found prevalence of plaque to be similar in patients with rheumatoid arthritis and controls.[84-86,89,91] Plaque frequently occurs in areas of transition or turbulence (e.g. internal carotid or carotid bifurcation) within the artery,[110] therefore variations in methodology across studies (i.e. area of plaque assessment within the carotid artery) may be influencing results.

Inflammatory markers, which are inherent to autoimmune diseases such as rheumatoid arthritis and systemic lupus, have been examined as potential factors in promoting atherosclerosis.[91,160,161,200] The pathogenesis of rheumatoid arthritis includes multiple proinflammatory agents including cytokines (e.g. tumor necrosis factor alpha and interleukin 1), chemokines and adhesion molecules, as well as active angiogenesis, which provides oxygen and nutrients needed for inflammatory cell recruitment.[152] Nonetheless, studies examining specific

markers have not consistently found associations with IMT and/or plaque. C-reactive protein was not significantly related to carotid IMT in one study,[85] while others found a positive association between C-reactive protein and IMT and plaque,[91] and between maximum C-reactive protein values and carotid IMT.[166] A positive association between soluble intercellular adhesion molecule and IMT and between soluble endothelial adhesion molecule and plaque have each been reported.[161] Increased erythrocyte sedimentation rate, a marker of systemic inflammation, was associated with increased IMT independent of other risk factors in some studies[91,160] but not others.[85] Interestingly, a study of 63 women with rheumatoid arthritis found a positive association with progression of IMT and markers of inflammation, such as C-reactive protein, white blood cell, platelet counts and erythrocyte sedimentation rate, which suggest that change in atherosclerosis rather than initiation of wall thickness may the link.[93] Nonetheless, inflammation in rheumatoid arthritis has been consistently associated with endothelial function,[166,201] one of the earliest markers of atherosclerosis, and initiating pathways by which systemic inflammation contributes to atherogenesis in patients with rheumatoid arthritis are quite likely (as reviewed by [166-168]).

Other factors characteristic of patients with rheumatoid arthritis and indicative of disease severity and activity are also important. A positive association between duration of disease and IMT has consistently been reported.[85,86,95,171,196,197] Research on pharmacological treatment is mixed, showing that glucocorticoids and disease-modifying antirheumatic drugs may be positively [169] or negatively [161] associated with atherosclerosis and cardiovascular mortality.[202] These incongruencies are possible because treatment can be interpreted as a marker for rheumatoid arthritis severity, contributing adversely to cardiovascular risk factors (e.g. hypertension) or conversely, exerting a protective effect by reducing rheumatoid arthritis-related systemic inflammation.[98,163,203-205] Class, type, effectiveness and side effects of medications influence when and how they are prescribed, consequently affecting their associations with atherosclerosis. Successful long-term management requires frequent assessment of disease activity and response to treatment.[152,206] Further, the overall therapeutic approach to rheumatoid arthritis treatment has changed overtime, where disease-modifying antirheumatic drugs, such as methotrexate, are now given earlier rather than later in the course of disease, and development of more effective therapies is a continual process.[152,206,207] Additionally, studies vary on whether current, cumulative, or multiple medication exposure is considered. Typical measures of disease

severity, such as swollen joint counts, Health Assessment Questionnaire[208] scores and disease activity have also revealed inconsistent results with some investigators showing independent positive associations with IMT,[85,88] and others finding no association between these markers and measures of subclinical atherosclerosis.[84] More recently, other disease aspects, such as hypothyroidism and uric acid concentrations, have been considered in relation to atherosclerosis.[171,196]

In sum, using IMT and plaque prevalence as measures for providing insight into the increased cardiovascular disease experienced by patients with rheumatoid arthritis needs further investigation. Additionally, carotid ultrasound studies of patients with rheumatoid arthritis thus far have not included outcome measures of lumen or interadventitial diameter. Mixed findings on the association of carotid atherosclerosis with inflammatory markers and related indicators of rheumatoid arthritis severity and activity also warrant further exploration. Our study will provide insight into vascular adaptation in women with rheumatoid arthritis by looking at carotid diameters in addition to IMT and plaque. Associations between indicators of rheumatoid arthritis disease, including inflammatory markers, and carotid outcomes will also be examined.

1.5.3 Negative Affect and Subclinical Cardiovascular Disease

There is good evidence linking negative affect and cardiovascular disease (reviewed by [184-187]), whether clinical events[55,57,209-215] or subclinical outcomes[214-220] are examined. Psychosocial traits and stressors influence cardiovascular disease via both behavioral and pathophysiological pathways.[184-187,188] Measures of negative affect, such as hostility, anger, anxiety and depression are of particular interest given previous associations found with subclinical cardiovascular disease. While associations with IMT have been studied extensively, examining negative affect and endothelial function has been done in a limited number of studies, several with small sample sizes.[221-224] Further, previous studies of brachial vasodilatation have primarily examined depression,[221-223,225] with one considering hostility in conjunction with induced stress,[224] and only one study looking at multiple psychosocial clusters.[128] Thus, further investigation regarding associations between FMD and several types of negative affect in a sizable sample of both men and women is warranted.

Studies of major depressive episodes or previously measured depression have more consistently found associations with measures of subclinical cardiovascular disease than studies examining depressive symptoms.[226-228] Research on the sample to be used here found depressive symptoms at baseline associated with three-year progression of IMT, but not IMT at baseline.[228,229] Other measures of subclinical cardiovascular disease (e.g. IMT & coronary calcification) also have a reported link with a history of two or more major depression episodes, but not with depressive symptoms.[226,227] Nonetheless, depressive symptoms are consistently reported after clinical cardiac procedures and events.[194,195]

Of particular interest here, is the association between depression and early endothelial dysfunction via FMD, which a few studies have examined.[128,221-223,225] A study of postmenopausal women looking at a cluster of multiple measures of negative affect reported a negative association between FMD and an anxiety/depression factor measured several years prior.[128] However, results of the individual scales showed that Trait Anxiety and Anger In (i.e. anger suppression), and not depression or social support, were the measures of the anxiety/depression cluster that were specifically associated with smaller diameter change. In three small studies ($n < 39$), impaired vasodilatation was associated with concurrent clinical depression or a history of major depression.[221-223] While another study found and inverse relationship between high depressive symptoms (perhaps more indicative of chronic depression) and FMD.[225]

While depression and anxiety are considered similar constructs in terms of their influence on chronic disease,[173,187] results from studies looking specifically at anxiety and subclinical measures of cardiovascular disease have been mixed. Sustained anxiety was positively associated with IMT progression in one study,[219] but Trait Anxiety and general anxiety were not in others,[218] including our own.[228,229] As mentioned earlier, Harris and colleagues found the anxiety component of a depression/anxiety cluster was associated with smaller brachial artery diameter change.[128]

Measures of anger and hostility have been positively associated with carotid artery IMT[214,215,217] and IMT progression[213,216,218,220] as well as cardiovascular risk factors and events,[209-211,214-216] including mortality.[212,213] In terms of endothelial-dependent vasodilatation, Harris and colleagues[128] found an inverse association with an aggregate Anger/Type A factor measured approximately 14 years prior to the women becoming postmenopausal. Individual

scales of Trait Anger and Type A personality, as well as Anger In, were associated with smaller diameter change, predominantly among non-hormone therapy users. A small study (n=38) looking at the effects of mental stress in both sexes found an inverse association between hostile affect and FMD.[224] It is not known whether the relationship between anger and/or hostility with FMD holds true for a larger sample of men and women, or when multiple psychosocial symptoms are measured closer in time, thus research addressing these concerns would be informative.

It's generally concluded that psychosocial traits and stressors influence cardiovascular disease via both pathophysiological and behavioral pathways.[184-187] Pathophysiological mechanisms include greater platelet activation,[230] cardiovascular reactivity (including increases in blood pressure and heart rate),[231] inflammation,[232,233] and excessive sympathetic nervous system and hypothalamic-pituitary-adrenal axis activity (as indexed by greater norepinephrine and plasma cortisol).[231,234] In particular, inhibition of cortisol improves FMD in depressed patients.[235] Substantial overlap exists in the biologic and functional pathways underlying these mechanisms and thus, many of these processes may be different manifestations of the same etiology. Pathways of the vascular endothelium, which is the measure of vascular health evaluated in our study, have been outlined by Harris and Matthews[188] and suggest mechanisms similar to those implicated in other studies. Further, genetic factors also contribute to psychosocial disposition,[189] and thus likely reflect intrinsic influences on associations between negative affect and cardiovascular disease. Even so, results are inconclusive on whether improvements are seen when psychosocial treatments are added to standard cardiovascular rehabilitation (e.g. [236,237]).

Negative affect has also been linked to adverse health habits, behavioral components of medical care, and environmental factors. Unhealthy behaviors linked to negative emotions include poor sleep, increased alcohol use, smoking, obesity and poor diet.[175-178,192] Individuals with negative dispositions are also less likely to seek or have access to medical care, and to adhere to medical interventions.[179-182] While it is not clear whether negative emotions mediate the association between social economic status and health,[192,193] individuals from lower and higher statuses clearly differ in how they describe their social environment and interactions with others.[238-240] Familial and twin studies suggest that both genetic and non-shared environmental factors influence negative affect.[189-191]

It can not be said with certainty whether insult to the vascular system or psychosocial traits occurs first since genetic and environmental predispositions underlie both; but the conditions appear to be fundamentally linked. Behavioral factors are highly influential,[175] but do not solely account for associations between psychosocial traits and cardiovascular disease; and psychosocial influences are evident even after controlling for traditional cardiovascular risk factors.[211,216 217] Further, the INTERHEART study, which included nearly thirty-thousand subjects from fifty-two countries, found a psychosocial factor on par with traditional cardiovascular risk factors in terms of population attributable risk for myocardial infarction.[55,57] The influence of negative affect is apparent in both healthy and diseased populations, with results more consistent in healthy ones.[173] Examining associations in healthy individuals supports the notion of non-traditional risk factors influencing cardiovascular health and minimizes confounding effects of cardiovascular symptoms and disease, which impact affect.

In sum, our study proposes to examine associations between negative affect and subclinical disease in healthy individuals. We will extend findings of previous FMD studies, which in the aggregate, have been limited by small sample sizes, the inclusion of only a single gender, and/or use of a single predictor, primarily evaluating depression. Further, by examining healthy participants, who have not shown clinical signs of cardiovascular disease, we can postulate that the connection is unlikely to be biased by knowledge of poor health or traditional cardiovascular risk factors.

1.6 HYPOTHESES AND SPECIFIC AIMS

Influences on early atherosclerotic disease will be explored via associations between ultrasound findings and putative markers of risk. Specifically, hypotheses will focus on traditional cardiovascular risk factors, diagnosis and indictors of rheumatoid arthritis, and measures of negative affect in conjunction with segment-specific IMT, carotid outcomes and FMD, respectively.

1.6.1 Traditional Risk Factors and Segment-Specific IMT

First, it is hypothesized that considering traditional cardiovascular risk factors in conjunction with individual segments of IMT may provide better insight into the mechanisms of atherosclerotic development than looking at associations with overall IMT (average or maximum IMT), as is commonly done when examining associations with markers of risk.[71] Variations in cellar processes and hemodynamics throughout the vessel, as well as distinctions in the risk factors themselves, provide the rational for looking at segments separately.

While the associations between segment-specific carotid IMT and traditional cardiovascular risk factors have been explored in a handful of populations, additional research is needed. Specifically, investigating risk factor associations with all three segments in the same statistical model has not previously been done. Further, associations will be considered in a healthy sample without clinical signs of cardiovascular disease. It is suspected that each IMT segment will be differentially associated with individual traditional cardiovascular risk factors.

Thus, the purpose of the first report (Chapter 2) is to evaluate atherosclerotic risk factors and IMT by carotid artery segment in a sample of 453 healthy women aged 46-58 years. In addition to using standard linear regression models, a repeated measures model is used to provide evidence of segment-specific pathways for development of IMT while accounting for the interdependence of IMT measures within each woman.

1.6.2 Rheumatoid Arthritis and Carotid Outcomes

Second, it is hypothesized that middle-aged women with rheumatoid arthritis will have a higher burden of atherosclerotic carotid disease as indicated by IMT, plaque and arterial diameters, independent of traditional risk factors for cardiovascular disease, and that rheumatoid arthritis-related factors will be independently associated with these ultrasound indicators of carotid atherosclerosis. We also have reason to believe that simultaneously examining carotid IMT, plaque, and diameters may provide new insight into evidence of preclinical atherosclerosis and vascular aging in patients with rheumatoid arthritis.

The reason is unclear as to why IMT of the carotid arteries in patients with rheumatoid arthritis has been found to be higher than matched controls in many but not all studies.[84-86,89-93]

Given that IMT, plaque and diameters are interdependent,[74,75,241,242] it is possible that the stage of atherosclerotic development and vascular aging should be considered. Perhaps arterial dilatation in patients with rheumatoid arthritis is initially maintaining their level of IMT, and hence varying results are reported across studies. We look to support this theory by including lumen and interadventitial diameters, as well as IMT and plaque prevalence, as outcome measures.

Additionally, it is posited that the carotid measures will be associated with inflammatory markers and related indicators of rheumatoid arthritis severity and activity. Women who participated in the cardiovascular rheumatoid arthritis study were well characterized, thus a variety of rheumatoid arthritis features will be examined, including phlebotomy/laboratory measures, medication use and disease-specific measures.

Thus there are two aims of the second study (Chapter 3). We plan to compare carotid IMT, plaque and arterial diameters in 93 middle-aged women with rheumatoid arthritis to 93 healthy women matched for age, race and menopausal status. The second purpose is to evaluate rheumatoid arthritis-related factors, including inflammatory markers, in patients with rheumatoid arthritis for an association with early indicators of carotid disease.

1.6.3 Negative Affect and Flow-Mediated Dilation

Third, it is hypothesized that measures of anger, hostility, anxiety and depression will be negatively associated with FMD. We propose to add to the literature on FMD and psychosocial traits by looking at associations between multiple measures of negative affect and FMD in both men and women.

There is no definitive answer on how negative affect is linked to cardiovascular disease and if one might precede the other. Nonetheless, studying generally healthy individuals implies that the connection is unlikely to be biased by knowledge of overt disease. Further, given that endothelial dysfunction occurs very early in the cardiovascular disease continuum, some knowledge can be gained by looking at associations with brachial artery FMD, rather than IMT as is frequently done.

The purpose of the third study (Chapter 4) is to examine whether a link exists between negative psychosocial indices and brachial artery vasodilatation in 332 healthy older men and

women. Specifically, we plan to evaluate if depression, anxiety, anger and hostility are associated with FMD, while controlling for traditional cardiovascular risk factors.

1.6.4 Specific Aims

In sum, the objective of my dissertation is to examine associations between measures of atherosclerosis and vascular health with putative risk markers. My dissertation will address the following three specific aims:

1. Evaluate the extent to which IMT locations within the carotid artery will be differentially associated with traditional cardiovascular risk factors.
2. Assess whether diagnosis of rheumatoid arthritis and indicators of the disease will be associated with measures of carotid atherosclerosis and vascular health.
3. Identify measures of negative affect inversely associated with endothelial-dependent FMD of the brachial artery in both men and women.

2.0 SEGMENT-SPECIFIC EFFECTS OF CARDIOVASCULAR RISK FACTORS ON CAROTID ARTERY INTIMA-MEDIAL THICKNESS IN WOMEN AT MID-LIFE

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2.1 ABSTRACT

Objective: We investigated associations between segment-specific carotid intima medial thickness (IMT) and cardiovascular risk factors collected prior to menopause for insight into mechanisms of atherosclerosis development.

Methods and Results: Participants were 453 healthy women (aged 46-58 years) enrolled in a dietary and physical activity randomized clinical trial. Ultrasound scan measures were taken approximately 2.7 years after baseline in the common (CCA), bifurcation (bulb) and internal (ICA) carotid artery segments. When scanned, 84% remained premenopausal. In linear regression models, adjusted for age, menopausal status and intervention group, measures independently ($p<.05$) and positively associated were: baseline weight ($\beta=0.007$ per 5 kg), SBP ($\beta=0.008$ per 10 mmHg) and age ($\beta=0.02$ per 5 years) with CCA IMT; smoking ($\beta=0.08$), weight ($\beta=0.009$) and SBP ($\beta=0.02$) with bulb IMT; and apoprotein B ($\beta=0.01$ per 0.1 g/L) with ICA IMT. Differential effects in a repeated measures model with all three IMT locations showed these risk factors to have segment-specific positive associations. The effect of weight was strongest in the CCA, smoking and SBP were specific to the bulb and apoprotein B was strongest in the ICA segment.

Conclusion: Analyses indicate that cardiovascular risk factors may differentially affect IMT in the CCA, bulb and ICA segments of healthy middle-aged women.

Key Words: atherosclerosis, women, carotid artery, ultrasound, cardiovascular risk factors

2.2 INTRODUCTION

Carotid intima medial thickness (IMT) is considered a marker of early atherosclerosis, predicts future risk of cardiovascular disease, and has been found to be high in individuals with coronary heart disease and myocardial infarction (IMT $\geq$ 0.73 mm and $\geq$ 1 mm, respectively).[1;2] Among women of all ages, cardiovascular risk factors, such as high blood pressure, body mass index and cholesterol, are associated with average and maximum carotid IMT.[3-7] However, associations

with specific locations of thickening within the carotid artery are not as definitive.[8-13] Considering risk factors in conjunction with specific segments of IMT may provide better insight into the mechanism of atherosclerotic development. It is likely that differences in hemodynamics, cellular processes, and the risk factors themselves affect the development and progression of disease.[14-22] Additionally, each site may have its own predisposing factors[12;19] and biomechanical and biochemical pathways[16;20;23;24] may be acting in combination to influence atherosclerosis.

The purpose of this report is to evaluate atherosclerotic risk factors and IMT by carotid artery segment in a sample of 453 healthy women age 46-58 years. In addition to using standard linear regression models, we use a repeated measures model to provide evidence of segment-specific pathways for development of IMT. Investigating risk factor associations with all three segments in the same model has not been done in previous studies

2.3 METHODS

2.3.1 Participants

Participants were recruited from the 535 women (92% Caucasian) who participated in the Women's Healthy Lifestyle Project, an intervention trial designed to evaluate the efficacy of diet and physical activity in preventing weight gain and elevations in other cardiovascular risk factors.[25-27] Women were enrolled from 1992-1994, randomized to either lifestyle intervention or an assessment-only control, and followed over 54 months. Beginning in 1994, carotid ultrasound scans were offered to all participants and obtained in 453 women (85%).

To be eligible for enrollment, women had to be aged 44-50 years, pre-menopausal (defined as less than three months amenorrhea in the six months prior to the screening interview) and have a diastolic blood pressure (DBP) < 95 mmHg, body mass index (BMI) between 10 and 34, fasting glucose < 7.77 mmol, low density lipoproteins (LDL) between 2.07 and 4.14 mmol and total cholesterol between 3.26 and 6.72 mmol. Exclusion criteria included hysterectomy, hormone therapy, and use of anti-hypertensive, lipid-lowering, insulin, thyroid or psychotropic medications. Further details of the clinical trial are available in previously published articles.[25-27]

Both the clinical trial and the carotid exams were approved annually by the Institutional Review Board of the University of Pittsburgh. All participants provided written consent.

2.3.2 Clinical Measures

Baseline clinical measures included: systolic blood pressure (SBP), DBP, weight, BMI (kg/m^2), waist circumference, percent body fat (from dual energy x-ray absorptiometry scanner) and fasting total cholesterol, high density lipoproteins (HDL), HDL2, HDL3, LDL, triglycerides, apoprotein B and glucose. Dietary intake (kilocalories per day) was assessed by questionnaire.[28] Leisure time physical activity (kilocalories in the past week) was measured by interview.[29] Serum insulin was assessed at the 6-month follow-up visit rather than baseline. Standard enzymatic assays were used to measure total serum cholesterol, HDLs, triglycerides and glucose; LDL was estimated by the Friedewald equation; apoprotein B was determined via turbidimetric measurement; and serum insulin was determined by radioimmunoassay. Smoking was categorized as current, former or never. Additional details of the clinical measures are available in previous publications.[25-27]

At each follow-up visit and at the time of their scan, women were assessed for hormone therapy use (any in the prior year) and hysterectomy, and categorized as pre-, peri- or postmenopausal (perimenopausal = missing a bleeding cycle or taking hormone therapy for 3-11 cycles in the last year; postmenopausal = no bleeding cycle or taking hormone therapy for ≥12 consecutive cycles, or hysterectomy). Since most women remained premenopausal at scan time, menopausal status was dichotomized as premenopausal versus peri/post menopausal for analyses.

2.3.3 Carotid Ultrasound Measures

Carotid arteries were examined using an ultrasound scanner (Toshiba SSA-270A, Tustin, CA) equipped with a 5-MHz linear array imaging probe. With participant in supine position, arteries were viewed in transverse and longitudinal projections. For the common carotid artery (CCA) segment, both near and far walls were examined 2 cm proximal to the bifurcation (bulb). For the bulb area and internal carotid artery (ICA), measurements were taken of the far walls only (since

near walls cannot be consistently visualized). Digitized images were used to trace the medial-adventitial and intima-lumen interfaces across 1-cm lengths and compute IMT for each segment..[30] Average IMT was calculated from the mean of both the right and left carotid arteries of the three segments (8 locations total).

Our IMT reliability study (n=15)[30] reported correlation coefficients of 0.96 between sonographers and 0.99 between readers for average IMT, and was greater than 0.87 for individual segments across sonographers and readers.

2.3.4 Statistical Analysis

Descriptive measures are summarized as mean ± standard deviation unless otherwise noted. Intervention and control groups were compared using standard *t*-tests, Wilcoxon tests and chi-square tests. Analyses are reported on the pooled population, given that risk factors were collected prior to the intervention and to maximize available data.

Pearson correlation coefficients were calculated between normally distributed cardiovascular risk factors and each IMT measure. Spearman correlations were calculated for non-normal risk factors. To determine which traditional cardiovascular risk factors were independently associated with each IMT segment, variables with a p-value of ≤ 0.15 in univariate correlations were included in multivariable linear regression models. More parsimonious multivariable models were identified with a stepwise selection procedure, using $p < 0.05$ as the selection cutoff. Models were adjusted for age, intervention group and menopausal status at the time of the scan. The three segment models (i.e. CCA, bulb and ICA) suggested that risk factor associations with IMT varied according to the IMT measurement location.

To formally test these observed differences, a single model utilizing all three IMT measures was developed. Since the three IMT measures taken from each woman were not independent, a repeated measures model was used. This modeled each IMT value (i.e. 3 lines of data per person; one for CCA IMT, one for bulb IMT, and one for ICA IMT) as a function of risk factors and a 3-level variable indicating location. The correlation between each woman's IMT values, which is accounted for in the repeated measures model, was modeled using a compound symmetric correlational structure.[31] Covariates in the repeated measures model included all significant multivariable regression predictors, IMT measurement location, and examined

possible location by risk factor interaction effects. Significant interaction effects would signify segment-specific effects. All analyses were implemented using the SAS system for windows version 8.2 (SAS Institute, Cary, NC).

2.4 RESULTS

The women scanned were similar in clinical characteristics to those not scanned (data not shown). Clinical characteristics for the 453 participants are presented in Table 2-1 and were similar across control and intervention groups.

Scans were done approximately 2.7 years (range 0.7-7.6 years) after the baseline visit, when 84% of all subjects were still premenopausal. There were no significant differences in IMT measures between treatment groups, thus the groups were combined for remaining analyses (Table 2-2). Mean average IMT was 0.68 mm in the pooled group; IMT was highest in the bulb and lowest in the ICA segment. For each measure, results include only participants for whom both the right- and left-sided IMT measurement was available. Those with missing IMT (n=9) were similar in available data to those included (data not shown).

2.4.1 Univariate Associations

In unadjusted correlations, average IMT was positively and significantly ($p < 0.15$) associated with typical cardiovascular risk factors of age, weight, BMI, waist circumference, percent body fat, SBP, DBP, lipid measures (i.e. cholesterol, LDL, apoprotein B and triglycerides), insulin and physical activity; while HDL and HDL 2 showed a negative association. Examining arterial segments individually, IMT at all three locations was positively associated with baseline weight, BMI and waist circumference. Additionally, higher CCA IMT was associated with older age and higher SBP, DBP, percent body fat, apoprotein B and insulin; higher bulb IMT was associated with higher SBP, DBP, percent body fat, lipid measures, insulin and physical activity, and lower HDL and HDL2; and higher ICA IMT was associated with older age, higher lipid measures and insulin and lower HDL. In unadjusted bivariate analyses, significant positive associations were

found between average IMT and smoking, hormone therapy and hysterectomy. By segment, significant positive associations were found between CCA IMT and smoking, hormone therapy and hysterectomy, and between bulb IMT and smoking. Correlations between IMT across the three locations were moderate (range r=0.32 to 0.43).

Table 2-1: Participant Characteristics (n=453)*

Characteristics at Baseline			
Age (years)	46.9 ± 1.9	HDL3 (mmol/L)	1.23 ± 0.22
Weight (kg)	67.3 ± 9.9	LDL (mmol/L)	2.97 ± 0.57
BMI (kg/m^2)	25.1 ± 3.3	Apoprotein B (g/L)	0.74 ± 0.16
Waist Circumference (cm)	78.5 ± 8.5	Triglycerides (mmol/L)†	0.80 [0.60-1.10]
Body Fat (%)	33.1 ± 4.6	Glucose (mmol/L)	5.4 ± 0.4
Systolic BP (mmHg)	110.3 ± 12.7	Insulin (pmol/L)‡	99.8 ± 54.8
Diastolic BP (mmHg)	68.2 ± 8.2	Dietary Intake (kcal/day)	904 [504-1817]
Cholesterol (mmol/L)	4.90 ± 0.63	Physical Activity (kcal/wk)	0 [0-805]
HDL (mmol/L)	1.53 ± 0.33	Smoking: Current	44 (9.8)
HDL2 (mmol/L)	0.28 ± 0.16	Former	175 (39.1)
Characteristics at the Time of the Scan			
Peri/Post Menopausal	74 (16.4)	Hormone Therapy Use	94 (20.8)
Hysterectomy	14 (3.1)	Time Since Baseline (yrs)	2.7 ± 1.1

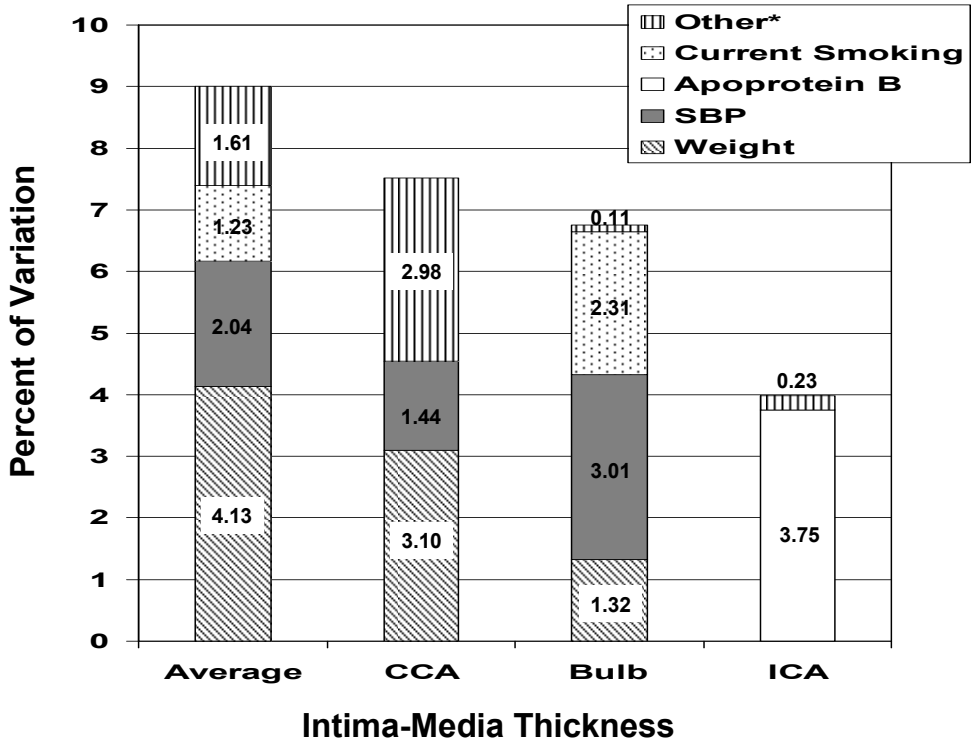
* Characteristics are summarized as mean ± standard deviation, median [inter-quartile range] or number (percentage). BMI=body mass index, BP= blood pressure, HDL=high density lipoproteins, LDL=low density lipoproteins.

† Triglycerides differed between control (0.75 [0.58-1.05]) and intervention (0.87 [0.62-1.13]) groups (p=0.02).

‡ Insulin was measured at 6 months after the baseline visit.

2.4.2 Multivariate Associations

Linear regression models were adjusted for intervention group, age and menopausal status at scan time. Risk factors showing significant univariate correlations with IMT (listed above) were included in respective models for average IMT and each location. Significant independent predictors of greater average IMT were higher weight, SBP, age and current smoking (Table 2-3). Higher weight, SBP, age and hysterectomy were independently associated with higher CCA IMT. Current smoking, weight and SBP were positively associated with bulb IMT. Higher apoprotein B was associated with higher ICA IMT. Comparing individual covariate percents of variation in each model (i.e. adjusted R^2) it was evident that weight was the largest contributor to average and CCA IMT, SBP was most prominent for bulb IMT and apoprotein B nearly the sole contributor to ICA IMT; Additionally, age at scan was 1.03% and 1.34% of the "other" category for average and CCA IMT, respectively (Figure 2-1).



CCA=common carotid artery, bulb=bifurcation, ICA=internal carotid artery
*Other=Intervention group, peri/postmenopausal status and age at scan for IMT locations, except CCA IMT for which hysterectomy is also included in the other variance

Figure 2-1: Percent of Variation in IMT in Average and Arterial Segments

Thus, differential effects could be seen across arterial locations. For example, a 10mmHg increase in SBP would produce a 0.008 mm increase in CCA IMT, a 0.022 mm increase in bulb IMT and no significant change in ICA IMT, although these comparisons do not take into account variations in wall thickness. To test the statistical significance of these observed differences, a single repeated measures linear model utilizing all three IMT measures (thus accounting for within woman and between women variability in IMT) was used to examine segment-specific associations.

2.4.3 Repeated Measures

The repeated measures model included significant multivariable predictors from the previous models (weight, SBP, smoking, apoprotein B and hysterectomy), control variables (intervention group, menopausal status and age), segment location (CCA, bulb, ICA), and risk factor by segment interaction terms. This was a single model with IMT as the dependent variable and multiple lines of data per woman, corresponding to each IMT segment. As expected, significant main effect associations with IMT were found across the three locations for weight (β=0.007 per 5 kg, $p<.001$), SBP (β=0.008 per 10 mmHg, $p=.008$), current smoking (compared to never β=0.037, $p=.005$) and apoprotein B (β=0.006 per 0.1 g/L, $p=.01$) with a trend for age at scan (β=0.02 per 5 years, $p=.08$). Results were the same for never and former smokers. Significant interaction effects (signifying segment-specific effects) were found between location and SBP ($p=0.02$), smoking ($p=0.01$) and apoprotein B ($p=0.02$). Specifically, simple effects estimates (Table 2-4) revealed a greater effect of weight and age in the CCA than in the ICA; and a greater effect for apoprotein B in the ICA than in the CCA. Smoking and SBP appeared to have an effect that was specific to the bulb segment. In addition, individual estimates showed significant segment associations between weight and apoprotein B and bulb location. There were no main or interaction effects for hysterectomy, which was likely a surrogate for age in this sample. Thus, significant and unique associations between baseline risk factors and segments exist even after accounting for the correlation in IMT at all three locations…

Table 2-2: Intima Media Thickness

Location	n	Control Mean ± SD	Intervention Mean ± SD	Pooled Group Mean ± SD	Pooled Group Range
Common Carotid Artery (mm)	451	0.68 ± 0.08	0.67 ± 0.08	0.67 ± 0.08	0.50 – 1.03
Carotid Bifurcation (mm)	452	0.74 ± 0.14	0.74 ± 0.16	0.74 ± 0.15	0.46 – 1.78
Internal Carotid Artery (mm)	447	0.62 ± 0.10	0.62 ± 0.10	0.62 ± 0.10	0.37 – 1.14
Average (mm)	444	0.68 ± 0.08	0.67 ± 0.08	0.68 ± 0.08	0.51 – 1.00

Table 2-3: Multivariable Regression Models of Intima-Media Thickness*

	Average		Common Carotid Artery		Carotid Bifurcation		Internal Carotid Artery	
Risk Factors	**β**	**C.I.**	**β**	**C.I.**	**β**	**C.I.**	**β**	**C.I.**
Intervention	-0.012	-0.026, 0.002	-0.011	-0.025, 0.004	-0.003	-0.031, 0.026	-0.007	-0.026, 0.012
Peri/Post Menopausal	0.003	-0.018, 0.024	-0.003	-0.026, 0.021	0.007	-0.036, 0.050	0.009	-0.020, 0.038
Age at Scan (5 yrs)	0.020†	-0.002, 0.038	0.024†	0.005, 0.042	0.011	-0.025, 0.047	0.002	-0.022, 0.026
Weight (5 kg)	0.008§	0.004, 0.012	0.007§	0.004, 0.011	0.009†	0.002, 0.017	-	-
SBP (10 mmHg)	0.009‡	0.003, 0.015	0.008‡	0.002, 0.014	0.022§	0.010, 0.033	-	-
Apoprotein B (0.1 g/L)	-	-	-	-	-	-	0.013§	0.007, 0.019
Current Smoking	0.029†	0.005, 0.052	-	-	0.079‡	0.031, 0.127	-	-
Hysterectomy	-	-	0.059†	0.010, 0.108	-	-	-	-
Adjusted R^2	0.10		0.09		0.07		0.03	

* Each of the four models included scan age, intervention group, menopausal status and any characteristic that was significantly correlated ($p<0.15$) with that IMT measure from univariate analyses. C.I.=95% Confidence Interval. A dash (-) indicates that the variable was not included/retained in the model for that segment because it was not significant.

†$p<0.05$; ‡$p<0.01$; §$p<0.001$

Table 2-4: Linear Repeated Measures Model of Intima-Media Thickness*

	Location					
	Common Carotid Artery		Carotid Bifurcation		Internal Carotid Artery	
Risk Factors	**β**	**C.I.**	**β**	**C.I.**	**β**	**C.I.**
Intervention Group	-0.012	-0.033, 0.008	-0.007	-0.027, 0.014	-0.006	-0.027, 0.015
Peri/Post Menopausal	-0.002	-0.034, 0.030	0.010	-0.022, 0.043	0.001	-0.031, 0.034
Age at Scan (5 yrs)	0.027†	0.001, 0.053	0.018	-0.008, 0.044	0.006	-0.020, 0.032
Weight (5 kg)	0.008‡	0.003, 0.014	0.009§	0.004, 0.015	0.004	-0.002, 0.009
SBP (10 mmHg)	0.006	-0.002, 0.015	0.016§	0.008, 0.025	0.002	-0.006, 0.011
Apoprotein B (0.1 g/L)	-0.000	-0.007, 0.007	0.007†	0.001, 0.014	0.011§	0.005, 0.018
Smoking: Current vs. Never	0.010	-0.025, 0.046	0.077§	0.041, 0.112	0.024	-0.011, 0.060
Current vs. Former	-0.001	-0.038, 0.035	0.073§	0.036, 0.110	0.023	-0.013, 0.060
Hysterectomy	0.031	-0.032, 0.094	-0.023	-0.086, 0.040	0.001	-0.063, 0.064

* This was a single model with IMT as the outcome measures and included all of the above covariates, IMT measurement location, and location by risk factor interaction effects. C.I.=95% Confidence Interval.
†$p<0.05$; ‡$p<0.01$; §$p<0.001$

2.5 DISCUSSION

Our research shows that cardiovascular risk factors may differentially influence atherosclerosis in carotid arterial segments in healthy middle-aged women. Further, repeated measures regression, which has not been used in previous studies of carotid segments, showed that these segment-specific associations were statistically significant when locations were directly compared.

Results of this study build on our previous findings of carotid atherosclerosis, where we reported on average IMT in a subset (n=292) of the study sample.[7] Similar associations between cardiovascular risk factors and average IMT were found. Additionally, segment-specific analyses were possible with the larger sample size.

2.5.1 Segment-Specific Carotid IMT

Unique to our study were the examination of IMT by carotid segment in healthy, primarily premenopausal women and the examination of risk factor and location interactions. Variations in methodology, including use of older women or heart clinic patients, make others' results of IMT by carotid segment challenging to compare.[8-13] Nonetheless, similarities between our results and those previously published include positive associations of SBP and body composition with CCA and bulb IMT,[10;11;13] lipids associated with bulb and ICA IMT,[9;11;13] and smoking with bulb IMT.[11] In contrast, neither lipids nor smoking were found to be associated with CCA IMT in our analyses.[9;11;13] Nor did we find older age associated with higher IMT across segments,[10] although this may be due to our narrow age range. Further, others have generally found the segments similarly associated with cardiovascular risk factors,[10;11;13] whereas we found distinct differences in risk factor and segment associations. Examining more diseased populations may have made it harder for others to tease out unique associations.

2.5.2 Theories on Differential IMT

Various mechanical and biochemical pathways and the risk factors themselves are most certainly acting in conjunction and possibly synergistically to determine the development and progression of atherosclerosis at any given site.[23;24] The moderate level of IMT we found in the CCA (compared to the ICA and bulb) is likely due to the lower sheer wall force[18;19;32] that is consistent with the laminar blood flow[33] in this location. Its association with higher weight is consistent with the positive relationship commonly found between body size and atherosclerosis and CCA IMT.[34] Diabetes, which is associated with obesity, is thought to decrease wall shear stress and is positively associated with CCA IMT.[35] Finally, aging, which itself is associated with increased CCA IMT,[3;11;13;32] is also inversely related to shear stress.[32]

Our positive association between apoprotein B and ICA IMT is consistent with research on sheer stress and lipids regarding influences on IMT. In the ICA segment, the smaller wall thickness, relative to the other segments, is likely attributable to the mix of laminar and oscillatory shear stress around the arterial wall.[19] Infiltration of lipids during extended contact of blood with the wall has been linked to areas of low shear stress,[36] which portions of this segment experience. Additionally, shear stress influences cellular processes such as increase in growth-regulatory factors,[16;22] expression of inflammatory molecules[24] and response of pro-oxidant processes,[33] which have also been linked to LDL and atherosclerosis,[37;38] although not specifically to the ICA segment.

Not surprisingly, the bulb, which connects the CCA and ICA segments, was associated with risk factors linked to each site. The bulb experiences the most oscillatory stress and turbulence as a result of reverse flow velocity components occurring during pulsatile flow[19;32;33] and consequentially exhibited greater wall thickness and was associated with higher SBP in our sample. The higher bulb IMT may also be a function of plaque, which is commonly found in this location,[39] and was evident in the bulb in 18.3% of our sample (post hoc; plaque defined as a distinct area protruding into the vessel lumen with > 50% thickness than surrounding area). The influence of shear stress on the extracellular factors of hypertension such as increased pressure,[16] decreases in vasoactive agents[17] and inflammatory effects,[14] would have the largest impact on the bulb segment. Research on increased residence time of blood[36] and adherence of platelets and macrophages to the arterial wall,[40] which influence lipid infiltration and plaque formation, is

consistent with our positive association between apoprotein B and bulb IMT. The increased residence time might also make the bulb more susceptible to the toxic components of cigarette smoke.[15]

Several study limitations should be mentioned. First, the wide time range between baseline and scan visits may have influenced results, although 91% of participants had scans within one standard deviation of the mean interval. Post hoc, we substituted 'time interval' and 'age at baseline' for the covariate 'age at scan' in regression models. Longer time interval replaced older age at scan as a significant predictor of higher average and CCA IMT, thus the model adequately adjusted for time. Second, risk factors explained only 3-9% of IMT segment variance, so other atherosclerotic mechanisms and pathways should continue to be examined. However, given the healthy status, young age, and low levels of IMT in our sample, the link between specific IMT segments and risk factors are noteworthy; and comparable to the 12-22% segment variance found by Wei and colleagues,[13] whose sample included diabetic subjects. Further, the enrollment exclusion criteria (i.e. requiring normal to high-normal ranges for DBP, BMI, glucose and lipids) likely reduced the effects of these risk factors and potentially increased the relative contribution of other risk factors (e.g. hysterectomy). Third, there may have been some influence of the intervention that was not detected in our analyses, since the clinical trial was successful in minimizing increased weight and LDL that traditionally occur with menopause.[25-27] Lastly, we examined a healthy, homogeneous sample of women, who may not be representative of the general population and thus may have underestimated associations. Future research should include other groups for a better understanding of segment-specific associations and their influences on clinical events.

In conclusion, cardiovascular risk factors traditionally associated with overall mean carotid IMT may differentially influence wall thickening of the carotid artery segments in middle-aged, healthy women, even after adjusting for other cardiovascular risk factors and wall thickness in all three segments.

2.5.3 Acknowledgements

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3.0 DO CAROTID ARTERY DIAMETERS MANIFEST EARLY EVIDENCE OF ATHEROSCLEROSIS IN WOMEN WITH RHEUMATOID ARTHRITIS?

Manuscript in preparation

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3.1 ABSTRACT

Objectives: To examine associations between rheumatoid arthritis (RA) diagnosis and characteristics and evidence of carotid atherosclerosis. We take a unique approach by evaluating lumen and interadventitial diameters, in addition to intima-media thickness (IMT) and plaque.

Methods: Ninety-three women with RA were matched with 93 healthy women by age, race and menopausal status. In cross-sectional analyses, common carotid artery measures were compared between groups and in association with measures of RA severity and activity.

Results: Mean age was 53.3 years. Median RA duration was 14 years. Lumen diameter (5.50 vs. 5.19 mm) and interadventitial diameter (6.92 vs. 6.61 mm) were significantly wider in patients than healthy women, respectively ($P < 0.001$ for both). Diagnosis of RA was independently associated with larger lumen ($\beta = 0.256$, $P < 0.01$) and interadventitial ($\beta = 0.261$, $P < 0.01$) diameters, after controlling for cardiovascular risk factors and IMT. Carotid IMT (0.70 vs. 0.71 mm) was similar, and prevalence of plaque in patients (21%) was higher but not statistically different than healthy women (15%). In patients with RA, we found positive associations between methotrexate dose and interadventitial diameter, between hypothyroidism and IMT, and between hypothyroidism and soluble endothelial adhesion molecule and plaque, independent of cardiovascular risk factors.

Conclusions: Women with RA have increased carotid artery diameters compared to healthy women. This may reflect premature vascular aging and be an early indicator of increased cardiovascular risk.

Key Words: rheumatoid arthritis, atherosclerosis, carotid arteries

3.2 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder that affects 1% of the U.S. population, particularly women of childbearing and early menopausal ages.[151,152] In patients with RA, cardiovascular disease is a major determinant of morbidity and is the leading cause of death.[157,159,243] To detect the burden of cardiovascular disease in RA, use of

noninvasive measures such as carotid ultrasound has been promoted to identify subjects with preclinical atherosclerosis.[84-96] Serum markers of inflammation are similarly being explored, given their link with cardiovascular disease in the general population and in patients with RA.[86,89,91,161,163,164,166-168,197,244] Ultimately, these measures might identify patients most appropriate for aggressive cardiovascular risk factor reduction and be used to monitor efficacy of interventions to reduce risk.

Carotid ultrasound measures of lumen diameter, interadventitial diameter, intima-media thickness (IMT) and plaque have been evaluated as early indicators of systemic atherosclerosis in non-RA populations and are informative measures of vascular health.[73-76] Changes in these four carotid structures are interdependent,[241,242] occurring as both part of the natural aging process and in response to insult or injury. Arterial dilatation (i.e. vascular remodeling) is considered to begin as an adaptive response to changes in wall shear stress concomitant with increased wall thickness,[63,74,75,108-110] but ultimately, larger carotid diameter is a marker of vascular aging and is associated with cardiovascular risk factors and increased risk of myocardial infarction.[61,63,75,111,112] IMT is increased in individuals with coronary heart disease and myocardial infarction, is associated with numerous cardiovascular risk factors, and predicts future risk of cardiovascular events.[1,2,4] Carotid plaque has been linked with specific cardiovascular risk factors and ischemic stroke.[60,107]

Thus far, carotid ultrasound findings in RA subjects have generally not reported outcome measures of lumen or interadventitial diameter. While studies typically find common carotid IMT to be higher in patients with RA compared to controls,[84-89,95] several studies have found no difference[90-92] or the opposite results.[94] Looking at carotid diameter in conjunction with IMT and plaque may provide new insight into these discrepancies. Further investigation of indicators of RA severity and activity with atherosclerosis, particular diameters measures, is also warranted.

The purpose of our study was twofold. We sought to i) compare carotid arterial diameters, IMT and plaque in women with RA to matched healthy women; and ii) evaluate the associations between RA-related factors and indicators of early carotid disease in patients with RA.

3.3 MATERIALS AND METHODS

3.3.1 Participants

Women with RA volunteered for a study of cardiovascular disease prevalence and risk factor assessment from a University-based outpatient rheumatology practice and the University of Pittsburgh Medical Center (UPMC) Arthritis Network Disease Registry. (Registry participants, who gave signed consent to be notified in writing of research opportunities, were comprised of RA patients from the University and two university-affiliated, community-based practices.) Eligible women (n=104) were greater than 30 years old and met American College of Rheumatology criteria for RA [245] for at least two years.

Healthy participants were selected from 535 women who participated in the Women's Healthy Lifestyle Project, an intervention trial examining cardiovascular risk factors during menopause.[25,27] At enrollment, these women were aged 44-50 years with no indication of cardiovascular disease. As part of an ancillary study, 85% of participants had carotid ultrasound scans an average of five years after enrollment, during which some changes in health status developed (e.g. hypertension, diabetes). Healthy participants eligible for the current analysis completed a scan (n=453) and were randomized to the assessment-only (i.e. non-intervention) arm of the study (n=275).

Patients with RA and healthy participants were matched by age (± 5 years), menopausal status and race. Arterial diameter measures were not available on all subjects because this protocol component was not initially included and was unobtainable post-hoc. The final sample consisted of 93 patients with RA and 93 healthy women with IMT and plaque measures, and 78 patients and 90 healthy women with diameter measures.

The RA cardiovascular study, intervention trial, and carotid scan protocols were approved annually by the Institutional Review Board of the University of Pittsburgh. All participants provided informed written consent.

3.3.2 Clinical Measures

Clinical data and traditional cardiovascular risk factors[81] were obtained through patient interview and physical examination (Table 3-1). Fasting blood samples were assayed at the Department of Epidemiology Nutrition Laboratory. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were averaged from two seated measurements; height and weight were used to calculate body mass index (BMI; kg/m^2). Women were assessed for hormone therapy use (any in the prior year), hypertension (SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg [246] and/or antihypertensive drugs), diabetes (glucose $\geq$ 6.99 mmol/L (126 mg/dL) [247] and/or medication) and hypercholesterolemia (cholesterol $\geq$ 5.17 mmol/L (200 mg/dL) [248] and/or lipid-lowering drugs). Menopause status was based on participant self-report, plus endogenous hormone levels in patients with RA. For analysis, menopause status was dichotomized as postmenopausal (no bleeding cycle or taking hormone therapy for $\geq$ 12 consecutive cycles, or hysterectomy) versus not (i.e. pre- or perimenopausal). Measures were collected within approximately one month and one year of the ultrasound scan for patients with RA and healthy participants, respectively, because protocols regarding timing of visits varied for the two studies.

3.3.3 Rheumatoid Arthritis Features and Inflammatory Markers

Patients with RA were also evaluated for the characteristics listed in Table 3-2. Soluble endothelial adhesion molecule (sE-selectin) was measured using a high sensitivity quantitative sandwich enzyme assay and determined colorimetrically; soluble intercellular adhesion molecule (sICAM-1) was measured by an ELISA assay and determined by colorimetric reaction (assays from R&D Systems, Minneapolis, MN; completed at the Laboratory for Clinical Biochemistry Research, University of Vermont, Burlington, VT). Additional laboratory variables were measured by routine methods

3.3.4 Carotid Ultrasound Measures

Arteries were examined using an ultrasound scanner (Toshiba SSA-270A, Tustin, CA) equipped with a 5-MHz linear array imaging probe. With participant in the supine position, left and right arteries were viewed at end diastole in transverse and longitudinal projections.

Common carotid near and far walls were examined 2 cm proximal to the bifurcation. Digitized images were used to trace the media-adventitial and intima-lumen interfaces across 1-cm lengths and IMT was computed as the average of both arteries. Lumen diameter was measured as the distance between the lumen-intima interfaces of the left common carotid artery, where the walls were parallel and there was no evidence of plaque. Interadventitial diameter was calculated as the distance between the adventitia-media interfaces.

Carotid arteries were evaluated for the presence of eccentric focal plaque in the bifurcation, internal and common areas. Plaque was defined as a distinct area protruding into the vessel lumen with ≥ 50% thickness than the surrounding area. Degree of plaque was categorized as no/small plaque (< 30% of vessel diameter), and medium/large/multiple plaques (≥ 30% stenosis). This categorization captured the severity distinction of our sample more appropriately than either dichotomizing as plaque "any versus none" or totaling the number of plaques.

Scans were completed by trained technicians in the same lab and read later by technicians blinded to study group. The lab has shown IMT, plaque and diameters to be reliable measures of carotid atherosclerosis. For each measure, the reproducibility coefficient was ≥ 0.82 and the intraclass correlation coefficient was ≥ 0.84.[30,249]

3.3.5 Statistical Analysis

In this cross-sectional study, patients with RA and healthy women were compared using unpaired tests. Univariate associations between clinical measures and carotid outcomes were examined via Pearson/Spearman correlation, *t*-tests and chi-square tests, as appropriate. Because of their skewed distribution inflammatory markers were both log transformed and divided into quartiles.

In multivariable analyses, dependent (outcome) measures were IMT, lumen diameter, interadventitial diameter (all linear regression), and plaque (logistic) with RA diagnosis as the

independent variable. Models were adjusted for cardiovascular risk factors determined via univariate associations. In diameter models, height was included to account for body size. To control for potential confounding between the two groups, diameter models controlled for IMT, while the IMT model was adjusted for interadventitial diameter. For RA-only regression, each RA characteristic was individually added to the above determined multivariable cardiovascular risk factor model for each outcome.

All analyses were implemented using the SAS system for windows version 8.2 (SAS Institute, Cary, NC). Values of $p \leq 0.05$ were considered significant.

3.4 RESULTS

The combined group was 97% Caucasian, 68% postmenopausal and 53.3 ± 3.9 years old. Patients with RA had higher BMI, blood pressure and triglycerides, and were more likely to be never smokers and have been diagnosed with hypertension (Table 3-1). Healthy women had higher fasting glucose levels than patients with RA. Patients with RA had larger lumen and interadventitial diameters than healthy participants. There was no difference in IMT between subjects. The higher plaque prevalence in patients with RA was not statistical different from healthy women.

For patients, average age at RA diagnosis was 37 years, with a median disease duration of 14 years, and 71% reported experiencing morning stiffness, lasting one hour on average (Table 3-2). Patients with RA who had diameter measures were generally similar to patients who did not have diameter measures (data not shown).

Table 3-1: Comparison of Healthy Women and Patients with Rheumatoid Arthritis (RA)*

Characteristic	Healthy Women (n=93)	Patients with RA (n=93)	P-value
Clinical Measures:			
Mean age, range (years)	53.2, 46.7 – 58.7	52.9, 42.0 – 60.2	0.62
Current/Former/Never smokers (%)	7 / 51 / 35	12 / 31 / 50	0.01
Diabetes	2 (2%)	3 (3%)	0.65
Glucose (mmol/L)	5.61 ± 0.57	4.98 ± 1.07	<0.001
Body mass index (kg/m^2)	25.8 ± 3.4	28.4 ± 6.5	0.001
Hypertension	11 (12%)	27 (29%)	0.004
Systolic blood pressure (mmHg)	110.5 ± 12.7	120.5 ± 17.9	<0.001
Diastolic blood pressure (mmHg)	70.8 ± 7.9	77.3 ± 10.4	<0.001
Hypercholesterolemia	51 (55%)	59 (63%)	0.23
Total cholesterol (mmol/L)	5.38 ± 0.78	5.30 ± 0.83	0.26
High density lipoproteins (mmol/L)	1.61 ± 0.36	1.52 ± 13.3	0.12
Low density lipoproteins (mmol/L)	3.22 ± 0.69	3.11 ± 0.78	0.34
Triglycerides (mmol/L)	0.94 [0.77, 1.39]	1.32 [0.98, 1.70]	<0.001
Current hormone therapy	36 (44%)	35 (38%)	0.36
Carotid ultrasound measures:			
Lumen diameter (mm)	5.19 ± 0.47	5.50 ± 0.60	<0.001
Interadventitial diameter (mm)	6.61 ± 0.54	6.92 ± 0.67	<0.001
Intima-media thickness (mm)	0.71 ± 0.08	0.70 ± 0.10	0.94
Presence of plaque	14 (15%)	19 (21%)	0.29

* Characteristics are summarized as mean ± standard deviation or median [inter-quartile range] unless otherwise indicated.

Table 3-2: Additional Characteristics for Women with Rheumatoid Arthritis*

Characteristic	
Age at rheumatoid arthritis diagnosis (years)	37.3 ± 10.9
Disease duration since diagnosis (years)	14 [7, 23]
Rheumatoid factor positive	64 (73%)
Morning stiffness	65 (71%)
Extra-articular disease	73 (78%)
Health Assessment Questionnaire Disability Index [208]	0.75 ± 0.57
Number of prior disease modifying antirheumatic drugs [DMARDs] (ever)	3.5 ± 1.9
Current nonsteroidal anti-inflammatory drug use	69 (75%)
Current prednisone use	38 (41%)
Daily prednisone dose (mg)	5.4 ± 3.5
Current methotrexate use	57 (61%)
Weekly methotrexate dose (mg)	14.5 ± 5.1
Current tumor necrosis factor inhibitor use	35 (38%)
Hypothyroidism	12 (13%)
Erythrocyte sedimentation rate [ESR] (mm/h)	10.0 [5.0, 24.5]
High sensitivity C-reactive protein [hsCRP] (mg/L)	5.6 [2.2, 13.0]
Fibrinogen (μmol/L)	9.3 [7.6, 10.8]
Plasminogen activator inhibitor type 1 [PAI-1] (μg/L)	19.3 [10.4, 45.0]
Soluble intercellular adhesion molecule [sICAM-1] (ng/ml)	271 [232, 335]
Soluble endothelial adhesion molecule [sE-selectin] (ng/ml)	40.3 [29.7, 64.5]

* Characteristics are summarized as mean ± standard deviation or median [inter-quartile range] unless otherwise indicated.

3.4.1 Regression Models – Rheumatoid Arthritis vs. Healthy Women

Based on univariate analyses, the following covariates were included in multivariable models: hypertension (positively associated with all carotid outcomes), hypercholesterolemia (positively associated with plaque) and glucose (positively associated with IMT). [Hypertension and

hypercholesterolemia were chosen over individual blood pressure and lipid measures that showed positive univariate associations but would not capture women under treatment. However, this principle was not possible for glucose because of the extremely small number of women meeting criteria for diabetes.] Age and race (both positively associated with IMT) were included as demographic characteristics in the models.

Table 3-3: Multivariable Regression Models of Carotid Artery Outcomes*

	Dependent Outcomes			
	Lumen Diameter (mm)	Interadventitial Diameter (mm)	Intima-Media Thickness (mm)	Plaque
Risk Factor	**β**	**β**	**β**	**OR**
Rheumatoid arthritis diagnosis	0.270‡	0.275‡	-0.015	1.66
Age (year)	0.012	0.010	0.002	1.12†
African American race	-0.199	-0.168	0.088†	2.61
Hypertension	0.274‡	0.290‡	-0.013	1.23
Hypercholesterolemia	-0.013	0.003	0.005	2.46†
Glucose (mmol/L)	-0.022	-0.027	0.014†	1.37
Height (cm)	0.017‡	0.017‡	–	–
Intima-media thickness (mm)	1.021†	3.084§	–	–
Interadventitial diameter (mm)	–	–	0.071§	–

* The first six risk factors were included in the model for each dependent outcome; additionally, height and intima-media thickness were included in the diameter models, while interadventitial diameter was included in the intima-media thickness model. β=regression coefficient, OR=odds ratio.
† $p<0.05$; ‡ $p<0.01$; § $p<0.001$

In regression analyses adjusted for these cardiovascular risk factors and IMT, RA diagnosis was positively associated with wider lumen and interadventitial diameters (Table 3-3). Thus, on average, diameters for patients with RA were 0.27 mm wider than controls. These results did not change if IMT was omitted from the models. In contrast, IMT was similar between groups, whether or not diameter was in the model. Similarly, RA diagnosis was not associated with the

presence of plaque in multivariable models. Results were essentially the same when other cardiovascular risk factors were considered as covariates, such as substituting SBP for hypertension or adding smoking to the models. Hypertension was the only cardiovascular risk factor associated with diameters, while African-American race (plus age when interadventitial diameter was omitted) was associated with IMT. Age and hypercholesterolemia were positively associated with presence of plaque

3.4.2 Rheumatoid Arthritis Factors and Carotid Outcomes

Unadjusted analyses in patients with RA showed average daily dose of prednisone was positively associated with lumen and interadventitial diameters (Spearman's r = 0.46, P = 0.008; and r = 0.47, P = 0.006, respectively). RA duration was positively associated with IMT (Spearman's r = 0.21, P = 0.04), but this relationship disappeared after controlling for age. Average weekly dose of methotrexate was positively associated with interadventitial diameter and IMT (Spearman's r = 0.33, P = 0.02; and r = 0.33, P = 0.01, respectively). The highest quartile of values for hsCRP, sE-selectin and PAI-1 were associated with a small but non-significant increase in diameter and plaque measures. There was a significant negative association between continuous sICAM-1 and IMT (Spearman's r = -0.23, P = 0.03).

In multivariable models in women with RA, no indicator of RA activity was associated with lumen diameter when individually added to the model controlling for cardiovascular risk factors. For RA women currently taking methotrexate, weekly dose was positively associated with interadventitial diameter (Table 3-4); however, this association was no longer significant when age was replaced with duration of disease and age at diagnosis, or when IMT was added to the multivariable model. Current prednisone use was associated with decreased IMT, while hypothyroidism was associated with increased IMT in separate models. These associations remained the same when interadventitial diameter was included, or if duration of disease and age at diagnosis were substituted for age. Plaque was positively associated with sE-selectin and hypothyroidism in individual models controlling for traditional cardiovascular risk factors. No other RA feature was significantly associated with carotid outcomes in multivariable models; however, other inflammatory markers and medications showed significant trends ($P < 0.15$).

Table 3-4: Multivariable Regression Models of Significant Rheumatoid Arthritis Factors Associated with Carotid Outcomes in Patients*

	Dependent Outcomes				
	Interadventitial Diameter (mm)	Intima-Media Thickness (mm)		Plaque	
Risk Factor	**β**	**β**	**β**	**OR**	**OR**
Age (year)	0.022	0.004	0.003	1.29	1.16
African American race	0.818	0.101	0.121†	3.65	–
Hypertension	0.166	0.013	0.008	0.98	1.43
Hypercholesterolemia	0.120	0.018	0.026	2.23	2.68
Glucose (mmol/L)	0.023	0.010	0.011	1.19	1.23
Height (cm)	0.019	–	–	–	–
Weekly methotrexate dose (mg)	0.040†	–	–	–	–
Prednisone user	–	-0.057‡	–	–	–
Hypothyroidism	–	–	0.085‡	7.25†	–
Log of sE-selectin (ng/ml)	–	–	–	–	3.09†

* The first five risk factors were included in each individual model, plus height for diameter; the remaining factors were individually added in respective models for each outcome (interadventitial diameter, intima media thickness and plaque). All significant associations are displayed, resulting in varying number of columns per outcome. β=regression coefficient, OR=odds ratio, sE-selectin=soluble endothelial adhesion molecule. Race was dropped from one plaque model because of missing data.

† $p<0.05$; ‡ $p<0.01$

3.5 DISCUSSION

This study is the first to describe wider carotid artery lumen and interadventitial diameters in women with RA compared with matched healthy women, independent of traditional cardiovascular risk factors and IMT. In the women with RA, positive associations were found between methotrexate dose and interadventitial diameter, between hypothyroidism and IMT, and

between sE-selectin and hypothyroidism and plaque, each independent of cardiovascular risk factors. These findings may indicate evidence of accelerated vascular aging and early atherosclerotic risk among women with RA

3.5.1 Vascular Adaptation or Rheumatoid Arthritis-Specific Effrect

Vascular remodeling is a dynamic, early response to risk factors, wall thickening and increased wall shear stress.[63,74,75,108-110] Concomitant increase of carotid diameters allows lumen cross-sectional area and arterial flow to be kept constant and maintains or decreases IMT by distributing it over a larger area. Since the artery has a limited capacity to dilate, continued increase in IMT and plaque will ultimately result in reduction of blood flow.[73,74,110,199] Arteries unexposed to risk factors and free of atherosclerosis maintain a normal size with aging in the general population.[75] Thus, enlarged diameters can be considered a sign of vascular adaptation and a marker for early atherosclerosis.

Alternatively, our results may reflect a unique, RA-related atherogenic effect on arterial diameters (rather than traditional vascular adaptation) that is specific to RA itself rather than a result of remodeling in response to thickened IMT. Perhaps the underlying autoimmune disease process, its treatment, or an interplay between the two accounts for a change in the evolution of atherosclerotic vascular disease in these patients. In contrast, diameter differences were not found among treatment groups or compared to controls in separate studies.[92,250] While we found links between RA-related factors and carotid outcomes, our study does not elucidate the pathway by which these changes occurred. However, published evidence to date suggests chronic systemic inflammation may lead to endothelial dysfunction and accelerated atherogenesis in patients with RA.[166-168,244]

3.5.2 Comparisons with Existing Literature

Our finding of similar common carotid IMT between groups is consistent with several studies of RA patients in the United States, where the average age was less than 50 or greater than 60 years old. A large study of RA men and women found no difference in carotid IMT compared with controls.[91] Another reported no difference in IMT between women with RA, women with

systemic lupus erythematosus, and controls, and found that patients with lupus had larger carotid artery diameters than controls.[92] In a small Italian study, internal carotid IMT was higher in patients with RA, while common carotid IMT was similar to controls.[90] Finally, Roman and colleagues found IMT in patients with RA to be less than controls.[94] Perhaps arterial dilatation in RA patients in these studies is maintaining a stable IMT.

Differences in study populations may explain some of the discrepancies between our IMT results and previous investigators, who found higher common carotid wall thickness in patients with RA compared to controls.[84-89,95] In contrast to the subjects we describe, these studies generally report on slightly older patients with a wider age spectrum (average age 55, range 36-85 years), and enrolled both men and women. Research indicates that IMT increases with age,[75,198] with menopausal status independent of age,[146] and is greater in men.[4] Thus, our sample of slightly younger women with a narrower age range may have been studied at an earlier point on an arterial remodeling continuum than those described elsewhere, with our RA women having vessel wall dilatation and thus preserved IMT. A study of IMT progression [93] in women with RA found an accelerated rate of annual wall thickening when compared to healthy controls. If followed over time, a divergence in IMT may be seen between our subject groups as well.

Given our results, it can not be said with certainty whether all women with RA have greater IMT than healthy controls, or if this difference is only observed in women of certain ages or ethnicities, at particular time points in the course of disease (RA or atherosclerosis), or in conjunction with health and population of controls. Based on patient ages, results suggest a curvilinear relationship, where IMT is similar between patients and controls at younger and older ages. Interestingly, carotid IMT in our healthy women (mean 0.71 mm) was much higher when compared to several previous studies (common carotid IMT 0.58-0.68 mm), while IMT in our patients with RA was similar (range 0.64-0.77 mm).[84-86] Thus, while there is evidence that IMT may differ between patients with RA and controls, the course of progression may be distinct from other conditions where increased IMT is seen at a very early stage of atherogenesis.

In regards to carotid plaque, several studies reported no difference in plaque prevalence,[84-86,89,91] while others have found increased carotid plaque in patients with RA.[88,90,92,94,95] Sample size may have limited our ability to detect between groups a statistically significant difference in plaque, which was clinically higher in our women with RA. Plaque frequently occurs in areas of transition or turbulence (e.g. internal carotid artery or

bifurcation),[110] therefore variations in methodology (i.e. area of plaque assessment) may be influencing results.

3.5.3 Rheumatoid Arthritis Factors

We found several RA-related risk factors associated with measures of carotid atherosclerosis. Others also have reported a positive association between duration of disease and IMT.[85,86,95,171,196,197] As was found here, RA medications may be positively [94,169] or negatively [161] associated with atherosclerosis. Incongruencies are possible because pharmacological treatment can be interpreted as a marker for RA severity, contributing adversely to cardiovascular risk factors (e.g. hypertension) or conversely, exerting a protective effect by reducing RA-related systemic inflammation.[163,203-205] Type, effectiveness and side effects of medications influence when and how they are prescribed, which may also affect their associations with atherosclerosis. Additionally, studies vary on whether current, cumulative, or multiple medication exposure is considered. Previous studies have linked hypothyroidism with IMT and plaque in patients with RA[171,196] and with accelerated atherosclerosis in non-RA patients.[251] Impaired lipid metabolism/profiles, obesity, high homocysteine levels and effects of thyroid hormones on vascular cells are all potential pathways for this link.

While the relationship between inflammatory markers and atherosclerosis in patients with RA has been inconsistent across studies, logical pathways by which systemic inflammation contributes to atherogenesis in patients with RA have been reviewed.[166-168] The cross-sectional nature of our study and others does not capture the changing inflammatory milieu over time in patients with RA. Further, the clinical course of RA is exceedingly variable, ranging from mild, self-limiting arthritis to rapidly progressing multi-system inflammation.[152] Like others,[85,94] we did not find a relationship between either CRP or ESR and atherosclerosis, although a positive association between these and IMT and/or plaque has been noted elsewhere.[91,93,166,197] Others have reported a positive association between sICAM-1 and IMT and between sE-selectin and plaque.[161] Similarly, we found a positive association between sE-selectin and plaque. Inflammation in RA has been most consistently linked to endothelial function, another early marker of atherosclerosis.[166]

3.6 CONCLUSIONS

While the strength of our study was the use of ultrasound measures novel for patients with RA, namely arterial lumen and interadventitial diameters, it was unfortunate that 18 subjects were missing these measures. Nonetheless, by examining carotid diameter we were able to demonstrate early signs of vascular aging and atherosclerotic risk in women with RA.

In conclusion, our study of patients with RA is unique and noteworthy. We used a carotid ultrasound technique not typically reported for detecting vascular aging in an RA sample. We found greater carotid arterial diameters in women with RA compared with matched healthy women, whereas IMT was similar. We propose that lumen and interadventitial diameters may be more sensitive measures of early atherosclerosis in women with RA than ultrasound measurement of IMT and plaque, and may explain previous discrepancies. Carotid diameter vascular remodeling may be an important step in atherogenesis in this autoimmune disease and should be included in measures of early atherosclerotic cardiovascular disease in patients with RA.

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4.0 IS BRACHIAL ARTERY FLOW-MEDIATED DILATION ASSOCIATED WITH NEGATIVE AFFECT IN MEN AND WOMEN?

Manuscript in preparation

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Running Head: Flow-Mediated Dilation and Negative Affect

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4.1 ABSTRACT

Background: A limited number of studies have examined associations between negative affect and endothelial dysfunction via brachial artery flow-mediated dilation (FMD).

Purpose: To examine which psychosocial measures are associated with FMD.

Methods: FMD was examined in 332 healthy older adults. Measures included Beck Anxiety and Depression Inventories, Cook-Medley Hostility Scale and Spielberger State-Trait Anger Expression Inventory (Anger In, Anger Out and Trait Anger).

Results: Mean age was 60.5 ± 4.8 years, 76% of participants were Caucasian and 50% were female. FMD was greater in women compared to men (6.17 vs. 4.07, $P < 0.001$). Women reported significantly greater Anxiety ($p < .001$) and men reported greater Hostility ($p=0.004$). In separate multivariable linear regression models controlling for cardiovascular risk factors, plus current hormone therapy for women, smaller FMD was associated with higher Anger In for women ($\beta=-0.223$, $p=0.04$) and with higher Hostility ($\beta=-0.108$, $p=0.02$) and Trait Anger ($\beta=-2.360$, $p=0.12$) for men.

Conclusion: Endothelial dysfunction, as indicated by less vasodilatation of the brachial artery, is positively associated with cognitive-type measures of negative affect in healthy older adults. Thus, associations between negative affect and cardiovascular health may be apparent very early in the disease process.

Key Words: flow-mediated dilation, endothelial dysfunction, anger, hostility, depression

4.2 INTRODUCTION

It has long been postulated that negative emotions play a role in the etiology of physical illness; and multiple physiological and behavioral pathways have been identified. In particular, there is strong evidence linking negative affect and cardiovascular disease (reviewed by[183-187,252]), whether clinical events [55,57,175,209-215] or subclinical outcomes [214-220] are examined. Further, certain psychological traits (e.g. anger and hostility,[172] and possibly depression[173]) can be considered chronic or dispositional (rather than acute or episodic); therefore their influence could be comparable to the enduring effects of some traditional cardiovascular risk factors such as

hypertension or obesity. Collectively, these emotions have been referred to as negative affectivity, with feelings of hostility, anger suppression and trait anger being categorized as cognitive-type, while aggression, anger expression and anxiety are more behavioral in nature.[173,174]

Identifying at risk individuals early in the disease progression allows for timely intervention. Associations between negative affect and cardiovascular disease are found more consistently in healthy populations.[173] Brachial artery flow-mediated dilation (FMD) is an easy to assess, non-invasive technique for evaluating endothelial dysfunction,[78,122,123] which is one of the earliest signs of insult in the cardiovascular disease continuum. It is associated with multiple cardiovascular risk factors and outcomes and has been suggested as a biomarker of disease, similar to IMT and coronary calcification (reviewed by [64,120,121]). Evidence of endothelial dysfunction has been found prior to the development of measurable atherosclerosis.[138] FMD is related to traditional cardiovascular risk factors,[78,113-117] provides a surrogate marker for health of the coronary arteries,[130,132,133] and is an independent predictor of cardiac risk and events in clinical populations.[65,134,135]

While numerous studies have examined associations between negative affect and subclinical cardiovascular disease,[214-220] only a limited number have examined the association with early endothelial dysfunction via FMD.[128,221-225] Three small studies ($n < 39$) found impaired vasodilatation associated with concurrent clinical depression or a history of major depression;[221-223] and one other found this association in coronary heart disease patients with high depressive symptoms.[225] Another, looking at the effects of mental stress, found an inverse association between hostility and FMD in healthy adults.[224] A larger study of postmenopausal women that considered multiple measures of negative affect via two psychosocial clusters, Anxiety/Depression and Type A/Anger, reported an inverse association between FMD and these two factors.[128] In the aggregate, these studies have been limited by small sample sizes, the inclusion of only a single gender in the one larger study, and/or use of a single predictor, primarily evaluating depression.

Further investigation regarding associations between FMD and several types of negative affect in both men and women is warranted. Studying generally healthy individuals, who have not shown clinical signs of cardiovascular disease, implies that the connection is unlikely to be biased by knowledge of poor health. We chose measures previously associated with subclinical

cardiovascular disease, specifically, depression, anxiety, hostility and anger. Our study examined whether a link exists between negative emotions and FMD in healthy older adults while controlling for traditional cardiovascular risk factors.

4.3 METHODS

4.3.1 Participants

Participants were drawn from the 464 men and women who enrolled from 1998-2000 in the Pittsburgh Healthy Heart Project, an observational study examining the bio-behavioral determinants of subclinical cardiovascular disease in healthy adults. The overall study protocol included ambulatory blood pressure monitoring, cardiovascular reactivity, interviewer- and self-administered questionnaires, anthropometric measurements, blood and urine sampling, and ultrasound measures, including brachial artery FMD. These assessments occurred over several visits during approximately a 5-month period with several of the measures repeated during a three year follow-up phase. Additional details of the study have been published.[228,253,254]

Eligibility criteria included community dwelling and 50-70 years of age. Additionally, women had to be postmenopausal, defined as cessation of menses, use of hormone therapy or a hysterectomy at least six months prior to enrollment. Exclusion criteria included current use of anti-hypertensive or lipid-lowering medications, history of heart disease or stroke, diabetes requiring insulin, other severe chronic disease (e.g. renal, liver, neurological), cancer treatment in past 6 months, or excessive alcohol consumption (5 or greater portions 3 or more times per week). Individuals were also excluded if they were unable to wear a portable blood pressure cuff for several days (e.g. body size, occupation) or had high blood pressure (systolic > 180 mmHg or diastolic > 110mmHg). Brachial artery ultrasound scans were not completed on 132 participants because of study drop out prior to scanning visit (n = 82), scheduling problem (n = 15), unreadable data (n = 11), study exclusion determined just after screening (n=10), ineligibility because of contraindication for brachial artery testing (n = 4), or other reason (n = 10). The final sample of those who completed a brachial artery scan included 168 men and 164 women.

Compared to participants who had a brachial artery scans (n = 332), those who were not scanned (n = 132) were statistically more likely to be current smokers, less likely to be married and had higher total cholesterol and high density lipoprotein levels (data not shown). Individuals who dropped out after the initial visit did not complete psychosocial measures; however, for those who did (n=37-53) symptoms of negative affect were similar to scan subjects.

All subjects provided informed written consent. The study was approved annually by the Institutional Review Board at the University of Pittsburgh.

4.3.2 Clinical Measures

A nurse collected clinical measures and medical history screening approximately 2 months prior to the ultrasound scan. This initial visit included a blood draw, anthropometric measures and blood pressure assessment after 12 hours fasting. Standard enzymatic assays used to measure total serum cholesterol, high density lipoproteins, triglycerides and glucose were completed at the Department of Epidemiology Nutrition Laboratory at the University of Pittsburgh. Low density lipoproteins were estimated by the Friedewald equation. Systolic and diastolic blood pressure was obtained while participants were seated for 30 minutes and calculated as the average of the second and third measurement. Height and weight were used to calculate body mass index (BMI: kg/m^2) and waist circumference was measured at the narrowest width. Smoking was categorized at enrollment as current, ever and never.

4.3.3 Flow-Mediated Dilation

Brachial arteries were examined using an ultrasound scanner (Toshiba SSA-270A and SAA-140A, Tustin, CA) equipped with a 7.5-MHz linear array imaging probe. All scanning was done in a dimly lit testing room with the participant in the supine position after 10 minutes of rest. A blood pressure cuff was placed distal to the right elbow with the arm extended out on a board and pillow perpendicular to the body. First, baseline brachial lumen diameter was computed as the distance between the lumen-intima interfaces 1-2 centimeters proximal to the antecubital fossa. Then the blood pressure cuff was inflated 30 mmHg above the participant's systolic pressure for two minutes. After release of the cuff, brachial artery images were obtained over three minutes

and digitized for later scoring. At each 30-second interval, end-diastolic images from three separate cardiac cycles were averaged from 140 pixel-width measurements taken across one centimeter. Absolute change in diameter was calculated as the peak (widest) of these diameter measures minus the baseline brachial diameter. FMD, the percentage change in brachial diameter, was calculated as the absolute difference divided by the baseline brachial diameter times one-hundred. Brachial artery FMD has been found to be a stable and reproducible technique.[123] Reproducibility in our lab (for 20 women with a second scan within 7 months) has previously been reported with an intra-class correlation of 0.72.[128]

4.3.4 Psychosocial Measures of Negative Affect

Measures of negative affect were completed on a computer via self-administered programs during three visits scheduled over approximately a four-month time span. These measures included the following published and validated scales: Beck Anxiety Inventory,[255] 21 items measuring the severity of anxiety symptoms; Beck Depression Inventory-II,[256] 21 items measuring symptoms of depression within the past week (rather than the past two weeks as per scale protocol); Cook-Medley Hostility Scale,[257] 50 true/false items assessing cynicism and mistrust (where one item was imputed from the other 49 because of an omission error on the computer presentation), and three subscales of the Spielberger State-Trait Anger Expression Inventory,[258] 44 items with four-point response scales measuring angry temperament, including Anger In (the extent to which feelings of anger are suppressed), Anger Out (the extent to which feelings of anger are expressed), and Trait Anger (general feelings of anger / predisposition to find a large range of situations to be annoying). Items were summed within each scale such that a higher score indicated more frequent or intense negative affect. In general, there were high positive correlations between the seven measures of negative affect.[228] Because scores for Anxiety, Depression and Trait Anger were positively skewed, these measures were log transformed for analyses.

4.3.5 Statistical Analysis

Baseline characteristics of men and women and univariate associations were evaluated via standard statistical methods. Associations between negative affect and FMD independent of traditional cardiovascular risk factors were examined using general linear regression models. Models were run separately on men and women because of gender differences in baseline brachial diameter size, the positive influence of hormone therapy on FMD in older women without clinical cardiovascular disease,[259] and to avoid constraining the way in which associations between FMD and cardiovascular risk factors were modeled across the two gender groups. Cardiovascular risk factors were chosen *a priori* based on existing FMD literature and included age, systolic blood pressure, BMI and ever smoking, as well as current hormone therapy use for women.[78,113-117,128,259] [While African American race is positively associated with cardiovascular morbidity and other measures of subclinical disease, it has not been linked with FMD.[113,128,259]] Each measure of negative affect was individually added as the independent variable to either the model for men or the model for women. Change in amount of variance in data that was explained by the model and the statistical significance of the psychosocial measures were examined.

To verify that linear analyses were appropriate and that outliers were not unduly influencing results, regression analyses were evaluated with scatter plots, quadratic terms, model residuals and influence diagnostics. In some cases, models were re-run to assess the impact of highly influential outliers on regression estimates.

Sex by negative affect interactions were considered in models combining both sexes to formally examine potential gender differences in the association between negative affect and FMD. These were generally not statistically significant however, and hence are not reported. Additionally, in the female-only models, interactions between hormone therapy use and negative affect were evaluated.

All analyses were implemented using the SAS system for windows version 9.1 (SAS Institute, Cary, NC). Values of $p \leq 0.05$ were considered significant.

4.4 RESULTS

4.4.1 Unadjusted Findings

Participants were 83% Caucasian, 49% female, 76% married, 76% had greater than a high school education, and 44% had a total household income of $50,000 or greater. The sample was normotensive with slightly elevated cholesterol, low density lipoproteins and triglyceride levels (see Table 4-1). Gender comparisons showed women had higher high density lipoproteins and smaller waist circumference as expected. Only 7% of the participants were current smokers, although males were more likely to be ever smokers. Fifty-two percent of the women were currently taking hormone therapy. In general, scores showed low levels of negative affect with moderate variance. Women had higher Anxiety scores, while men had higher Hostility scores.

FMD scores range from -3.6% to 31.7%, with women generally showing greater vasodilatation as expected. Absolute change in brachial diameter was similar across sexes. In looking at univariate associations and cardiovascular risk factors for women, age showed a significant negative association with FMD ($r=-0.25$, $p=0.001$) and those on hormone therapy had greater vasodilatation than those not currently taking hormones (6.74 vs. 5.35, respectively, $p=0.095$). Measures of blood pressure, lipids, glucose and ever smoking were not significantly associated with FMD in overall or sex-specific correlations.

In stratified univariate analyses between psychosocial scales and FMD, there were negative associations with Anger In (Spearman correlation coefficient: $r=-0.18$, $p=0.02$) for women and with Hostility ($r=-0.14$, $p=0.06$) and Trait Anger ($r=-0.14$, $p=0.08$) for men, but the latter were not statistically significant. There were also positive associations between FMD and Anxiety ($r=0.17$, $p=0.03$) and Depression ($r=0.13$, $p=0.08$) for women.

Table 4-1: Participant Characteristics*

	All (N=332)	Men (n=168)	Women (n=164)
Age (years)	60 ± 5	60 ± 5	60 ± 4
Systolic blood pressure (mmHg)	130 ±15	130 ±15	130 ± 15
Diastolic blood pressure (mmHg)	80 ± 9	82 ± 9	79 ± 9†
Total cholesterol (mg/dL)	214 ± 36	208 ± 31	220 ± 39‡
High density lipoproteins (mg/dL)	54 ± 16	47 ± 12	61 ± 16§
Low density lipoproteins (mg/dL)	132 ± 33	131 ± 28	133 ± 38
Triglycerides (mg/dL)	119 [84, 170]	125 [91, 189]	113 [79, 158]†
Glucose (mg/dL)	90 [86, 95]	92 [87, 98]	89 [84, 94]§
Waist circumference (cm)	92 ± 12	98 ± 9	86 ± 12§
Body mass index (kg/m^2)	28 ± 5	28 ± 3	28 ± 6
Ever smoker	164 (49)	94 (56)	70 (43)†
Beck Anxiety Inventory (0-63)	4 [2, 7]	3 [1, 5]	5 [2, 8] §
Beck Depression Inventory II (0-63)	3 [1, 6]	3 [1, 6]	3 [1, 6]
Cook-Medley Hostility Scale (0-50)	12.2 [8.2, 17.3]	13.3 [9.2, 18.4]	11.2 [7.1, 15.3]‡
Spielberger: Anger In (8-32)	14 [12, 17]	14 [13, 16]	14 [12, 17]
Spielberger: Anger Out (8-32)	13 [11, 15]	13 [11, 15]	13 [11, 15]
Spielberger: Trait Anger (10-40)	15 [13, 17]	15 [13, 17]	14 [13, 16]
Baseline brachial diameter (mm) ^	3.54 ± 0.67	3.97 ± 0.50	3.09 ± 0.42§
Absolute change in diameter (mm)	0.17 ± 0.15	0.16 ± 0.15	0.18 ± 0.14
Flow-mediated dilatation (%)	5.11 ± 4.82	4.07 ± 3.96	6.17 ± 5.38§

* Data is reported as Mean ± SD, Median [IQR] or as n (%). Comparisons are between men and women.

^ Adjusted for height.

† $p<0.05$; ‡ $p<0.01$; § $p<0.001$

4.4.2 Multivariable Regression

In men, the amount of variation in the data that was explained by a model of just the covariates of age, systolic blood pressure, BMI and ever smoking (i.e. traditional cardiovascular risk factors) was 0.9% (with an Adjusted R^2 of -0.016; Table 4-2). None of these cardiovascular risk factors was significantly associated with FMD (all $p > 0.15$). When measures of negative affect were added to this multivariable model in individual analyses, hostility was the only measure to substantively affect the amount of variation in the data accounted for by the model, increasing the Adjusted R^2 to 0.011. Men with higher Hostility had significantly smaller FMD (β=-0.108, p=0.02), and those with higher Trait Anger showed a trend toward less vasodilatation (β=-2.360, p=0.12). Removing residual outliers from models did not change associations, although exclusion of influential data points (n=10-17) caused slight variations (Hostility: β=-0.097, p=0.08 and Trait Anger: β=-3.413, p=0.049).

In women, the amount of variation in the data that was explained by a model of just the covariates of age, systolic blood pressure, BMI, ever smoking and hormone therapy was 11.0% (with an Adjusted R^2 of 0.080, or 8.0%). Age was inversely associated with FMD (β=-0.358, p=0.0002), and the other cardiovascular risk factors were non-significant. When measures of negative affect were individually added to this multivariable model, women with higher Anger In had significantly smaller FMD (β=-0.223, p=0.04). Additionally, Anger In explained 3.9% of the variance beyond the covariate-only model when all women were included and explained an additional 5.5% when influential data points were excluded.

While hormone therapy was not significant in multivariable models, the interactions of hormone therapy with Anger In and Anxiety were marginally significant ($p < 0.10$). Specifically, women currently taking hormones showed a significant inverse association between Anger In and FMD (β=-0.579, p=0.10), while this association was not significant in women not taking hormones. Conversely, women not on hormones showed a positive association between Anxiety and FMD (β=7.70, p=0.04).

Table 4-2: Multivariable Generalized Linear Regression Models of Flow-Mediated Dilation for Each Measure of Negative Affect by Sex*

	Men			Women		
Individual Models	**β**	**C.I.**	**R^2**	**β**	**C.I.**	**R^2**
Base model ^			-0.016			0.080
Base + Anxiety †	-0.493	-1.246, 0.259	-0.012	0.671	-0.532, 1.874	0.083
Base + Depression †	-0.243	-1.004, 0.519	-0.020	0.512	-0.437, 1.461	0.081
Base + Hostility	-0.108‡	-0.200, -0.015	0.011	0.046	-0.095, 0.186	0.077
Base + Anger In	-0.084	-0.269, 0.102	-0.021	-0.223‡	-0.437, -0.008	0.119
Base + Anger Out	-0.140	-0.379, 0.099	-0.017	0.125	-0.161, 0.411	0.098
Base + Trait Anger †	-2.360§	-5.342, 0.621	-0.010	1.182	-2.920, 5.284	0.096

* C.I.: Confidence Interval, R^2: Adjusted R^2
^ Base model = age, systolic blood pressure, BMI and ever smoked (all $p > 0.15$ for men); plus current hormone therapy in models for women (age $p < 0.001$, all others $p > 0.15$).
† log transformed
‡ $p<0.05$
§ $p<0.15$

4.5 DISCUSSION

After controlling for traditional cardiovascular risk factors in healthy older adults, we found impaired brachial artery vasodilatation in men with high general anger and hostility, and in women with high anger suppression. Our research bolsters evidence of an association between negative affect and cardiovascular health and implies that these links may be evident very early in the disease continuum. We used a larger sample of both men and women than previous studies of negative affect and FMD, and our results suggest that cognitive-type measures of negative affect in particular may influence endothelial function.

Overall, FMD is a useful measure for evaluating the influences of negative affect on endothelial dysfunction, a good indicator of cardiovascular health. The vascular endothelium is critical in vascular growth, vasoprotection and vasoregulation. FMD is thought to reflect nitric oxide bioavailability and the effects of shear stress on the endothelium.[124-126] Impaired vasodilatation is associated with cardiovascular risk factors, such as increased age, systolic blood pressure, smoking and BMI, and with lipid-lowering medication;[78,113-117] and is a surrogate marker for health of the coronary arteries.[130,132,133] FMD is impaired in patients with coronary artery disease,[78,130] is an independent predictor of cardiac risk and events,[65,134,135] and has been shown to improve with treatment.[136,137]

4.5.1 Anger / Hostility

Our study supports prior evidence of a link between anger/hostility and subclinical cardiovascular disease. In examining FMD, Harris and colleagues[128] found an inverse association between an aggregate Anger/Type A measure and vasodilatation in postmenopausal women. Trait Anger and Type A personality (and not Anger Out and Hostility measures), as well as Anger In, were individually associated with smaller diameter change. Similarly, a small study (n=38) looking at the effects of mental stress found an inverse association between hostile affect and FMD in men and women.[224] Other subclinical measures show similar relationships, including positive associations between measures of anger and hostility with carotid artery intima-media thickness (IMT),[214,215,217] IMT progression[213,216,218,220] and coronary calcification[260] Additionally, hostility and anger show a positive association with cardiovascular risk factors and events,[209-211,214-216] including mortality.[175,212,213]

4.5.2 Depression / Anxiety

In contrast, we did not find an inverse association between measures of depression and/or anxiety with FMD that others have reported.[128,221-223,225] Three of these studies[221-223] looked at current or history of major depression, which was not evaluated in our sample, and one examined high depressive symptoms in coronary heart disease patients,[225] thus possibly more indicative of chronic depression. In contrast, depression and anxiety in our older adults was skewed toward

lower scores, and associations, if any, were in the opposite direction for women. Interestingly, in the large FMD study of healthy women[128] the individual scales of Trait Anxiety and Anger In, and not depression or social support, were the measures of the Anxiety/Depression cluster that were specifically associated with smaller diameter change. Other measures of subclinical cardiovascular disease have also reported a link with a history of two or more major depression episodes, but not with depressive symptoms.[226,227,229] Similarly, it has been reported that sustained anxiety was positively associated with IMT progression in one study,[219] but Trait Anxiety and general anxiety were not in others,[218] including our own.[228,229] Thus, chronic anxiety/depressive symptomatology may be more influential in early endothelial dysfunction.

It may also be that anxiety/depressive symptomatology is linked with future morbidity or disease progression rather than concurrently measured subclinical cardiovascular disease. Notably, in our same sample of healthy older adults, higher depressive symptoms were related to 3-year change in carotid IMT,[228] but not baseline IMT.[229] In the Harris et al. study,[128] their scales were administered an average of 13.6 years (while still premenopausal) and 1.5 years prior to FMD measurement. Additionally, depression and anxiety are consistently noted following cardiac surgery for clinical events. [194,195] In the Sherwood et al. study, associations between high depressive symptoms and FMD were reported in patients with established cardiovascular disease.[225]

4.5.3 Etiology and Environment

Psychosocial traits and stressors influence cardiovascular disease via both pathophysiological and behavioral pathways.[183-187,252] Pathways involving the vascular endothelium, in particular, have been outlined by Harris and Matthews.[188] Pathophysiological mechanisms and physiologic responses include greater platelet activation,[230] exaggerated cardiovascular reactivity (including increases in blood pressure and heart rate),[231] associations with inflammatory and hemostatic markers[232,233] and excessive sympathetic nervous system and hypothalamic-pituitary-adrenal axis activity (as indexed by greater norepinephrine and plasma cortisol).[231,234] Inhibition of cortisol improves FMD in depressed patients.[235] Substantial overlap exists in the functional and biologic pathways underlying these mechanisms and thus, many of these processes may be different manifestations of the same etiology.

Lifestyle behaviors, environmental situations and genetic factors also influence the association between negative affect and vascular health. Negative emotions have been linked to adverse health habits, including poor sleep, increased alcohol use, smoking, obesity and poor diet.[175-178,192] Individuals with negative dispositions are also less likely to adhere to medical interventions and to seek or have access to medical care.[179,180] While it is not clear whether negative emotions mediate the association between health and social economic status,[192,193] individuals from higher and lower statuses clearly differ in how they describe their social environment and interactions with others.[238-240] A study of young adult twins also suggests that non-shared environmental factors influence negative affect.[189] Behavioral factors are highly influential,[175] but do not solely account for associations between psychosocial traits and cardiovascular disease; and psychosocial influences are evident even after controlling for traditional cardiovascular risk factors.[211,216,217] Genetics likely play a role, predisposing both psychological factors and physiological vulnerabilities.[189-191]

Given the cross-sectional nature of the analyses reported here, a causal relationship between vascular system dysfunction and psychosocial traits can not be established. Furthermore, genetic and environmental predispositions underlie both entities. Our study did reduce some of the confounding influences that may impact such associations by evaluating healthy individuals. In showing that the influence of negative affect on cardiovascular health is not limited to individuals with established cardiovascular disease, this study suggests, in any case, that such associations may develop early in the disease process. Hostility, anger, depression and anxiety are enduring and relatively stable psychosocial characteristics, and as such are appropriate for examining influences on subclinical cardiovascular disease in both men and women.

4.5.4 Considerations

While there is an overlap in psychosocial risk factors such as hostility, anger, anxiety, depression and other constructs,[173,189,261] a relationship with FMD was not found with all measures in our study. Additionally, post hoc attempts to create aggregate measures of negative affect did not produce stronger associations. It is feasible that some psychosocial measures have stronger construct validity or allow for greater variance even among mildly affected individuals (as our

sample was); or that our use of concurrently measured psychosocial traits influenced some results.

Alternately, it is possible that attitudinal or cognitive measures of hostility and anger, as well as major clinical depression, might be more influential early in the disease process (i.e. endothelial dysfunction), while depressive symptomatology may be more compatible later with atherosclerotic progression[228] and clinical outcomes.[55,57] Considerable overlap has been reported in the constructs of hostility, cynicism and anger.[173,174] While depression and anxiety are often considered together, the intercorrelation of emotions vary among samples,[173] and the levels of depression and anxiety in our participants were lower than other healthy groups (while the anger measures were comparable). Of note, people who score high on measures of Anger In and Hostility tend to ruminate a lot about others, but are not necessarily more physically aggressive or violent than others in the general population, and it may be this particular trait that is most congruent with early subclinical cardiovascular disease.

Of mention, in our analyses no correction was made for multiple comparisons; thus our results should be interpreted with caution. However, the literature suggests this may not have been necessary given our hypotheses.[262,263] While an interaction effect between sex and negative affect was not found, we reported slight variations in which scales were associated with FMD in each gender. This may have been due to the influence of hormone therapy,[113,128,259] to the wider variance in respective scales for men and women, to the influence of age on FMD in women, or to measurement "noise" in the paradigm of cognitive-type constructs of anger/hostility.

4.5.5 Conclusions

In conclusion, hostile, mistrustful attitudes and a tendency to harbor unexpressed anger were shown to be associated with impaired vasodilatation of the brachial artery in healthy older adults. This study adds the measure of FMD to the literature on the association of negative affect and cardiovascular health, confirms that such associations may be present for men as well as women, and suggests this connection may be apparent very early in the disease process.

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5.0 DISCUSSION AND CONCLUSIONS

5.1 OVERVIEW OF FINDINGS

Following is a review of the main findings of the complete papers presented in Chapters 2-4. A brief accounting of how these findings compare and contrast to the existing literature is also presented. Each synopsis is completed with the theories supporting the findings and conclusions that were drawn.

5.1.1 Traditional Risk Factors and Segment Specific IMT

In the first paper, we evaluated the extent to which intima media thickness (IMT) in three locations within the carotid artery were differentially associated with traditional cardiovascular risk factors. Starting with separate linear regression models, adjusted for age, menopausal status and intervention group, we found baseline weight, systolic blood pressure and age independently and positively associated with common carotid artery IMT. In the bifurcation area, we found smoking, higher weight and higher systolic blood pressure independently associated with higher IMT. IMT in the internal carotid artery was independently and positively associated with apoprotein B. Then using a multivariable repeated measures mixed model of all three IMT locations, we were able to show differential effects whereby these risk factors had segment-specific associations. Specifically, the effect of weight was strongest and aging was limited to the common carotid artery, smoking and systolic blood pressure associations were specific to the bifurcation area of the vessel, and the effect of apoprotein B was strongest in the internal carotid artery segment.

Similarities between our results and previous research include positive associations of body composition and systolic blood pressure with common carotid and bifurcation IMT,[10,11,13]

lipids associated with internal carotid and bifurcation IMT,[9,11,13] and smoking with IMT in the bifurcation.[11] However, others have generally found the segments similarly associated with cardiovascular risk factors,[10,11,13] whereas we found distinct differences in risk factor and segment associations in healthy middle-aged women.

Our findings that risk factors differentially influence IMT locations can be substantiated based on previous research of arterial hemodynamics, shear stress and vascular biology. Each area of the carotid arterial vessel experiences variations in hemodynamics and cellular processes, which affect the development and progression of disease.[14-22] Additionally, the risk factors themselves are likely acting in conjunction and possibly synergistically with various biomechanical and biochemical pathways to determine the development and progression of atherosclerosis at any given site.[16,20,23,24] Further, each site may have its own predisposing factors.[12,19] Thus, the pathophysiology of the arteries and the biomechanics of the individual risk factors support our findings of significant and targeted associations between traditional cardiovascular risk factors and carotid arterial segments even after accounting for the correlation in IMT at all three locations.

5.1.2 Rheumatoid Arthritis and Carotid Outcomes

In the second paper, we assessed whether diagnosis of rheumatoid arthritis and indicators of the disease were associated with measures of carotid atherosclerosis and vascular health. Lumen diameter and interadventitial diameter were significantly wider in patients with rheumatoid arthritis than in healthy women. After controlling for age, African American race, hypertension, hypercholesterolemia, glucose, height and IMT, diagnosis of rheumatoid arthritis continued to be associated with larger lumen and interadventitial diameters. Prevalence of plaque was higher in patients but not statistically different than healthy women. Carotid IMT was similar between groups. In patients with rheumatoid arthritis, we found positive associations between methotrexate dose and interadventitial diameter, between prednisone use and hypothyroidism and IMT, and between hypothyroidism and soluble endothelial adhesion molecule and plaque, independent of cardiovascular risk factors. And while additional indicators of rheumatoid arthritis severity or activity were not significantly associated with carotid outcomes in multivariable models, other inflammatory markers and medications showed significant trends.

Our finding of similar IMT between groups is consistent with several studies of rheumatoid arthritis patients conducted in the United States, [91,92,94] but in contrast to others' findings, [84-89,95] although others have reported on slightly older patients with a wider age spectrum, and enrolled both men and women. We suspect that our sample of slightly younger women with a narrower age range may have been studied at an earlier point on an arterial remodeling continuum than those described elsewhere, with our women with rheumatoid arthritis having vessel wall dilatation and thus preserved IMT. Alternatively, differences in study results may be due to a unique, rheumatoid arthritis-related modification of atherogenesis whereby the progression of disease is different from other conditions where increased IMT is seen early.

Like others we found measures of rheumatoid arthritis severity and inflammation, including disease duration, medication use and soluble endothelial adhesion molecule, positively related to carotid atherosclerosis.[85,86,94,95,161,169,171,196,197] Literature reviews suggest chronic systemic inflammation may lead to endothelial dysfunctions and accelerated atherogenesis in patients with rheumatoid arthritis.[166-168,244] Links with measures of rheumatoid arthritis activity may be particularly interesting given that traditional cardiovascular risk factors are not consistently associated with atherosclerosis in patients with rheumatoid arthritis.[156,158,170,171]

Given our results, it can not be said with certainty whether all women with rheumatoid arthritis have greater IMT than healthy controls, or if this difference is only observed in women of certain ages or ethnicities, or at particular time points in the course of disease (rheumatoid arthritis or atherosclerosis). Nonetheless, we believe that lumen and interadventitial diameters may be more sensitive measures of early atherosclerosis in rheumatoid arthritis than ultrasound measurement of IMT and plaque. These findings, as well as associations of disease severity with carotid outcomes may indicate evidence of accelerated vascular aging and early atherosclerotic risk among women with rheumatoid arthritis.

5.1.3 Negative Affect and Flow-Mediated Dilation

In the third paper, we identified measures of negative affect inversely associated with endothelial-dependent flow-mediated dilation (FMD) of the brachial artery in both men and women. Univariate correlations between psychosocial scales and FMD showed a significant negative association with anger suppression for women, and a trend for a negative relationship

with hostility and general feelings of anger for men. After controlling for traditional cardiovascular risk factors of age, systolic blood pressure, body mass index and ever smoking, FMD was associated with higher hostility and general anger scores in individual multivariable models for men. In women, after also controlling for current hormone therapy, we found decreased FMD was independently and significantly associated with higher anger suppression scores. We did not find significant associations between measures of anger expression, depressive symptoms or anxiety with FMD in using these multivariable models.

Our study is consistent with others who have found a link between anger/hostility and subclinical cardiovascular disease,[213-218,220,260] including reduced vasodilatation in postmenopausal women[128] and in combination with mental stress task.[224] In contrast, we did not find a statistically significant association between measures of anxiety/depression with FMD as previously reported.[128,221-223,225] However, previous reports examined major depression or prior symptomatology, [221-223] while we evaluated concurrent depressive symptoms. Results of other measures of subclinical cardiovascular disease have also shown an association with a history of major depression episodes, but not with depressive symptoms.[226,227,229] However, depressive symptoms show positive associations with IMT progression and in heart patients.[1,5,7,194,195] Previous results for anxiety and subclinical disease have been mixed.[128,218,219]

Both behavioral and biological influences contribute to the association between negative affect and disease. Positive associations have been found between negative affect and poor health behaviors (e.g. smoking, unhealthy diet, inactivity) including treatment non-adherence and low socio-economic status.[175-182,192,193] Pathophysiological mechanisms by which negative affect may be linked to cardiovascular disease include excessive sympathetic nervous system and hypothalamic-pituitary-adrenal axis activity,[231,234] greater platelet activation,[230] inflammation,[232,233] and exaggerated cardiovascular reactivity such as increased blood pressure and heart rate.[231] Genetic and environmental influences are also important aspects to the relationship between negative emotions and cardiovascular disease.[189-191]

We conclude that attitudinal or cognitive measures of anger and hostility (and major clinical depression reported in other studies [221-223]) might be more influential early in the disease process (i.e. endothelial dysfunction), while depressive symptomatology may be more compatible later in atherosclerotic progression[157] and with clinical outcomes.[55,57,194,195] In sum, our study found hostile attitudes and a tendency to harbor unexpressed anger were associated

with impaired vasodilatation of the brachial artery in healthy older adults, and thus supports evidence of a link between anger/hostility and subclinical cardiovascular disease in men and women.

5.2 GENERAL DISCUSSION

These three papers illustrate that ultrasound measures of subclinical cardiovascular disease and vascular health can be used to assess relationships with putative risk markers including, traditional cardiovascular risk factors, diagnosis of rheumatoid arthritis and measures of negative affect. Evaluating subclinical cardiovascular disease, especially in otherwise healthy samples, suggests associations are present early in the disease process. Several common themes can be drawn from the three papers just presented, including the use of ultrasound measures in healthy samples, the influences of aging and hemodynamics, and the use of female participants. There are also unique characteristics and specific contributions to the literature for each paper that can be appreciated. Finally, potential weaknesses of each study will be considered.

5.2.1 Common Themes

While we used three different study samples and evaluated a variety of ultrasound measures and risk markers, several common themes can be drawn from the three papers just presented. Mutual topics include the application of ultrasound to assess subclinical measures of disease and vascular health, the use of healthy adult participants, the influence of aging on the cardiovascular system, the focus on samples of women, and the implications of hemodynamics on the adaptation of the vasculature.

5.2.1.1 Noninvasive Ultrasound Measures. We used ultrasound measures to examine the carotid artery in two studies and looked at the brachial artery in the third. We showed that multiple outcomes show associations with putative markers of risk, suggesting that insult to the vasculature is not limited to a single vessel. However, we also learned that insults may not be universally influential given the differential associations seen by IMT segment in healthy

middle-aged women, and in diameters but not IMT when comparing women with rheumatoid arthritis to healthy women. While not fully "interchangeable" because of their particular assessments, measures of IMT, plaque, diameters and FMD could have been substituted for one another in the three studies. That is, each of these ultrasound tools is not uniquely associated with the putative maker of risk examined, but rather indicative of underlying cardiovascular disease and vascular health.

5.2.1.2 Healthy Samples. By design all three studies looked at subclinical cardiovascular disease and vascular health, thus by definition outwardly healthy participants and pre-clinical measures of disease were evaluated. In all three cases, we utilized a "healthy heart" sample or controls, so that putative risk factors could be examined without the influence of known disease. For studies of negative affect in particular, associations with cardiovascular disease are more consistently found in healthy populations, presumably because fewer confounder effects exist.[173] In showing that the influence on cardiovascular health is not limited to individuals with established cardiovascular disease, we support targeting interventions to vulnerable individuals early in the disease process.

5.2.1.3 Vascular Aging. While one of the goals of cardiovascular disease prevention is to promote healthy aging, the passage of time still has a seemingly inevitable influence on the vascular system. It has been postulated that increasing age contributes both to changes in the cardiovascular structure and function as well as interactions with and resulting from an increased exposure time to various risk factors.[58,59,264] In the first paper, we found aging was associated with IMT in the common carotid artery even when controlling for cardiovascular risk factors and IMT in the other segments. In the rheumatoid arthritis paper, in the absence of other risk factors, aging and the duration of disease were associated with IMT. Finally, in the third paper aging showed an inverse relationship with FMD of the brachial artery in women, possibly indicative of their younger biologic cardiovascular age as compared to men. Thus, we showed that aging has an effect on multiple vascular beds and on various indicators of atherosclerosis and vascular health. Lakatta and Levy suggested that the combination of age-associated endothelial dysfunction, intima media thickening, arterial stiffening and pulse pressure widening along with risk factor exposure and genetic makeup determine an individual's overall vascular aging profile and lead to the most unsuccessful cardiovascular aging.[59]

5.2.1.4 Female Samples. An extensive review of MEDLINE studies from 1966 through 1998 showed that women have been underrepresented in clinical trial of cardiovascular disease.[69] In a detailed review of 724 manuscripts from specific publications and years, Meinert and colleagues concluded that male participants outnumbered females 3.66 to 1 in heart disease clinical trails.[69] Further, while the representation of women in cardiovascular clinical trials has increased due to a few large, single-sex trials, women are still underrepresented in the evaluation of particular heart conditions, and there has been no change over time in the sex composition in the majority of cardiovascular disease studies.[68,69] The American Heart Association has concluded that men and women differ with respect to disease processes, clinical presentations and outcomes and thus has established guidelines for cardiovascular disease prevention specific to women.[43,70,265] Differences between genders are often attributed to the effects of estrogen on the vascular system, including lowering lipid concentrations, increasing nitric oxide secretion and vasodilation, and altering fibrinolytic, coagulation and antioxidant systems.[266] Thus, the assessment of vascular health and aging in women was of particular interest here.

The first two papers we presented used female-only samples. In Chapter 2, we applied methodology to the Women's Healthy Lifestyle Project, a diet and exercise intervention trial in premenopausal women. While the novel associations we found were unlikely to be gender-specific, the original study likely contributed to the recent gains sited in participation of women in clinical trials. In our second paper, it was natural to focus on the vascular health of women, because rheumatoid arthritis is more common in women than men. Thus, from both a logistic perspective (in terms of recruitment and statistical power) and application of the findings, using a sample of only women was most effective. In the third paper, we were able to assess the associations of negative affect in both men and women. And although sex-specific interactions were not found, we reported differential associations for men and women. While the issue of adequate representation of women in cardiovascular research remains debatable[267] and sex difference exist in willingness to participate may explain some discrepancies,[268] making use of female samples is worthwhile.

5.2.1.5 Adaptation of the Vasculature. Hemodynamics and cellular processes of the vascular system influence the adaptive and moderating responses of the arterial vessels. Shear stress is inversely related to age, systolic blood pressure, body mass index, carotid diameter, IMT and plaque[18,19,109,140,141] and positively related to FMD.[127] The greater the viscosity and velocity

of the blood the greater the shear stress, resulting in insult and adaptation of the endothelial cells and vascular wall.[269,270]

Two of the papers portray the continually changing environment of the carotid arteries and the ability of the vasculature to respond. When considering carotid segments, we saw that the high oscillatory blood flow inherent to the bifurcation produces greater IMT and plaque than is found in the common carotid artery, which is prone to laminar blood flow. This "negative" response mirrors the "positive" vascular adaptation seen in the women with rheumatoid arthritis. These patients had larger arterial diameters than healthy women, presumably to moderate tensile stress of the dilated artery and maintain constant blood flow in response to arterial wall thickening.

5.2.2 Unique Characteristics and Contributions to the Literature

In addition to the common themes, each paper possesses unique characteristics allowing the breadth of research and knowledge gained by this investigation to be appreciated. The paper on rheumatoid arthritis has the biggest potential for clinical application as rheumatologists continue to tease out specific aspects of the disease most relevant to cardiovascular disease development. Our findings that hypothyroidism, medication use and inflammatory markers were associated with carotid diameters, IMT and plaque make these important rheumatoid arthritis-related characteristics for clinicians to track.

The segment-specific IMT paper showed that proper statistics applied to a dataset can facilitate new insight and add certainty to associations that are found. Epidemiologists need to keep current on and take advantage of advances in statistical software and techniques to ensure that they are gleaning the most accurate conclusions from their results. We demonstrated that a repeated measures statistical model can be applied to data that is biologically correlated, rather than data with multiple time points, thus improving upon conclusions drawn from separate regression analyses.

The paper on negative affect and FMD was the only one of the set that looked at the brachial artery and included both men and women. When feasible, studies should enroll both men and women (and multiple race/ethnicities), increasing the chance that findings will be applicable to a wider population.

In addition to their unique characteristics, each paper made an individual contribution to the literature on subclinical cardiovascular disease and vascular health with regards to putative markers of risk. First, in looking at carotid IMT by segment, we found that traditional cardiovascular risk factors were differentially associated with each segment even after accounting for the correlation in wall thickness between segments. Thus, when comparing and evaluating IMT results between studies it is important to consider which segments are included in measures of arterial thickening.

Second, we raised the possibility that vascular adaptation may explain mixed results in the literature comparing IMT in patients with rheumatoid arthritis to healthy controls. Our findings of greater carotid artery diameters but similar IMT in women with rheumatoid arthritis compared to healthy women, suggest that the timing of assessment (i.e. chronological age, rheumatoid arthritis duration and vascular aging) is important. Therefore, it is important to consider where in the disease continuum patients may be and what rheumatoid arthritis-related or atherosclerotic adaptations may be influencing wall thickness.

Third, in finding an inverse association between hostile attitudes and anger suppression with brachial artery FMD, we demonstrated that cognitive-type measures of negative affect may influence endothelial function. It has been suggested that insult to the endothelium occurs prior to measurable atherosclerosis,[138] therefore future studies may want to consider using FMD in addition to or instead of the more commonly used measures of subclinical cardiovascular disease.

5.2.3 Limitations and Weaknesses

While each study made important contributions to the literature, they were not without limitations. Ideally, the study of IMT segments would have included men and had greater ethnic diversity. Having a broader pool of participants would have increased the generalizability of the results. Nonetheless, we chose to make use of a female-only dataset in an effort to balance the existing research on cardiovascular disease, which has often evaluated only male subjects. The field now clearly recognizes that the development and course of disease in women may differ from men,[43,70] therefore, applying these analyses to a broader sample would be beneficial.

In women with rheumatoid arthritis we found soluble endothelial adhesion molecule positively associated with plaque prevalence. However, no other statistically significant association with markers of inflammation or in conjunction with carotid diameters or IMT was seen in multivariable models. Review of the literature suggest chronic systemic inflammation may lead to endothelial dysfunction,[166-168,244] so it is unknown whether we examined these markers at the wrong time point in the disease continuum or if repeated measures of systemic inflammation (rather than a single reading) would have shown stronger results.

In the third paper, where distinct associations between negative affect and FMD were reported, a larger sample size, younger population or greater variance in negative symptoms might have produced more robust results. Interestingly, our participants reported levels of anger and hostility that were similar to others, but lower levels of depression and anxiety compared to other normal populations. While we provided valid and well substantiated results, we had postulated stronger associations and a greater number of scales with significant findings.

Finally, all three papers evaluated cross-sectional associations with ultrasound measures of cardiovascular disease, which are not clinical endpoints themselves. A causal pathway between the markers of risk and the outcomes studied can not be established. Further, it can not be said with certainty which individuals will experience acute cardiovascular events or develop overt signs of disease. Established guidelines regarding how to monitor or treat individuals with subclincial disease are lacking. Nonetheless, our findings contribute to the broader knowledge of vascular aging and cardiovascular health.

5.3 PUBLIC HEALTH SIGNIFICANCE

The research summarized here has several public health implications. These include appreciation of multiple risk markers, analytic methods and subclinical measures of cardiovascular health. Additionally, further evaluating the specific needs of women with heart disease (as previously discussed) and addressing primordial and primary prevention are critical.

5.3.1 Putative Markers of Risk

It is important for both clinicians and scientists to consider the global picture of heart health when treating patients and designing research studies. While traditional cardiovascular risk factors are clearly linked to both clinical and subclinical cardiovascular outcomes, our research showed that other aspects of health, including other chronic conditions (such as rheumatoid arthritis) and psychosocial symptoms (such as negative affect) are associated with indicators of heart health. Thus, a large range of putative markers of risk should be factored into evaluation, prevention and treatment of disease.

5.3.2 Statistical Evaluation

We also showed the importance of appropriate and thorough statistical evaluation in epidemiologic studies. While it should be standard practice to include various statistical methods to thoroughly evaluate ones findings, they are sometimes overlooked in the haste to report results or because findings match hypothesis. While somewhat time consuming, additional statistical evaluation can strengthen ones conclusions or reveal potential pitfalls, and thus are critical to include in one's own results and important when considering those stated by others.

As mentioned already, the use of a mixed regression model, allowed for an impartial evaluation of the three carotid artery segments, thus providing an unbiased assessment of segment-specific associations. While similar results were found using individual multivariable models, there was greater overlap amongst IMT locations. In other words, several risk factors were associated with more than one segment. In contrast, the mixed model was able to tease out more distinction relationships.

In making good use of regression diagnostics and model residuals, as was done in the FMD and negative affect paper, the chance of spurious results based on a few outliers was decreased. Given that multiple models were run to individually assess associations for each measure of negative affect for each gender, the possibility of finding chance correlations was increased. Further, while several outliers existed (as evidenced by residual plots and influential diagnostics) the remaining observations showed limited variance. Thus, the additional analyses

added confidence to the reported results by showing consistent associations across multiple analytic evaluations of each measure of negative affect.

5.3.3 Application of Ultrasound Measures

Ultrasound measures of subclinical cardiovascular disease and vascular health and adaptation are useful as research benchmarks and as screening tools for specialty populations. Noninvasive measures of disease are ideal for research studies because they have minimal adverse outcomes (if any) to study subjects, while also providing the opportunity to evaluate individuals without overt disease. When evaluating the effects of an intervention study with specialty populations (e.g. patients with rheumatoid arthritis, obese individuals), evidence of vascular adaptation (i.e. changes in carotid diameters and IMT) would be particularly applicable. Using FMD to track pharmacological treatments in hypertensive patients has also been suggested.[271,272] The subclinical disease measures used here, particularly IMT, may also be applicable for noninvasive diagnostic and prognostic testing of high risk individuals or in cases where standard screening tools are inconclusive.[273] FMD has the potential for use in patient identification for early prevention, although additional research is needed to determine the efficacy of endothelial function testing in cardiovascular disease risk assessment.[72,274] In sum, use of ultrasound measures of disease is warranted for both clinical and research settings.

5.3.4 Cardiovascular Disease Prevention

In addition to the specific public health implications mentioned, one should also consider the more global perspective of cardiovascular disease prevention. According to the Centers for Disease Control and Prevention National Center for Health Statistics, life expectancy would rise by nearly seven percent if all forms of major cardiovascular disease were eliminated.[44,45] Given the multiple genetic, environmental and behavioral influences on the development of cardiovascular disease, the road to prevention can be quite daunting.[53,54] Granted, secondary prevention can sometimes be viewed as more cost effective (i.e. because higher risk patients garner more benefits), and easier for the medical community and insurance companies to track

and target. However, for fundamental lifestyle changes to occur community programs, societal changes, and support from medical and health care institutions are needed.[53,54,150,165,275,276]

The World Health Organization states that the most important causes of heart disease are due to "modifiable risk factors" of physical inactivity, unhealthy diet and tobacco use, and that 80% of cardiovascular disease deaths could be avoided by changing these behaviors.[42] Psychosocial factors, such as perceived stress and depression, may be similarly influential.[55,57] While medications are very effective in secondary prevention,[148] to achieve a major reduction in disability and deaths due to cardiovascular disease the focus needs to be on changes in the modifiable risk factors not a cure.[42] Therefore it is recommended that individuals avoid tobacco use and second-hand smoke, engage in 30 minutes of daily physical activity, maintain a healthy body weight, and choose a diet rich in fruits, vegetables, whole grains and nuts, while avoiding foods high in salt, sugar and fat.[42,53,148] Unfortunately, only three percent of those surveyed in 2000 met all four Healthy Lifestyle Indicators of nonsmoking, healthy weight, five fruits / vegetables and regular physical activity.[277] These healthy behaviors go hand-in-hand with a positive psychosocial disposition and are directly linked to traditional cardiovascular risk factors, suggesting that the umbrella of cardiovascular research is quite large and rightfully so given the enormous public health implications.

5.4 FUTURE RESEARCH AND APPLICATIONS

As just mentioned the three papers suggest several theoretical implications including, utilizing appropriate statistical methods and carefully evaluating others work when drawing conclusions and making comparisons. Beyond these, the works described lay the groundwork for future research in terms of both immediate "next steps" and broader applications.

The logical progression for each of the individual studies would be to follow up on the unanswered questions by broadening their scopes and carrying through on conclusions drawn. Thus, the study of segment-specific IMT risk factors could readily be applied to other existing datasets. By choosing studies of males, varying ages and multiple ethnicities, the generalizability of the reported results could be demonstrated. For the second paper the obvious next step would be to test our theory that vascular adaptation accounted for the difference in

diameter and similarities in IMT between patients with rheumatoid arthritis and healthy women. Thus, the women with rheumatoid arthritis would be brought back for an additional carotid ultrasound scan(s) so that the progression of IMT and diameters could be tracked. These data could then be compared to the healthy controls, who had multiple carotid scans as part of their original carotid study. Similar to the first paper, the study of negative affect would benefit by testing if similar associations were found in additional populations, particularly younger participants and those with more profound negative dispositions. In recruiting healthy, motivated volunteers, our study may have been limited by selection bias.

From a broader perspective, research on putative marker of risk and subclinical cardiovascular disease and vascular health could go in many directions. Of those who die of coronary heart disease, half of men and nearly two-thirds of women have no prior symptoms,[43,47] leaving much to be discovered. The three studies presented here evaluate only a small fraction of the multitude of factors affecting cardiovascular disease. Future studies should continue to evaluate other markers of risk and make use of ultrasound sound measures as surrogate markers of disease as well as endpoints in themselves. For example, researchers could use these subclinical measures to track the effects of a treatment or intervention study by assessing vascular health and adaptation in hypertensive, obese, systemic lupus or similar samples. This could be done by obtaining a baseline scan, implementing medications or healthy lifestyle program, and then checking follow-up scans to evaluate treatment success.

While research applications are most promising, ultrasound measures also have some potential for clinical practice. For example, the measures employed here could be included in the battery of clinical tests used to evaluate cardiovascular disease in both at risk and high risk populations. Exercise treadmill testing and stress cardiac imaging can accurately diagnosis and risk-stratify individuals with known and suspected cardiovascular disease. Nonetheless, new approaches could supplement current screening tools.[273,278] This is especially true in women for whom the symptom presentation often differs from that traditionally seen in men. Use as a screening tool may also be helpful in identifying at risk individuals for whom intervention early in the disease process would be beneficial. The measures of common carotid IMT and carotid plaque are the most likely candidates for clinical application given their high reproducibility and clear link with cardiovascular morbidity and mortality; while evaluating endothelial dysfunction via FMD may provide an even early assessment of vascular health.

A third implication of this dissertation is the need to include psychosocial measures in large epidemiologic studies of cardiovascular disease. The psychosocial measure in the INTERHEART study included primarily questions on perceived stress and depression.[57] Given the associations we and others have reported, questions on anger and hostility should be included in a world-wide epidemiologic study with cardiovascular disease measures and outcome events. A study of this type and magnitude would have the potential to notably substantiate current research.

5.5 CONCLUSIONS

Subclinical ultrasound measures are useful for studying associations early in the disease process. At one of the earliest stages of cardiovascular disease, we looked at endothelial dysfunction via vasodilatation of the brachial artery and found it inversely associated with hostile attitudes and tendency to harbor unexpressed anger. In finding wider carotid artery diameters in women with rheumatoid arthritis compared to healthy women, we suggest that these may be more sensitive measures of early atherosclerosis in rheumatoid arthritis than ultrasound measurement of IMT and plaque, and that vascular adaptation may explain previously reported discrepancies. We also showed that cardiovascular risk factors may differentially affect IMT in the common carotid, bifurcation and internal carotid artery segments of healthy middle-aged women when wall thickness in all three segments is simultaneously taken into account. Thus, the works described here were successfully able to evaluate early signs of atherosclerosis and vascular health and aging in conjunction with putative markers of risk.

The path to cardiovascular morbidity and mortality occurs in conjunction with a range of factors. Traditional cardiovascular risk factors are most obvious, but do not completely explain the story, thus other avenues must be considered. We were able to demonstrate that diagnosis and characteristics of rheumatoid arthritis and measures of negative affect were associated with subclinical cardiovascular disease. Insult to the cardiovascular system does not occur in a vacuum and both public health programs and epidemiologic research should target an array of risk factors, particularly those that are modifiable. Healthy lifestyle behaviors, genetic predispositions, negative emotions and environmental conditions are all acting together to

influence cardiovascular disease and vascular aging. Therefore, when evaluating cardiovascular health it is important to consider psychosocial traits and co-morbid disease conditions and disorders in combination with traditional cardiovascular risk factors.

Research evaluating relationships with endothelial dysfunction, atherosclerosis and vascular health are informative measures regarding influences early in the disease process. In addition to evaluating specific relationships, we validated the more general influences of hemodynamics and vascular aging, utilized a range of statistical methods, demonstrated the importance of critical evaluation, and supported the inclusion of women in cardiovascular research. We evaluated the public health implications of our research and endorsed the need for individuals to adopt a healthy lifestyle. In conclusion, evaluating associations between putative markers of risk and subclinical cardiovascular disease and vascular health provides insight into the broader epidemic of heart disease.

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