INFLAMMATION AND CHARACTERISTICS OF ATHEROSCLEROTIC PLAQUE

by

Natalie Christine Suder

BS Biology, Pennsylvania State University, 2012

MPH Infectious Diseases and Microbiology, University of Pittsburgh, 2014

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This dissertation was presented

by

Natalie Christine Suder

It was defended on

August 24th, 2017

and approved by

Maria Mori Brooks, PhD
Professor and Vice Chair of Education, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Suresh Mulukutla, MD
Assistant Professor of Medicine, Division of Cardiology
School of Medicine
University of Pittsburgh

Trevor Orchard, MBBCh, MMedSci
Distinguished Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Rebecca Thurston, PhD
Professor of Psychiatry
School of Medicine
University of Pittsburgh

Christina Wassel, PhD, MS
Associate Professor, Department of Pathology and Laboratory Medicine
College of Medicine
University of Vermont

Dissertation Advisor: Emma Barinas-Mitchell, PhD
Assistant Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Emma Barinas-Mitchell, PhD

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Natalie Chrisine Suder, DrPH

University of Pittsburgh, 2017

ABSTRACT

Atherosclerosis, or the accumulation of lipids within the arterial wall, is the underlying process responsible for the majority of cardiovascular disease (CVD). Inflammation is one of the essential forces driving the development and progression of atherosclerosis. Several cells, chemokines, and co-stimulatory molecules of the innate and adaptive immune response have been associated with atherosclerosis and cardiovascular risk. Another important risk factor associated with atherosclerotic CVD risk is psychosocial stress, and it has been widely hypothesized psychosocial stress influences CVD through increased inflammation. Within atherosclerotic plaque, greater inflammatory content is associated with plaque instability and greater likelihood of plaque to result in a cardiovascular event via erosion or rupture.

B-mode ultrasound is an imaging technique that allows for the measurement and characterization of atherosclerotic plaque in the superficial arteries such as the carotid and femoral arteries. The measurement and characterization of plaque in subclinical stages affords us the opportunity to better understand the processes contributing to the development of atherosclerosis and for the identification of individuals who may be at risk of having a cardiovascular event.

In this dissertation, the associations between the circulating burden of inflammation and coagulation with measures of plaque presence, burden, and characteristics in the carotid and femoral arteries are evaluated with the intention of better understanding the inflammatory

mechanisms contributing to plaque instability. Additionally, we assessed inflammatory burden as a potential mediator of the relationship between perceived everyday discrimination, a measure of psychosocial stress, with carotid plaque characteristics.

Overall, we did not find any independent associations between inflammatory and coagulation burden with measures of femoral plaque presence, burden, and characteristics among healthy older adults. However we did find several associations between inflammatory and coagulation burden with various measures of carotid plaque burden and characteristics independent of traditional CVD risk factors among women in midlife. We also identified inflammatory burden as a partial mediator of the relationship between everyday discrimination and carotid plaque height.

The findings presented in the following chapters have public health significance because they highlight important associations between circulating inflammatory and coagulation burden with measures of carotid plaque burden and characteristics among women, and suggest that increased inflammatory and coagulation burden may serve as mechanisms contributing to the progression and complication of atherosclerotic plaque among women at midlife. Furthermore, our results suggest that increased inflammatory burden and greater atherosclerotic plaque height may be mechanisms through which experiences of discrimination increase cardiovascular risk among midlife women.

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PREFACE

There are several individuals that deserve acknowledgement for their contributions to this research. First, I would like to thank my advisor, Emma Barinas-Mitchell, for her constant encouragement and commitment to me as a student. Without her guidance, and the assistance of those at the University of Pittsburgh Ultrasound Research Laboratory, this work would not have been possible.

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1.0 DISSERTATION OVERVIEW AND OBJECTIVES

Cardiovascular disease (CVD) is the leading cause of death globally, and the majority of CVD morbidity and mortality is attributed to atherosclerotic disease. Atherosclerosis is a chronic inflammatory disease occurring throughout the lifetime that results in the accumulation of lipids within the arterial wall. A variety of inflammatory cells, chemokines, and co-stimulatory molecules of the innate and adaptive immune system are associated with the development and progression of atherosclerotic plaque.

The subclinical measurement of atherosclerotic plaque via various imaging techniques has helped shape our understanding of the processes contributing to various manifestations of CVD. Subclinical measures of plaque allow for the assessment of atherosclerotic disease progression in different stages and for the identification of individuals at risk of cardiovascular events or complications. The purpose of this dissertation is to evaluate the associations between circulating markers of inflammation and coagulation with measures of carotid and femoral plaque presence, burden, and characteristics identified via B-mode ultrasound. The long-term goal of this dissertation is to shed light on the potential mechanisms responsible for plaque instability, and help identify characteristics that may put individuals at risk of developing plaque that is vulnerable to erode or rupture.

AIM 1: (a) To determine whether there is an underlying structure to seven circulating biomarkers of inflammation and coagulation (CRP, IL-6, ICAM-1, fibrinogen, D-Dimer, PTX-3, and Lp(a)). (b) To measure the associations between groups of the circulating biomarkers of inflammation and coagulation with measures of femoral artery plaque presence, burden and characteristics in healthy older adults.

AIM 2: (a) To determine whether there is an underlying structure to six circulating biomarkers of inflammation and coagulation (CRP, IL-6, fibrinogen, D-Dimer, PTX-3, Factor VII, and Von Willebrand Antigen). (b) To measure the associations between the groups of circulating biomarkers of inflammation and coagulation with measures of carotid artery plaque presence, burden and characteristics in midlife women.

AIM 3: (a) To measure the association between perceived everyday discrimination and carotid artery plaque presence, burden, and characteristics in midlife women. (b) To determine whether this hypothesized relationship is mediated by circulating markers of inflammation and coagulation.

2.0 GENERAL INTRODUCTION

2.1 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD), the world's leading cause of mortality, is responsible for over 30% of all deaths globally, and it is estimated that the annual costs of CVD are upwards of US $860 billion (1, 2). From 2010 to 2030, the annual number of CVD deaths is expected to increase from 18.1 million to an estimated 24.2 million globally, with over 75% of all deaths predicted to occur in low- and middle-income countries (3). The leading causes of CVD mortality, coronary heart disease (CHD) and stroke, are responsible annually for 7.4 million deaths and 6.7 million deaths, respectively (1). In the U.S., CVD mortality steadily declined from the mid-1990s through the early 2000s, but has experienced a slight increase since 2011 (4). CVD remains the leading cause of death in non-Hispanic white and non-Hispanic black Americans, while cancer has eclipsed CVD as the leading cause of death in non-Hispanic Asian, Pacific Islander, and Hispanic Americans (4, 5). The prevalence of CVD increases with age. In the U.S., 6.3% of men and 5.6% of women aged 40-59 years are affected by CHD compared to 19.9% of men and 9.7% of women aged 60-79 years. Similarly, stroke prevalence in adults between ages 40-59 is 1.9% in men and 2.2% while for those aged 60-79 years the prevalence increases to 6.1% in men and 5.2% in women.

In the U.S., the incidence and prevalence of CVD differs greatly by sex and geographic region. Longitudinal studies suggest that women are more likely to have cerebrovascular disease as a first event while men are more likely to suffer an event from CHD, and that in general, CVD develops approximately ten years later in women compared to men (6, 7). CVD prevalence and mortality also vary geographically across the U.S., with the highest burden seen in the southeastern states (i.e. the "stroke belt") (8, 9). Additionally, despite progress leading to a steady decrease in CVD mortality rates in the U.S., gender and racial/ethnic disparities in cardiovascular health have persisted. Non-Hispanic blacks experience the highest age-adjusted CVD mortality rates with an estimated 316.9 deaths per 100,000 persons, followed by non-Hispanic whites with an estimated 243.5 deaths per 100,000 persons (10). Racial/ethnic disparities in CVD burden in the U.S. are predominately attributed to disparities in CVD risk factors by poverty status(11).

In addition to its role as the leading cause of death worldwide, CVD also contributes tremendously to global morbidity. According to the World Health Organization (WHO), CHD and cerebrovascular disease (stroke) are the fourth and sixth leading causes of disability-adjusted life years (DALYs) lost in the world, contributing to 62.5 million and 46.6 million DALYs lost, respectively (12). Peripheral artery disease (PAD), the third leading cause of cardiovascular morbidity, is estimated to affect over 202 million people (13). The prevalence of PAD in U.S. adults increases steeply with age such that the prevalence in men and women under 60 is less than 5%, but in men and women 80 years or older the prevalence increases to 26% and 20%, respectively (14). Individuals diagnosed with PAD experience reduced functionality and mobility, and have a three times greater risk of mortality due to cardiovascular event such as myocardial infarction (MI) or stroke (13). The global prevalence of PAD has increased greatly

over the last decade by 13.1% in high income countries and 28.7% in low and middle income countries. Consequently, morbidity caused by PAD has steeply increased during the last 20 years, particularly in women (14).

Although CVD represents various diseases affecting the heart and blood vessels, atherosclerosis is the underlying disease process responsible for the majority of CVD manifestations including CHD, cerebrovascular disease, and PAD (5). Therefore, the majority of the global burden of CVD morbidity and mortality can be attributed to atherosclerotic CVD.

2.1.1 Traditional Risk Factors for Atherosclerotic CVD

In addition to age, sex, and race/ethnicity described above, several important modifiable and non-modifiable risk factors for atherosclerotic CVD have been identified. Traditional risk factors for atherosclerotic CVD include high total or low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL) cholesterol, diabetes, cigarette smoking, and high systolic blood pressure (SBP) (15). Each of these factors is included in the Framingham Risk Score, which is commonly used in order to assess one's 10-year risk of a coronary event (16). While individuals with atherosclerotic disease in one location within the vasculature are more likely to have atherosclerosis in another location within the vasculature (17, 18), evidence suggests the relative importance of traditional CVD risk factors may vary slightly by disease location. For example, individuals with PAD, which results from atherosclerotic disease in the femoral artery, are often diabetic, smoke tobacco, and have a heightened inflammatory state compared to those with stable angina (19). Alternatively, high SBP is considered the most important modifiable risk factor for cerebrovascular disease which results from atherosclerotic disease in the carotid artery

(20). Additional risk factors associated with atherosclerotic CVD include but are not limited to: family history, obesity, physical inactivity, fasting glucose, and metabolic syndrome (21).

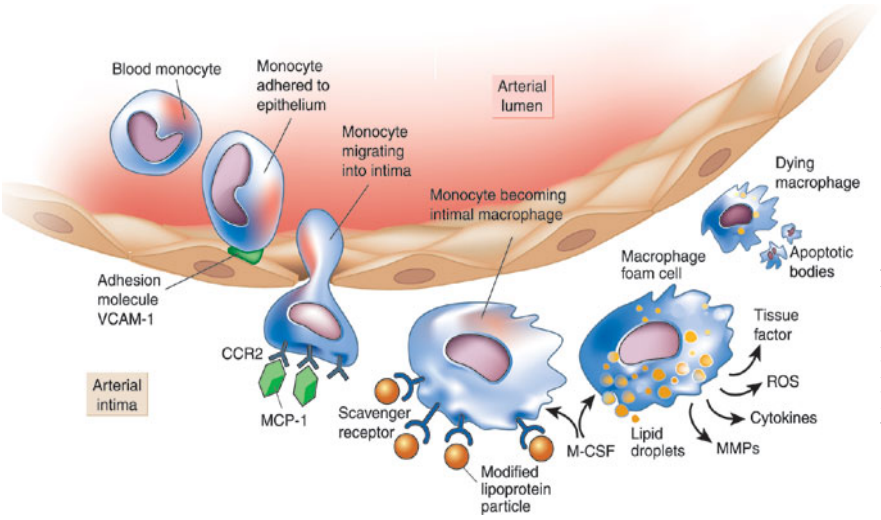
2.2 ATHEROSCLEROSIS AND PLAQUE

Atherosclerosis is a chronic inflammatory disease that occurs throughout the lifetime, which results in the hardening and thickening of arteries due to the buildup of plaque (lipids, immune cells, calcium, and other substances) within the arterial wall (13, 22, 23). While atherosclerotic processes are initiated at a young age, it typically remains clinically silent throughout the majority of its progression. For example, initial lipid accumulation in the intimal layer of the vascular wall takes place in the first decade of life, but CVD events do not usually occur until middle-age (24). The progression of plaque can eventually lead to narrowing or a local or distal blockage of an affected artery resulting in symptoms such as angina, silent cerebral infarcts, and claudication, or serious clinical events such as myocardial infarction (MI), ischemic stroke, and acute limb ischemia.

2.2.1 The Role of Inflammation

Atherosclerotic plaque development is a multi-step process that is partially driven by inflammation. A variety of inflammatory cells, chemokines, and co-stimulatory molecules employed by both the innate and adaptive immune response are involved in plaque development and progression (22). Atherosclerotic plaque development is initiated following injury to the vascular endothelium that triggers an inflammatory response and allows for lipoprotein

penetration of the endothelium (22, 25). Once injury occurs, local macrophages release pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), resulting in the recruitment of leukocytes to the site of inflammation and the expression of cellular adhesion molecules along the vascular endothelium (25). Intracellular adhesion molecule 1 (ICAM-1) expressed on the leukocytes, and ICAM-1 and vascular cell adhesion protein 1 (VCAM-1) expressed on the vascular endothelium allow for diapedesis, the transendothelial migration of leukocytes from the circulation to the site of tissue damage, to occur (Figure 2.1) (23, 26).



Relevant Acronyms:
CCR2 = Chemokine receptor type 2
M-CSF = Macrophage colony-stimulating factor
MCP-1= Monocyte chemoattractant protein-1
ROS = Reactive oxygen species
VCAM-1 = Vascular cell adhesion molecule-1

Obtained from Libby, Nature, 2002.

Figure 2.1 Mononuclear Phagocytes in Atherogenesis

Activation of the vascular endothelium and monocyte-endothelial cell interactions allow for lipids to also migrate through the vascular endothelium (27). It is not clear precisely why this initial injury to the vascular endothelium occurs; however, branches and bifurcations throughout the vasculature are especially prone to atherosclerotic plaque development. Turbulent blood flow and altered shear stress caused by recirculation are common in the area surrounding bifurcations in the vasculature (Figure 2), and these phenomena have been associated with endothelial

changes, at the gene expression, cellular, and molecular level, that precede plaque initiation and development (28).

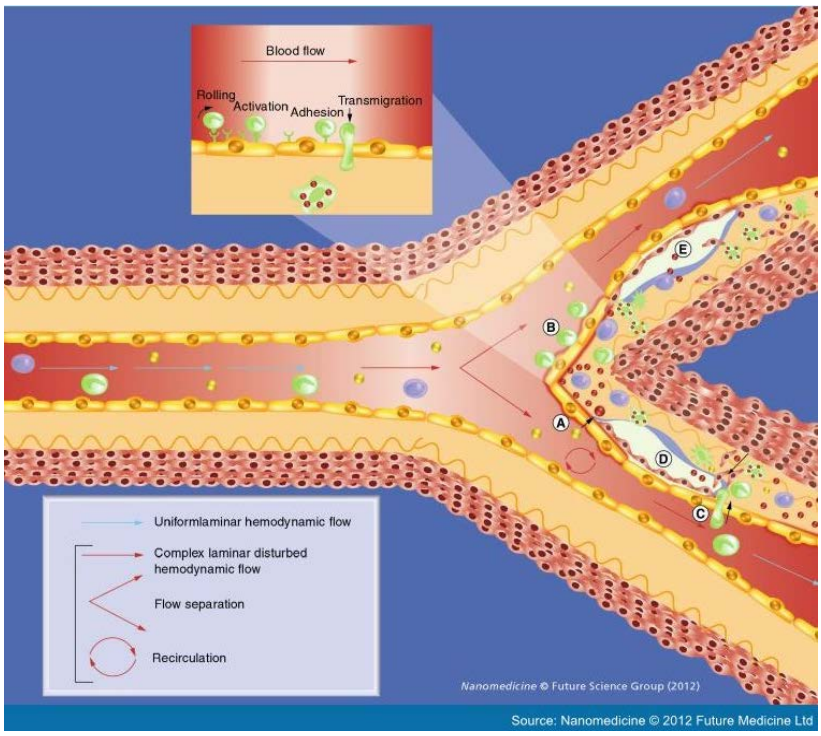


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Figure 2.2 Atherogenesis Surrounding the Bifurcation

Once lipids have crossed the vascular endothelium and begun accumulating within the intima of the vessel wall, macrophages internalize the lipids utilizing receptor-mediated endocytosis and micropinocytosis (29). Foam cells are formed when the uptake of lipids by macrophages results in an imbalance in the amount of cholesterol retained and released by the cell. Activated macrophages and T cells secrete interleukin 6 (IL-6) in order to recruit additional lymphocytes and neutrophils to the developing plaque (22). The pro-inflammatory state also results in the migration of smooth muscle cells from the media to the intima of the vascular wall (23, 26). Here, smooth muscle cells become activated and proliferate, resulting in further inflammation. IL-6 also signals the production and release of C-reactive protein (CRP), an acute phase reactant, by the liver.

In addition to promoting the expansion of plaque lesions, inflammation also contributes to the weakening of the fibrous tissue "capping" plaques, which makes the plaque more vulnerable to erosion or rupture (30). Specifically, interferon gamma (IFN-γ) secreted by T cells disrupts collagen synthesis, and matrix metalloproteinases (MMPs) secreted by T cells and macrophages break down collagen within plaque (22). Additionally, neutrophils are rich in tissue proteases such as lipocalin, which has been identified in hemorrhaged plaques via histology (31). Plaques may fuse together as they expand over time, further increasing the likelihood of complications (32).

2.2.2 Overview of Inflammatory and Coagulation Markers Assessed

IL-6 is a pro-inflammatory cytokine that is secreted by T cells and macrophages in response to infection or tissue damage in order to activate lymphocytes and increase antibody production (33). IL-6 also induces the production of acute-phase proteins by the liver. IL-6 is associated with higher odds of PAD and incidence of PAD in cross-sectional and prospective cohort studies of older adults (34, 35). Elevated serum IL-6 is independently associated with the presence of symptomatic plaques in the carotid artery among older adults (36), and greater extent of coronary stenosis among those in mid-life (37). It has also been associated with low echogenicity and greater complexity of plaques in the carotid and coronary arteries (38, 39).

CRP is an acute-phase reactant that is synthesized by hepatocytes in response to inflammation, specifically, IL-6 secretion by macrophages and adipocytes (33). CRP binds to dead or dying cells in order to activate the complement system so that bacteria and the affected cells may be cleared by macrophages. CRP found in the blood plasma is a well-established non-specific marker of inflammation that has been associated with increased CVD risk such that a

circulating CRP level greater than 3mg/dL is considered a CVD risk factor (40). Similar to IL-6, higher CRP is associated with higher odds of PAD and incidence of PAD over time independent of traditional CVD risk factors (41, 42). Additionally, among those with PAD, CRP is associated with faster functional decline and increased mortality (43). Elevated CRP is an independent predictor of carotid plaque presence and burden in men, but the relationship among women is only marginally significant after adjustment for traditional CVD risk factors (44, 45). CRP is negatively correlated with echogenicity of carotid plaques assessed via ultrasound; however this association is only borderline statistically significant following adjustment for CVD risk factors (39).

Intracellular Adhesion Molecule (ICAM)-1 is a transmembrane protein expressed on activated leukocytes and endothelial cells in the vasculature (33). ICAM-1 facilitates transendothelial migration of leukocytes across the vascular endothelium so that they may reach the site of injury or tissue damage. Increased ICAM-1 expression is induced by the expression of inflammatory cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF). Multiple studies have reported an association between increased circulating ICAM-1 and PAD in hemodialysis patients (46, 47); however, less is known about this relationship in the general population.

Fibrinogen is an acute phase protein synthesized in hepatocytes in response to inflammation which aids in thrombogenesis (48). During clot formation fibrinogen is converted to fibrin, which polymerizes with platelets in order to form the clot. Multiple longitudinal studies have reported an association between increased circulating fibrinogen levels and incident PAD among both healthy adults and diabetic patients (41, 49, 50). Among individuals with PAD, circulating fibrinogen is associated with increased risk of coronary and all-cause cardiovascular

mortality (51, 52). In longitudinal cohort studies, elevated fibrinogen levels have been associated with carotid plaque presence and greater carotid stenosis (53-55); however, the evidence regarding the association between fibrinogen and carotid plaque echogenicity remains mixed especially in women (53, 55-57).

D-Dimer is a fibrin degradation product that is present in the blood after a clot has been degraded (58), which is measured in order to determine the presence of a thrombus in the vasculature. While D-Dimer is not associated with PAD after adjustment for CVD risk factors, it is independently associated with reduced functional performance, increased severity of PAD, and increased risk of mortality in those with PAD (59-61). The evidence regarding the associations with carotid artery plaque are limited. However, one histologic study of individuals undergoing carotid endarterectomy found that higher plasma D-dimer levels were cross-sectionally associated with lower echogenicity plaques and greater plaque burden among participants (62), and another study examining diabetic patients found that participants with higher D-dimer levels were more likely to have ulcerated carotid plaques (57).

PTX-3 is an acute phase protein synthesized in macrophages, dendritic cells, myeloid cells, and endothelial cells (33). PTX-3 initiates the classical pathway of the non-specific complement response and is a pattern recognition receptor. In addition to its role in inflammation, some studies suggest that PTX-3 plays a role in coagulation by initiating the expression of Tissue Factor, an activator of the coagulation cascade (63, 64). Few studies have examined the association between PTX-3 and PAD (65). In a small study of patients undergoing revascularization for PAD, circulating levels of PTX-3 were positively correlated with severity of PAD (65). Additionally, in a sample of hemodialysis patients, PTX-3 has been found to be cross-sectionally and longitudinally associated with PAD (66, 67). The relationship between

PTX-3 and femoral plaque characteristics remains unclear; however, one study found that among older adults PTX-3 was associated with increased severity (plaque score) of carotid and femoral plaque assessed via B-mode ultrasound (68).

Lp(a) is a plasma lipoprotein produced in the liver whose primary function remains unclear; however it is hypothesized to be a causal genetic risk factor of CVD based on its strong association with traditional CVD risk factors and premature coronary heart disease (69, 70). Lp(a) was found to be independently associated with symptomatic PAD and lower ABI in the population-based KORA study as well as a cohort of male patients suffering from intermittent claudication (71). A recent study of participants in the Multiethnic Study of Atherosclerosis similarly found an independent association between Lp(a) and PAD, but when the sample was categorized by race/ethnicity the relationship between Lp(a) and PAD remained significant only among Hispanic American men and women (72).

Factor VII is a serine protease found in circulating blood that binds to Tissue Factor in order to initiate coagulation, and it is commonly measured as a marker of coagulation activity (73). While multiple cohort studies have found significant longitudinal associations between elevated Factor VII levels and CVD (74, 75), the results in this area of the literature remain mixed (76-78). Investigators from the Cardiovascular Health Study, within which no association between circulating Factor VII and CVD was observed, speculate that the variability in Factor VII testing between laboratories may contribute to the inconsistent findings (76). Among patients undergoing carotid endarterectomy, elevated Factor VII is associated with symptomatic carotid plaque (79); however, the relationship between circulating Factor VII with carotid plaque presence, burden, and characteristics remains unknown.

Von Willebrand Factor Antigen (vwAntigen) allows for the measurement of von Willebrand Factor (VWF) protein in the blood plasma. VWF is a protein produced by endothelial cells and megakaryocytes that binds to several proteins involved in the coagulation cascade, and also binds to platelets in order to facilitate clotting (80). Among patients with transient ischemic attack or ischemic stroke, elevated VWF levels are associated with the presence of calcification in the carotid artery. (81)

2.2.3 Plaque Erosion and Rupture

As atherosclerosis progresses throughout the vasculature, the majority of plaques will remain clinically silent. In fact, plaques may repeatedly rupture or experience erosion of the fibrous cap only to heal without causing symptoms or occlusion of the vessel (31). However, plaques that have eroded, ruptured, or expanded to an extent that they have caused severe narrowing or blockage of the vessel locally or downstream can cause serious symptoms and life-threatening events (25). While thrombosis is the primary cause of acute cardiovascular events, the evidence regarding the proportion of acute events that may be attributed to plaque erosion vs. plaque rupture is limited (82). The few studies comparing the prevalence and characteristics of erosion and rupture suggest that rupture is a more common cause of acute cardiovascular events compared to erosion; however, gender differences may exist with erosion being a more common cause of acute events in women (83, 84). Symptoms like chronic angina may occur when occlusive plaques limit blood flow, without causing an acute event due to rupture or erosion. Although individuals with stable angina are at increased risk of a cardiovascular event compared to the general population, their risk is lower than individuals who have previously suffered an

acute event (85, 86). The likelihood that a plaque will result in symptoms or an event may be influenced by numerous factors and characteristics.

The risk of serious complications caused by a specific plaque is heavily dependent upon the location of the plaque within the vasculature. First, the occlusion of the coronary or carotid arteries by plaque results in more serious health consequences, such as MI and stroke, compared to occlusion of the femoral artery. Additionally, plaques are more likely to result in vessel occlusion in the carotid or coronary arteries than the larger, lower extremity vessels (87). Moreover, characteristics of plaques typically vary based on vascular bed. A histologic study of individuals who underwent carotid and femoral endarterectomy found that femoral artery plaques contained significantly more calcium and less lipid content compared to carotid artery plaques, and that plaques found in the carotid and coronary arteries are of higher American Heart Association (AHA) lesion grade than plaques found in the femoral arteries(88). According to an autopsy study of individuals with carotid and femoral atherosclerosis, carotid plaques were more likely to contain foam cells and lipid core while femoral plaques were dominated by fibrous content, suggesting that femoral plaques may be more stable than those found in the carotid (89). These differences in characteristics could be due to the fact that the carotid and coronary arteries are narrower and experience more turbulent blood flow than the femoral artery making them more susceptible to endothelial injury, and that atherosclerosis typically develops later in the femoral arteries compared to the carotid and coronary arteries (89, 90).

There are also numerous features that may indicate how far along a plaque has progressed, and how prone it may be to erosion or rupture at a given time. Typically, plaque components that are associated with stability of the plaque are thick fibrous cap, high collagen content, high smooth muscle content, low macrophage content, and small fatty core (91). In

contrast, components that are associated with plaques that are more vulnerable to rupture or to erode are thin fibrous cap, lipid-rich necrotic core, intraplaque hemorrhage, greater inflammatory burden (i.e. high macrophage content), and neoangiogenesis. However, plaques may contain several of the above listed components, and those components may change over time (92). Therefore, plaques often cannot simply be identified as "stable" or "vulnerable". One characteristic of plaque that is particularly complicated in terms of interpreting the risk of a serious health event is calcification. For many years, calcification has been utilized as a marker of progressive atherosclerosis within the general population. However, among those with cardiovascular disease, calcification of plaque may be an indicator of the body's effort to stabilize existing plaque (93, 94). Due to the complex and dynamic nature of changes in atherosclerotic plaque over time, the mechanisms triggering acute cardiovascular events via plaque erosion or rupture are still unclear. This dissertation aims to shed light on the potential inflammatory and thrombogenic mechanisms that may influence the likelihood of plaque to erode or rupture.

Epidemiologic studies using histologic specimens and magnetic resonance imaging (MRI) in order to study the relationships between specific components of carotid plaque and risk of cerebrovascular events have found extremely consistent results. In 2013, Howard et al. found that lipid rich necrotic core (LRNC) and inflammatory burden are associated with significantly increased odds of a cerebral event in the previous six months in a retrospective histologic study of over 1,600 individuals who underwent carotid endarterectomy in the Athero-Express and Oxford Plaque Studies (OR = 1.38 and OR=1.32, respectively) (95). Conversely, fibrous content and calcification were found to be associated with significantly reduced odds of cerebral event in the previous six months (OR = .71 and OR=.70, respectively). Another histologic study of the

Athero-Express and Oxford Plaque Study that used immunohistochemical staining in order to identify specific plaque components found that participants in the highest quartile of predicted stroke risk had greater odds of plaques containing thrombus (OR=1.42), high macrophage content (OR=1.41), and high microvessel density (OR=1.49), and less fibrous content (OR=.65) compared to individuals in the lowest quartile of predicted stroke risk (96). Using MRI to characterize plaque components, Takaya et al. found in 2006 that asymptomatic individuals with thin or ruptured cap (HR=17.0), intraplaque hemorrhage (HR=5.2), and lipid rich necrotic core (HR=1.6) had a higher hazard of a cerebrovascular event over the mean 38.2-month follow-up time (97). Similarly, in 2013 Esposito-Bauer et al. found that lipid-rich necrotic core and thin/ruptured fibrous cap identified via MRI are associated with the development of cerebral ischemia over 41-month follow-up in 83 asymptomatic individuals with ≥ 50% stenosis of the internal carotid artery (ICA) (98). Lastly, a study comparing 41 patients suffering acutely from transient ischemic attack (TIA) to 40 asymptomatic patients matched by percent carotid stenosis found that symptomatic patients were significantly more likely to have carotid plaques with a rupture to the fibrous cap. Symptomatic patients were also more likely to have plaques with intraplaque hemorrhage and luminal thrombus; however, these differences were not statistically significant (99).

2.3 MEASURING AND CHARACTERIZING PLAQUE

Because symptoms and complications of CVD typically present themselves only after the disease has developed for many years, the study of subclinical CVD provides valuable insight into the pathologic processes that lead to CVD events. Additionally, measurement of subclinical CVD

allows for the assessment of an individual's risk of developing CVD complications, and offers an opportunity for prevention.

The measurement of plaque presence and extent is a direct measure of atherosclerotic burden, and is a well-established indicator of CVD risk in longitudinal epidemiologic studies (100-102). There are numerous methods that can be utilized to measure plaque presence, some of which also allow for the characterization of plaque that is identified (see Table 2.1). There are strengths and limitations of each method, and the ideal method of plaque measurement is highly dependent upon the population being studied. Invasive techniques utilized to measure plaque include: intravascular ultrasound (IVUS), optical coherence tomography (OCT), intravascular magnetic resonance (IVMR), and near infrared spectroscopy (NIRS) (103). These techniques all offer high-resolution images of the vessel wall and plaque structure, and allow for several plaque components to be identified. However, they are all invasive, relatively expensive, and time-consuming, making them undesirable for the study of subclinical atherosclerosis in the general population.

There are several noninvasive methods of plaque assessment that also allow for the collection of high-resolution images and the characterization of plaque including: computed tomography (CT), MRI, positron emission tomography (PET), and single positron emission computed tomography (SPECT) (103, 104). While these techniques are valuable methods of assessing plaque morphology and specific plaque components, individuals undergoing CT, PET, and SPECT are exposed to radiation. Additionally, contraindications and aversion to MRI make it impractical for the study of plaque presence in certain populations (105). Duplex ultrasound is another noninvasive method of assessing plaque in the superficial arteries with high temporal resolution (103). Ultrasound is quick, inexpensive, and functional making it a desirable method

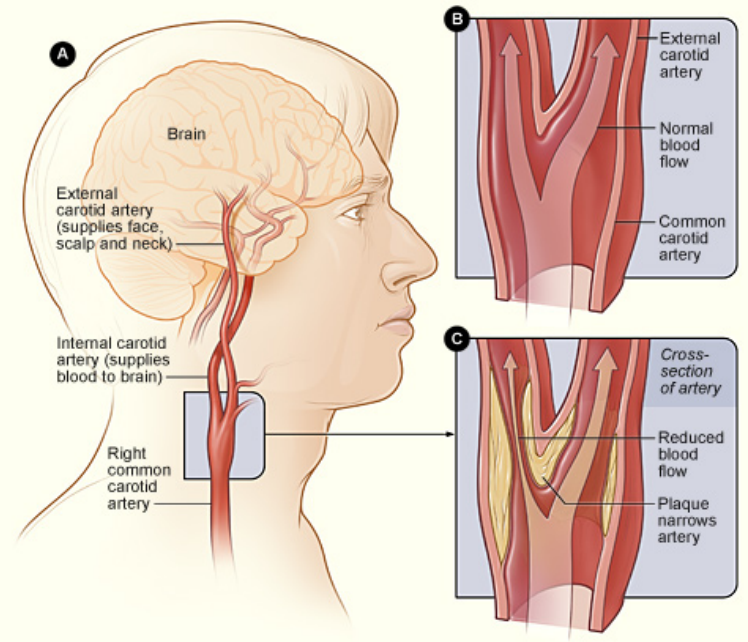
of plaque assessment in large studies. While ultrasound cannot be utilized to visualize plaque in the coronary arteries, it can assess plaque in the carotid and femoral arteries both of which are associated with atherosclerosis in the coronary arteries (90, 106-108). Reliability of determining plaque presence within- and between-sonographers ranges from k=0.72-0.78 (109), and a reproducibility study of plaque scoring based on the number and size of lesions found a between-sonographer intraclass correlation coefficient of 0.86 (110, 111).

More advanced methods of B-mode ultrasound such as 3D or 4D B-mode ultrasound allow for the measurement of plaque volume, identification of surface area deformation and temporal resolution (112). Additionally, high-risk plaque components such as intraplaque vascularization and ulceration may be assessed via contrast-enhanced ultrasound (113). Although traditional 2D B-mode ultrasound does not allow for the visualization of specific plaque components, it does allow for crude markers of plaque characteristics, such as echogenicity, to be measured. Between sonographer agreement regarding plaque characteristics such as thickness and echogenicity range from k=.54-.73 (109). This dissertation focuses on the detection and characterization of atherosclerotic plaque using images obtained via 2D B-mode ultrasound of the femoral arteries of older adults from the San Diego Population Study and the carotid arteries of midlife women in the MS Heart Study.

2.3.1 Detection and Measuring Plaque Using 2D B-Mode Ultrasound

Carotid plaque measured via B-mode ultrasound is a well-established measure of subclinical atherosclerosis (Table 2.2) (100-102). In addition to its widespread use in epidemiological studies, carotid B-mode ultrasound is also used clinically in order to screen individuals who may be at risk of stroke (101). The carotid artery is primarily of interest because

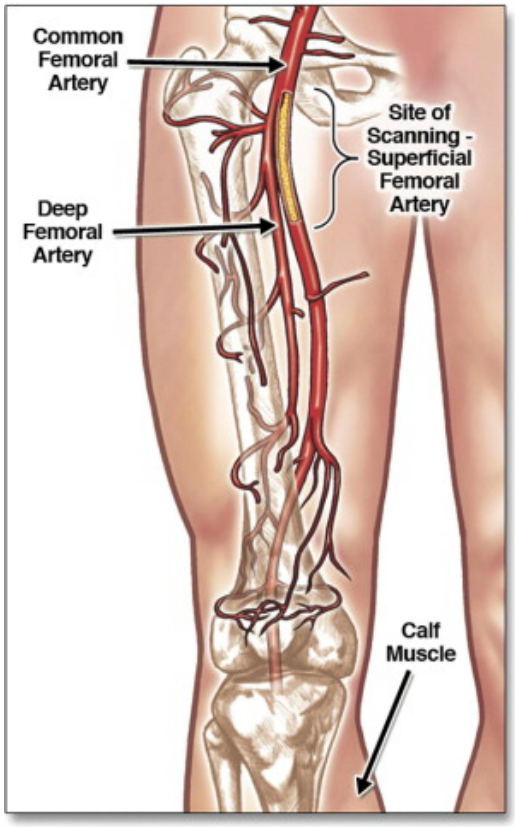
the carotid artery supplies the brain with oxygen rich blood. Typically, ultrasound is used to assess the common carotid, the bifurcation, and the internal carotid artery. Atherosclerosis in the external carotid that supplies the face with blood is not usually measured (Figure 2.3).



Obtained from: https://www.nhlbi.nih.gov/health/health-topics/topics/catd

Figure 2.3 Carotid Artery Disease

Similar to the carotid, the femoral artery is a superficial artery that may be easily assessed via ultrasound for the presence of atherosclerotic plaque. Due to the fact that femoral plaques do not directly lead to major acute cardiovascular events like carotid or coronary plaques do, it has been less commonly assessed throughout the literature (Table 2.3). However, atherosclerosis in the femoral artery can lead to PAD, and can cause symptoms such as numbness and pain in the legs (114). And in severe cases where the vessel is occluded, circulation to the leg may be cut off resulting in gangrene and amputation. Typically, the bifurcation of the common femoral artery into the deep and superficial femoral arteries is assessed for atherosclerosis (Figure 2.4) (115, 116).



Obtained from: McDermott, JACC, 2008.

Figure 2.4 Location of Femoral Imaging

2.3.1.1 Measurement of Carotid Plaque

Carotid plaque presence is first assessed via ultrasound by sonographers who examine specific segments of the artery for plaque (101). Sonographers obtain images of the various carotid segments of interest, which may be assessed for plaque presence by the sonographer in real-time or by a trained ultrasound reader at a later time. The definition used to identify plaque via ultrasound in a research setting is not entirely consistent throughout the literature. However, the most commonly used definitions are based on the Mannheim consensus and American Society of Echocardiography Carotid Intima-Media Thickness Task Force consensus criteria,

which define plaque presence as a focal wall thickening that is at least 50% greater than the surrounding IMT or as a focal structure that protrudes >1.5mm into the vessel lumen distinct from the adjacent boundary (101, 117).

There is also heterogeneity among studies regarding measures of plaque burden. Some measures are based on the number of segments of the carotid containing plaque, while others consider the size of plaque in addition to the number of segments containing plaque (106, 118, 119). Other common measures of plaque burden throughout the literature include percent luminal stenosis, area, height, length, and maximum thickness (119-123). Percent luminal stenosis is sometimes described categorically as "plaque grade", which can be determined in each segment of the carotid (110, 121). Plaque area is frequently measured, and a great deal of evidence suggests that greater plaque area is associated with increased cardiovascular risk (120, 124-126). For example in the BioImage Study, among those with carotid plaque, greater plaque area is associated with a 2.36 times higher hazard of a major adverse cardiac event over 2.5 years of follow-up (120). And in the Tromso study, each standard deviation increase in square-root-transformed plaque area is associated with a higher hazard of ischemic stroke over 10 years of follow-up (HR=1.23 and 1.19 in men and women, respectively) (124).

2.3.1.2 Epidemiologic Studies of Carotid Plaque Presence

Plaque in the carotid artery is significantly correlated with atherosclerosis in the coronary arteries (PPV=91%), and improves risk prediction for CVD event when added to traditional risk factors (107, 127). Two retrospective investigations of the Rotterdam Study population have demonstrated that carotid plaque presence is associated with significantly higher odds of stroke (all subtypes) (OR=2.44) and cerebral infarction (OR=2.70) after six years of follow-up, and significantly associated with increased hazard of MI (HR = 1.72) after 10 years of follow-up

(100, 102). Multiple prospective studies of asymptomatic individuals have found that carotid plaque presence is associated with a significantly increased hazard of composite vascular outcomes (stroke, MI, CVD death) over time (120, 128). Additionally, sex differences in the risks associated with carotid plaque presence has been documented. Although women are less likely than men of the same age to have carotid plaque (129, 130), according to the Tromso Study, carotid plaque presence is associated with a 1.56 times higher risk of MI in men and 3.95 times higher risk of MI in women after five years while adjusting for covariates (125).

2.3.1.3 Measurement of Femoral Plaque

Similar to plaque in the carotid artery, femoral plaque may be identified in real-time by a sonographer or via images read by a trained ultrasound reader at a later time. Segments of the femoral artery that may be assessed for plaque include the common femoral artery (CFA) proximal to the bifurcation of the CFA and the superficial femoral artery (SFA), the bifurcation, and the SFA distal to the bifurcation. The presence of plaque in these segments is determined identically to plaque in the carotid artery, typically using the Mannheim consensus or American Society of Echocardiography consensus criteria (117).

2.3.1.4 Epidemiologic Studies of Femoral Plaque Presence

Among individuals with PAD, atherosclerotic plaque presence in the femoral artery is associated with reduced functional performance (116). Additionally, plaque in the femoral artery is associated with the presence of atherosclerosis in other arterial beds (90, 131). Because atherosclerosis in the larger, lower extremity vessels typically develops later then atherosclerosis in the smaller arteries, individuals with femoral plaque often have plaque in the carotid and coronary arteries (90). For example, an autopsy study conducted by Dalager et al. in 2007 found

that among individuals with plaque in the superficial femoral artery, 100% had plaque presence in the coronary arteries (90). Femoral plaque presence is also associated with cardiovascular events. According to Davidsson et al., plaque in the femoral artery was associated with significantly higher odds of a cardiovascular event (OR=1.99) over 10-year follow-up in a population-based sample of men (132). Additionally, femoral plaque presence was found to be associated with significantly higher odds of coronary death (OR=7.07) in a cross-sectional autopsy study, and a significantly higher hazard of a MACE (HR=5.92) over 4-6 years of follow-up in individuals with systemic lupus erythematosus (90, 115).

2.3.2 Characterizing Plaque Using 2D B-Mode Ultrasound

Echogenicity, or the ability to bounce (reflect) an ultrasonic wave, is a frequently utilized surrogate marker of plaque components that may be assessed in plaque via 2D B-mode ultrasound. Based on histologic studies of plaques that had formerly been assessed via carotid ultrasound, predominately echolucent plaques are associated with higher lipid and macrophage content compared to echogenic plaques, while echogenic plaques are associated with more fibrous content and calcification (Table 2.4) (133-136). There are several measures that can be collected in order to assess the echogenicity of a plaque. Most simply, echogenicity can be operationalized as a binary variable, in which case a plaque can be categorized as either echolucent or echogenic by automated software or the individual reading the plaque image (137, 138).

Additionally, echogenicity may be operationalized as an ordinal or continuous measure. Gray-Weale Scale, a scoring method first proposed in 1988, can be used by ultrasound image readers to subjectively categorize plaque echogenicity into four levels: Type I – predominantly

echolucent with a thin fibrous cap, Type II - echolucent with echogenic areas, Type III – echogenic with echolucent areas, or Type VI – uniformly echogenic (139). Plaques categorized as more echolucent (Types I and II) are associated with unstable characteristics including intraplaque hemorrhage and ulceration in histologic studies; however, this subjective scoring method is limited by moderate inter-observer agreement (139, 140).

The most commonly used continuous measure of echogenicity is grey-scale median (GSM). B-mode ultrasound allows for the intensity of reflected ultrasound waves to be indicated by a corresponding shade of grey within an ultrasound image (141). GSM of a plaque may be determined once it has been outlined, and samples of the blood and adventitia have been selected by a reader (Figure 2.5). The selection of blood and adventitia portions within the image is necessary for normalization, so that images collected from different ultrasound machines under various gain settings may be compared (142). Once a plaque has been outlined, automated or semi-automated software can determine the median grey-scale value of the identified plaque. Because GSM values may be affected by image quality and the angle at which a plaque image is obtained, it is important that standardized procedures are utilized when obtaining images particularly for longitudinal studies of plaque.

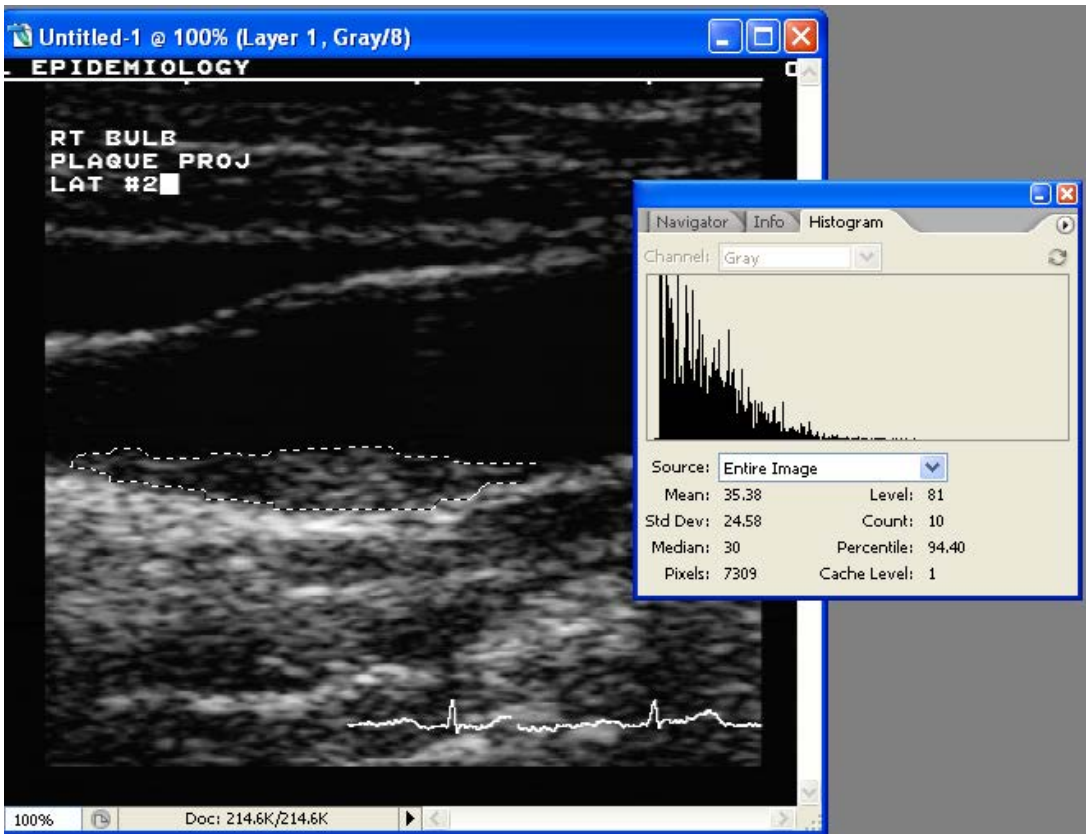


Figure 2.5 Plaque in the Right Carotid Bulb

Multiple histologic studies have attempted to determine correlations between GSM and specific components of plaque. In individuals undergoing carotid endarterectomy (CEA), GSM has been significantly negatively correlated with lipid content and necrotic core content, and significantly positively correlated with fibrous content (134, 135). Additionally, a histologic study of over 100 CEA patients found that individuals with the lowest quartile of GSM values had 3.76 higher odds of having unstable plaque (containing hemorrhage, thrombus, neovascularity, or cap rupture) (136).

Other methods of continuously measuring plaque echogenicity exist, such as percent white or plaque density; however, these measures are less commonly utilized and have less histologic evidence supporting their association with specific plaque characteristics (143, 144).

Lastly, bright areas of echogenicity accompanied by an acoustic shadow, an area in which ultrasound waves are strongly absorbed by solid structure, can be used to determine whether or not a plaque is calcified or not (145, 146).

2.3.2.1 Epidemiologic Studies of Carotid Plaque Characteristics

Prospective cohort studies have demonstrated the risks associated with echolucent plaque in the carotid artery (Table 2.5) (137, 138, 147-150). A 2001 study conducted by Gronholdt et al. found that asymptomatic individuals with echolucent plaque in the carotid artery, defined as having a plaque with GSM <74, had a statistically significant 3.1 times higher risk of ischemic stroke over time compared to those with echogenic (GSM ≥74) plaque (137). Similarly, in a study conducted by Reiter et al. of nearly 600 asymptomatic individuals with carotid plaque, individuals with carotid plaque GSM values in the lowest quartile compared to those in the highest quartile had a statistically significant 1.71 times higher hazard of a major adverse cardiovascular event (MACE) over three years (148). Calcification has also been found to be predictive of CVD events, particularly in studies comparing those with calcified plaque to those with no plaque. In the CAPE study, elderly adults with calcified plaque in the carotid artery had significantly higher rates of CVD events (RR=2.35 CI=1.5-3.8) and overall mortality (RR=2.72, CI=1.4-5.2) after 11-year follow-up compared to elderly adults with no plaque (151). This study did not compare rates of CVD events among those with calcified and non-calcified plaque. In the Atherosclerosis Risk in Communities (ARIC) study, calcification identified via acoustic shadowing has been associated with higher risk of stroke compared to those who have plaques without acoustic shadowing in women; however, this relationship was not maintained after adjustment for CVD risk factors and was not present in men (147).

2.3.2.2 Epidemiologic Studies of Femoral Plaque Characteristics

Plaque in the femoral arteries may be characterized using B-mode ultrasound identically to plaque in the carotid arteries. Echogenicity, GSM, Gray-Weale Scale, and calcification of femoral plaque may all be determined using appropriate software or by the image reader. A recent prospective study conducted by Schiano et al. found in individuals diagnosed with PAD that the presence of echolucent femoral plaques defined by Gray-Weale Scale criteria was associated with a significantly higher hazard of a MACE (HR= 7.24, CI=3.23-16.22) compared to echogenic plaque after adjusting for possible confounders, and that continuously measured GSM of femoral plaques was independently associated with a significantly lower hazard of a MACE (HR= 0.96, CI=0.95-0.98) (149). Although literature examining the associations between femoral plaque characteristics and cardiovascular events is limited, the results of the Schiano et al. suggest that the relationship is consistent to that seen in the carotid artery (Table 2.5).

2.4 PSYCHOSOCIAL STRESS AND CARDIOVASCUALR DISEASE

A broad range of psychosocial exposures are associated with increased risk of CVD (152-154). These exposures include negative emotional states such as depression, anxiety or hostility (155-157), as well as acute traumatic life stressors such as abuse or trauma (158-160). Additionally, social factors including but not limited to social support (161), loneliness (162), and discrimination (163) are associated with cardiovascular risk. In longitudinal studies, measures of psychosocial stress are predictive of incident CHD and MI in those with existing CHD (154). Psychosocial distress (including depression, anxiety, and perceived stress) has also been longitudinally associated with fatal and non-fatal stroke (164, 165).

The associations of specific psychosocial stressors with measures of subclinical atherosclerosis vary greatly depending on the type of stressful exposure and the subclinical outcome. This dissertation will focus on one well-established psychosocial risk factor, perceived discrimination, and its potential association with atherosclerotic plaque in the carotid artery. Additionally, the evidence supporting increased inflammation as a mechanism linking psychosocial stress to atherosclerosis will be explored.

2.4.1 Inflammation as a Mechanism Linking Psychosocial Stress and CVD

Although it was initially believed that stress exerted suppressive effects on the immune system, current research suggests that under normal conditions stress temporarily shifts immunoregulation toward an anti-inflammatory response (166). However, in the case of chronic or long-term stress, the dysregulation of the immune system results in increased inflammation, which is believed to contribute to atherosclerotic disease development and progression (152, 153). There are three main mechanisms by which stress results in a heightened inflammatory state described in the literature: increased susceptibility to secondary infection, decreased anti-inflammatory feedback, and local effects of stress hormones (153).

First, stress alters the modulation of the immune system from a Th1 cell to a Th2 cell-driven response, increasing one's susceptibility to secondary viral or bacterial infections (166). Th1 and Th2 cells are variations of T Helper cells which serve different functions in the immune response. Th1 cells initiate the response to intracellular pathogens (i.e. viruses, intracellular bacteria) via the secretion of inflammatory cytokines, while Th2 cells initiate B cell activation for the response to extracellular pathogens (i.e. parasites, extracellular bacteria) (167). Evidence suggests that Th1 cells play a role in plaque development via the secretion of the inflammatory

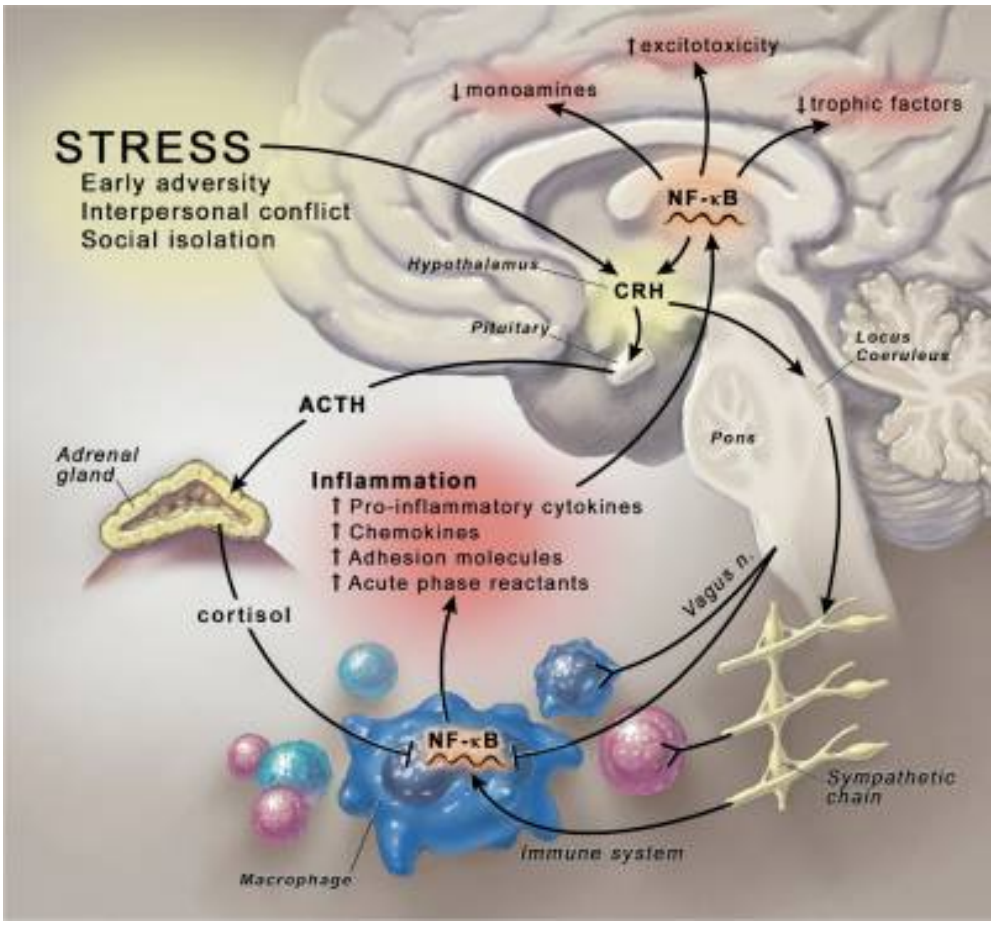
cytokines such as interferon gamma (IFN-γ), and they are commonly found in plaque lesions (168). Th2 cells are rarely found within plaque lesions (166, 168). Based on mouse models, Th2 cells are hypothesized to counter the atherogenic effects of Th1 cells via the downregulation of IFN- γ; however, this are of research is limited to animal models and remains controversial (169). When the immune system is chronically shifted from a Th1 to a Th2 cell-driven response due to stress, it increases the susceptibility of an individual to common viral or bacterial infections (153). This susceptibility can result in infections which ultimately lead to inflammation.

Additionally, decreased anti-inflammatory feedback due to the exhaustion of the hypothalamus and adrenal medulla contributes to the heightened inflammatory state under chronically stressful conditions. Typically, stress response results in the release of glucocorticoids (i.e. cortisol) via the activation of the hypothalamic-pituitary-adrenal (HPA) axis, and the release of catelcholamines (i.e. epinephrine) via stimulation of the adrenal medulla by the autonomic nervous system (ANS) (152, 153). Both glucocorticoids and catecholamines inhibit pro-inflammatory cytokines by mediating the shift in immune response from a Th1 to Th2 cell-driven response (170). However, under chronically stressful conditions evidence suggests that the chronic activation of the HPA axis and the ANS result in increased inflammation via both the exhaustion of the hypothalamus and adrenal medulla as well as resistance to the anti-inflammatory signaling of glucocorticoids and cathelcholamines on a cellular level (153) (Figure 2.5). Therefore, there is reduced anti-inflammatory feedback occurring during chronic or long-term stress, which allows for Th1 cells to dominate the immune response.

Lastly, stress hormones like catelcholamines have direct pro-inflammatory effects locally upon release by the adrenal medulla. Catelcholamines induce the production of IL-6 by adipose

cells (170). IL-6, a potent pro-inflammatory cytokine, triggers the acute phase inflammatory response including the production of CRP by hepatocytes (171).

Additionally, the results of a limited number of studies suggest that specific autoimmune diseases may be exacerbated by the effects of chronic stress via dysregulation of Th1 and Th2 cells and chronic HPA activation (172-174). These studies suggest that individuals with autoimmune diseases such as rheumatoid arthritis and multiple sclerosis may be at an even greater risk of developing a heightened inflammatory state under stressful conditions compared to the general population.



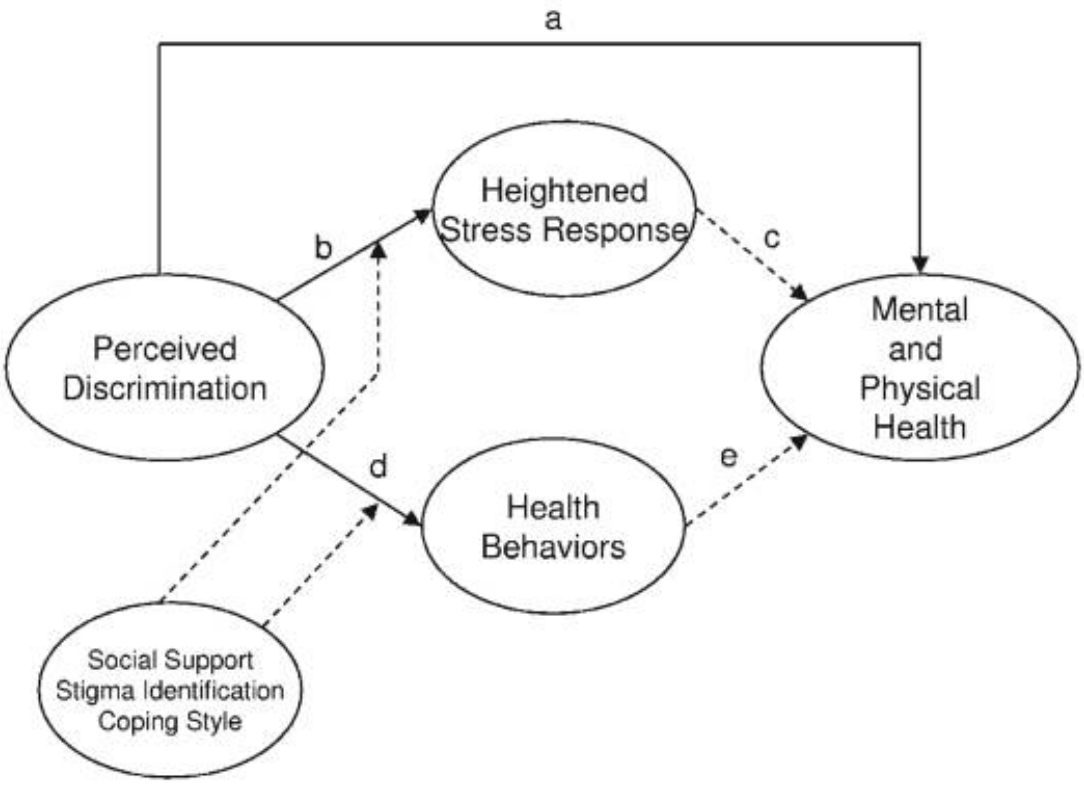
Obtained from: Miller et al. Biological Psychiatry. 2009.

Figure 2.6 Stress Induced Activation of Inflammatory Response

2.4.2 Perceived Discrimination as a Psychosocial Risk Factor for CVD

Discrimination, defined as the unjust or prejudicial treatment of different categories of people, is an unpredictable and uncontrollable stressful experience that has been linked to a broad range of negative health outcomes and behaviors (175). In order to assess the health effects of discrimination in epidemiologic studies, questionnaires are typically utilized to measure perceived discrimination via self-report. Due to the well-documented racial/ethnic disparities in CVD across the U.S., perceived discrimination has gained attention over the last two decades as a potential risk factor contributing to the disproportionate effect of CVD on the African American population (176). Perceived discrimination is associated with numerous lifestyle and risk factors causally implicated in CVD including smoking (177), physical inactivity (178), alcohol and substance abuse (179, 180), high blood pressure (181, 182), poor sleep (176), and depression (183, 184). In 2015, Everson-Rose et al. first reported that perceived discrimination was longitudinally associated with increased hazard of an incident cardiovascular event (HR=1.05, 95% CI = 1.01-1.60) after adjustment for CVD risk factors, chronic stress, and depressive symptoms in the Multi-Ethnic Study of Atherosclerosis (MESA) (185). However, after stratifying by sex, the association was only seen in male participants. In addition to providing evidence of the harmful effects of perceived discrimination on physical health and health behaviors, these studies have somewhat consistently reported that the type and attribution of discrimination are of lesser importance compared to the frequency and consistency of the perceived discrimination. These results support the idea that long-term or consistent exposure to discrimination may result in chronic stress that negatively impacts cardiovascular health; however, additional studies investigating the mechanisms through which perceived discrimination may influence physical health are needed.

There are three main pathways through which perceived discrimination is hypothesized to affect mental and physical health outcomes such as CVD (Figure 2.5) (175). First, perceived discrimination may exhibit direct negative effects on physical and mental health (Path A). Perceived discrimination may also result in chronic heightened stress response such as those described in the previous section, which may result in chronic systemic inflammation (Path B). Lastly, negative health behaviors may increase among those who perceive that they are discriminated against as a method of coping with this stressful experience (Path C).



Obtained from: Pascoe and Richman. Psychological Bulletin. 2009.

Figure 2.7 Hypothesized Pathways by which Perceived Discrimination Influences Health Outcomes

There are multiple validated questionnaires that are commonly utilized to measure the frequency and type of perceived discriminatory experiences. These questionnaires may focus on major experiences like being denied housing or a bank loan as well as more minor experiences like feeling as though you receive poor service at restaurants or stores. The Everyday Discrimination Scale (EDS) is one of the most widely utilized measures of perceived discrimination (186). The EDS is a brief questionnaire that has been validated across racial/ethnic groups for the measure of "chronic or episodic, but generally minor" experiences of perceived discrimination (To be added in appendix) (187). For example, the EDS includes items intended to measure experiences such as being treated with less respect than other people, being treated as though you are dishonest, and being treated as though you are not smart (186). Each of the 9-items on the EDS are then rated on a 4-point Likert-type frequency scale ranging from "never" to "often". The EDS also includes an item which allows participants to identify the most important characteristic to which they attribute their experienced discrimination including race, ethnicity, gender, age, income level, language, physical appearance, sexual orientation, or other. Overall, the majority of research regarding the role of perceived discrimination in CVD development has focused on perceived racial discrimination in African American populations; however, evidence suggests that self-reported discriminatory experiences may be related to cardiovascular health in various populations (188-190).

2.4.2.1 Epidemiologic Studies of Perceived Everyday Discrimination and Subclinical CVD

The number of studies aimed at examining the hypothesized association between everyday discrimination and subclinical CVD has increased greatly in recent years. Most of these studies have focused on the effects of everyday discrimination on blood pressure (181, 191-195), but more recently, studies have begun to incorporate measures of subclinical atherosclerosis

including coronary artery calcium (CAC) (196, 197), intima-media thickness (IMT) (188, 198), and adventitial diameter (AD) (188). Only one study conducted in the Study of Women's Health Across the Nation (SWAN) has specifically examined the association of EDS with plaque (198). This study was conducted in a limited sample of 334 participants from the Pittsburgh site, and found only a marginal association between EDS score and carotid plaque index among African American participants in unadjusted models.

The results of the remaining studies focusing on subclinical outcomes are mixed (See Table 2.6). Of the six studies that have examined the association between the EDS and blood pressure, the three conducted in midlife or older adults found no statistically significant association between the EDS and blood pressure even before adjustment for covariates (191-193). Conversely, three studies conducted among children and young adults have found significant associations between EDS score and SPB after adjustment for demographics, physical activity, and BMI (181, 194, 195). In one of these studies conducted by Klimentidis et al., EDS score was found to be significantly associated with SBP among African American children (β=0.20, p=0.041) (181). The two studies focusing on CAC as a subclinical outcome have included only African American participants, and have found mixed results. Lewis et al. reported that chronic discrimination measured by the EDS was associated with higher odds of CAC (OR=2.6 95%CI=1.0-6.47) in SWAN participants after adjustment for covariates (196), while Everage et al. has shown that EDS score was associated with lower odds of CAC (OR=0.93 95%CI=0.87-0.99) among CARDIA participants after adjustment for covariates (197). Additionally, two studies among SWAN participants have examined the relationship between EDS and cIMT. Among SWAN participants from the Pittsburgh site, Troxel et al. found only marginal associations between EDS score and cIMT before adjusting for covariates in their

cross-sectional analysis (198). However, a recent study conducted by Peterson et al. in the complete SWAN cohort found that among Caucasian women cumulative unfair treatment assessed via the EDS over six time-points was associated with increased cIMT (β =0.03 p=0.009) and adventitial diameter (β =0.02, p=0.013) after adjustment for covariates (188).

There are two major limitations to the literature examining the associations between perceived everyday discrimination and subclinical CVD. First, many of these analyses have been conducted in the same cohort study participants. Although the samples in which these analyses have been conducted consist of participants enrolled in reputable cohort studies, it is possible that these cohorts are not representative of the entire general population. Therefore, similar studies are necessary in samples consisting of participants in various age, sex, and racial/ethnic groups. Additionally, several of the analyses described above do not account for depression or negative affect. Depression is highly correlated with both perceived discrimination and CVD, so it is important to take into account measures of depression and negative affect in these analyses as they may confound the hypothesized relationship between perceived discrimination and subclinical CVD (183, 184, 199). Lastly, it is worth mentioning that many of the studies were conducted only in African American populations or sub-samples of existing cohort studies. The literature consistently has suggested that the attribution of discrimination (racial/ethnic vs. other types) may not be of importance in regards to the relationship of perceived discrimination and subclinical CVD. It may be beneficial for future studies to include a more generalizable study population in order to help elucidate the effects of perceived discrimination on different measures of clinical and subclinical CVD, and to help determine whether this relationship differs by racial/ethnic group.

2.5 SUMMARY

CVD, the leading cause of death globally, is primarily caused by atherosclerosis, a chronic disease that results in the accumulation of lipids over time within the arterial wall. Various inflammatory cells, chemokines, and co-stimulatory molecules are involved in the initiation of atherosclerotic lesion development following arterial injury; however, less is known about the potential involvement of these inflammatory cells in the progression, erosion, and rupture of atherosclerotic plaque.

Numerous types of psychosocial stressors are associated with increased CVD risk. One specific cause of psychosocial stress, perceived discrimination, has been shown to be associated with CVD risk factors, unhealthy behaviors that contribute to CVD, subclinical CVD measures, and incident CVD. One mechanism through which psychosocial stress is hypothesized to influence CVD risk is increased inflammation due to chronic stress.

Imaging via B-mode ultrasound allows for the direct identification, measurement, and characterization of atherosclerotic plaque in subclinical stages. Assessment of plaque in the subclinical phase can help shape our understanding of the processes that contribute to the various manifestations of CVD. This dissertation aims to determine whether there is an underlying structure to several circulating biomarkers of inflammation and coagulation that have previously been associated with CVD risk in order to determine whether groups of these biomarkers are associated with measures of carotid and femoral plaque presence, burden, and characteristics identified via B-mode ultrasound. Furthermore, this dissertation is intended to measure the association between perceived discrimination and carotid plaque, and to determine whether this hypothesized relationship is mediated by circulating markers of inflammation and coagulation. These analyses may shed light on potential mechanisms responsible for atherosclerotic plaque

instability, and may help identify characteristics that put individuals at risk of developing atherosclerotic plaque that is vulnerable to erode or rupture.

2.6 TABLES

Table 2.1 Imaging Techniques Used to Visualize Atherosclerotic Plaque

Technique	Plaque Characteristics Identified	Advantages	Limitations
Invasive Imaging Techniques			
Intravascular Ultrasound (IVUS)	Plaque distribution, severity, cross-sectional area, characterization of plaque (lipid core and spotty calcification	High resolution images of vessel wall and plaque structure	Intra- and interobserver subjectivity, invasiveness, limited spatial resolution, and limited temporal resolution
Optical Coherence Tomography (OCT)	Plaque composition (fibrous, fibrofatty, and fatty), thick fibrous cap, macrophages, neoangiogenesis, and collagen formation	10 times higher image resolution compared to IVUS	Requires blood-free imaging field, intra- and interobserver variation, invasiveness, and limited tissue penetration
Intravascular Magnetic Resonance (IVMR)	Early atherosclerosis and more advanced plaque formations and plaque composition (lipid, fibrous, and calcified tissues)	High resolution of plaque structure and composition	Invasiveness and need for occlusion balloon
Near Infrared Spectroscopy (NIRS)	Thin fibrous cap, lipid core, and macrophages	High resolution of plaque structure with reliability	Invasiveness, limited tissue penetration and cardiac motion artifact

Table 2-1 Continued

Noninvasive Imaging Techniques

Computed Tomography (CT)	Plaque morphology (eccentric pattern, outward remodeling, and spotty calcification), coronary plaque burden, cap thickness, and macrophages	High spatial and temporal resolution, real time, quire fast, operator-independent, and excellent calcium detection	Radiation exposure, contrast, difficult to distinguish thrombus, blooming artefacts by calcium and claustrophobia
Magnetic Resonance Imaging (MRI)	Plaque morphology, plaque composition, lipid-rich necrotic core, intraplaque hemorrhage, neoangiogenesis, macrophages, flow measurement, and quantification of stenosis	No radiation, high soft tissue contract, can be repeated over time, functional, operator independent, with or without contrast, and many plaque components detected	Low resolution, system fibrosis due to contrast agent, time-consuming metal implants contraindicated, claustrophobia, cardiac motion artefact, and limited spatial resolution
Ultrasound	Plaque morphology, intima media thickness, flow velocities, and neoangiogenesis (contract US)	High temporal resolution, cheap, easy to use, no radiation, bedside/large availability, fastest, and functional	Limited sensitivity and specificity, interobserver variability, calcium and air artefacts, limited spatial resolution, and penetration
Positron Emission Tomography (PET)	Plaque inflammation, macrophages, and neoangiogenesis	High sensitivity and specific targets are detected	Limited resolution, radiation exposure, expensive, limited availability, myocardial uptake of FDG, and cardiac motion artifact
Single Positron Emission Computed Tomography (SPECT)	Plaque inflammation, apoptosis, lipoprotein accumulation, chemotaxis, angiogenesis, proteolysis, and thrombogenicity	High sensitivity, low cost, and more spatial resolution as compared with PET	Limited resolution, nonspecificity, radiation exposure, limited availability, and cardiac motion artifact

Table 2.2 Carotid Plaque Presence as a Predictor of CVD Outcomes

Year	Author	Study	N	Follow-Up	Outcome	Measure of Association
1991	Salonen et al.	KIHD	1,277	2.5 yrs	MI	Small plaque RR=4.15 (1.51-11.47) Large plaque RR=6.71 (1.33-33.91)
2001	Held et al.	APSIS	558	Med. 3 yrs	CV death, MI, revascularization	CVD death/MI RR=1.83 (.96-3.51) Revascularization RR=1.17 (.70-1.96)
2002	Hollander et al.	Rotterdam	4,217	Avg. 5.2 yrs	Stroke and cerebral infarction	Stroke OR=2.44 (1.42-4.20) Cerebral Infarct OR=2.70 (1.27-5.77)
2004	Van der Meer et al.	Rotterdam	6,389	7-10 yrs	MI	Mild plaque HR=1.33 (.84-2.09) Severe Plaque HR=1.72 (1.72-3.47)
2005	Rosvall et al.	MDCS	5,163	Med. 7 yrs	Stroke	HRR=1.75 (1.11-2.75)
2006	Stork et al.		403	48 months	CVD death	RR=1.16 (1.03-1.31)
2007	Cao et al.	CHS	5,020	Med. 11 yrs	Composite Vascular Outcome (IS, MI, or vascular death)	Intermediate Risk HR=1.41 (1.15-1.72) High Risk HR=1.38 (1.14-1.67)
2007	Johnsen et al.	Tromso	6,226	Avg. 5.4 yrs	MI	Men RR=1.56 (1.04-2.36) Women RR=3.95 (2.16-7.19)
2007	Prabhakaran et al.	Northern Manhattan	1,118	Avg. 2.6 yrs	Composite Vascular Outcome (IS, MI, or vascular death)	HR=2.6 (CI=1.3-5.3)

Table 2-2 Continued

2009	Cournot et al.		2,561	Avg. 6 yrs	CVD death, MI	RR=2.81 (1.84-4.29)
2010	Nambi et al.	ARIC	13,145	Med. 15 yrs	CVD death, MI, revascularization	Addition of plaque to risk score increase AUC from .742 to .751
2011	Plichart et al.	Three-City	5,895	Med. 5.4 yrs	CVD death, MI, revascularization	Plaque present HR=1.5 (1.0-2.2) >2 plaques present HR=2.2 (1.6-3.1)
2011	Polak et al	Framingham Offspring	2,965	Avg. 7.2 yrs	Composite Vascular Outcome (CVD death, MI, stroke, heart failure)	HR=1.92 (1.49-2.47)
2011	Xie et al.		3,258	5 years	MI	≥3 plaquesHR=3.46 (1.89-6.35)
2013	Polak et al.	MESA	6,562	Avg. 7.8 yrs	CHD	HR=1.67 (1.33-2.10)
2015	Baber et al.	BiolImage	5,808	3 yrs	MACE	HR=2.36 (CI=1.13-4.92)

Table 2.3 Femoral Plaque Presence as a Predictor of CVD Outcomes

Year	Author	Study Name	N	Follow-Up	Outcome	Measure of Association
2001	Held et al.	APSIS	558	Med. 3 yrs	CVD death, MI, revascularization	CVD death/MI RR=.86 (.43-1.71) Revascularization RR=1.93 (1.10-3.39)
2008	Dalager et al.		100	Cross-sectional autopsy	Coronary death	OR=7.07 (2.40-20.81)
2011	McDermott et al.	WALCS III	427	Cross-sectional	Functional performance	Slower paced usual walk p-trend<.0001 Fastest-paced walk p-trend=.023
2014	Frerix et al.		190	4-6 yrs	Composite CVD event	HR=5.92 (1.55-22.67)
2014	Barone-Rochette et al.		410	Cross-sectional	Traditional risk factors	OR=10.13 (5.94-17.30)
2016	Avramovski et al.		101	4 yrs	Composite CVD event	HR=2.93 (1.43-6.01)

Table 2.4 B-Mode Ultrasound Characteristics of Plaque Verified by Histology

Year	Author	N	US Measured Characteristics	Histologically Verified Characteristics	Measure of Association
1997	El-Barghouty et al.	52	Echogenicity	Fibrous tissue, intraplaque hemorrhage, lipid content, calcification	Echo-rich plaques associated with fibrous tissue (p=.03) and calcification (p=.01). Echolucent plaques associated with lipid content (p=.01).
1997	Gronholdt et al.	246	GSM	Lipid content, fibrous content	Correlation between lipid content and GSM: r=-.351 (p<.05) Correlation between fibrous content and GSM: r=.411 (p<0.001)
2005	Grogan et al.	48	GSM	Necrotic core area	Correlation between GSM and necrotic core: r=-0.9 (P<0.001)
2014	Salem et al	126	Plaque area, GSM	Composite Instability: hemorrhage, thrombus, inflammation, neovascularity, foam cells, cap rupture	Plaque Area> 95 OR=4.15 (1.34-12.8) GSM<25 OR=3.76 (1.14-12.39)

Table 2.5 B-Mode Ultrasound Measured Characteristics of Plaque as Predictors of CVD Outcomes in Epidemiologic Studies

Year	Author	N	Artery	US Measured Characteristic	Outcome	Follow-Up	Measure of Association
2001	Gronholdt et al.	346	Carotid	Echogenicity	Stroke	Avg. 4.4 yrs	Echolucent Plaque RR=3.1 (1.3-7.4)
2001	Hunt et al.	13,123	Carotid	Calcification/ Acoustic Shadowing	Stroke	8 yrs	Women HR=4.01 (2.28-7.06) Men HR=2.23 (1.32-3.79)
2004	Honda et al.	286	Carotid	Echogenicity	Coronary event risk	30 months	Echolucent plaque OR=7.0 (2.3-21.4)
2008	Reiter et al.	574	Carotid	GSM	MACE	Avg. 3.2 yrs	Lowest vs. highest GSM quartile HR=1.71 (1.09-2.66)
2012	Schiano et al.	246	Femoral	Echogenicity, GSM	MI, stroke	30 months	Echolucent plaque HR=7.24 (3.23-16.22) GSM HR=.96 (.95-.98)
2015	de Kreutzenberg et al.	581	Carotid	Calcification	MACE	Avg. 4.3 yrs	Echogenic plaque HR=3.71 (2.09-5.59)
2015	Thompson et al.	374	Carotid	Calcification	Mortality CVD event	11 yrs	Mortality RR= 2.72 (1.4-2.51) CVD Event RR= 2.35 (1.5-3.8)

Table 2.6 Epidemiologic Studies of Perceived Everyday Discrimination and Subclinical CVD

Year	Author	Study	N	Outcome	Follow-Up	Measure of Association
2003	Troxel et al.	SWAN (Pittsburgh)	334	cIMT, Plaque	Cross-sectional	EDS score marginally associated with cIMT in full sample (p=0.06), and marginally associated with plaque index >1 in African American women (n=109)
2006	Brown et al.	SWAN	3,300	Blood Pressure	Cross-sectional	Marginal negative correlation between perceived discrimination with SBP and DBP in the total sample. No significant correlations among race/ethnicity subgroups
2006	Cozier et al.	Black Women's Health Study	30,330	Incident Hypertension	4 Years	IRR marginally significant for personally mediated racism (IRR=1.1 CI=1.0-1.3), no major differences based on neighborhood (predominately white, black, immigrant)
2006	Lewis et al.	SWAN (African American Participants from Pittsburgh & Chicago)	181	CAC	5 Years	Chronic discrimination assessed by EDS marginally associated with CAC after adjustment for covariates (OR=2.6 CI=1.0-6.47). Attribution of discrimination does not matter.
2012	Sims et al.	Jackson Heart Study	4,939	Hypertension	Cross-sectional	No associations seen with EDS; however discrimination burden (composite of multiple measures) was associated with greater likelihood of hypertension (OR= 1.09 CI=1.02-1.16). Relationship was marginally significant after covariate adjustment.

2012	Klimentidis et al.	Children recruited to UAB for metabolic cohort study	294	Blood Pressure	Cross-sectional	Perceived racial discrimination associated with SPB among African American participants (p=0.041) after adjusting for demographics, physical activity, and body fat.
2012	Everage et al.	CARDIA	1,362	CAC	Cross-sectional	EDS associated with lower odds of CAC (OR=0.93 CI=0.87-0.99) after adjustment for covariates.
2013	Gregoski et al.	Normotensive young adults in the Southern US	587	Ambulatory BP	Cross-sectional	Significant interaction between EDS and race/ethnicity in the prediction of daytime SBP (B=0.43 p=0.02)
2015	Goosby et al.	OURHealth Study	58	Blood Pressure	Cross-sectional	EDS positively associated with SBP (B=0.34 p=<0.05), DPB (B=0.47 p=0.05), and CRP (B=0.34 p<0.05) after adjustment for demographics and BMI
2016	Peterson et al.	SWAN	1,056	cIMT, Adventitial Diameter	SWAN Visit 0 - Visit 10	In Caucasian women, cumulative unfair treatments measured by EDS associated with cIMT (B=0.03 p=0.009) and adventitial diameter (B=0.02, p=0.013) after adjustment for covariates. No relationship seen among other racial/ethnic groups.

3.0 MANUSCRIPT 1: ASSOCIATIONS OF INFLAMMATORY AND COAGULATION BIOMARKERS WITH MEASURES OF FEMORAL PLAQUE PRESENCE, BURDEN, AND CHARACTERISTICS

Natalie C. Suder[1], Emma Barinas-Mitchell[1], Matthew A. Allison[2], Michael H. Criqui[2], Joachim H. Ix[2,3], Nancy S. Jenny[4], Christina L. Wassel[4]

[1]Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA

[2]Division of Preventive Medicine, University of California-San Diego, La Jolla CA

[3]Division of Nephrology, University of California-San Diego, La Jolla, CA

[4]Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT

3.1 ABSTRACT

Background: Atherosclerosis is a chronic inflammatory disease that results in the accumulation of plaque within the vessel wall of medium- and large-sized arteries. Biomarkers of inflammation including C-reactive protein (CRP), interleukin (IL)-6, and intracellular adhesion molecule (ICAM)-1, have been previously associated with atherosclerotic disease in the lower extremity vessels. We utilized an exploratory factor analysis (EFA) to identify distinct factors derived from circulating inflammatory and coagulation biomarkers, then examined the associations of these factors with measures of plaque presence, burden, and characteristics in the femoral artery.

Methods: The San Diego Population Study (SDPS) is a prospective, population-based, multi-ethnic cohort of 1103 men and women averaged age 70 at a follow-up exam taking place from 2007-11. An EFA was conducted in order to identify the underlying structure of circulating inflammatory and coagulation markers collected during the SDPS follow-up exam including CRP, ICAM-1, IL-6, fibrinogen, D-dimer, Lipoprotein-a (LP(a)), and pentraxin-3 (PTX3). Regression analysis was utilized to measure associations between the identified grouping of biomarkers (factors) and plaque measures (presence, total number, total area, average grey-scale median, and presence of calcification) obtained via B-mode ultrasound of the right and left superficial and common femoral arteries.

Results: Two biomarker factors emerged from the EFA: Factor 1 composed of CRP, IL-6, and fibrinogen, and Factor 2 composed of D-dimer and PTX-3. In unadjusted models, Factor 2 was associated with increased odds of plaque presence (OR=2.19 CI=1.47-3.25) and a greater number of plaques (OR=2.36 CI=1.64-3.32) in the femoral artery; however, these associations did not remain statistically significant after adjustment for CVD risk factors. No statistically

significant associations were identified between Factor 1 and characteristics of femoral plaque in this population.

Conclusions: In this multi-ethnic, population-based cohort of predominately healthy aging men and women, two distinct groupings of biomarkers of seven circulating markers of inflammation and coagulation were identified via EFA. These factors were differentially associated with femoral plaque presence and burden, but neither factor was associated with femoral plaque characteristics. Additional studies utilizing this technique to assess populations with more progressive CVD may help elucidate the relationships between various biomarkers of inflammation and coagulation with specific atherosclerotic processes contributing to CVD.

3.2 INTRODUCTION

Atherosclerosis is a chronic inflammatory disease that occurs throughout the lifetime, and is the underlying disease process responsible for the majority of cardiovascular disease (CVD) morbidity and mortality (13, 23, 26, 200). Peripheral artery disease (PAD), one manifestation of atherosclerosis, affects an estimated 8.5 million individuals in the U.S. (201). PAD is associated with reduced functionality, increased risk of cardiovascular events, as well as cardiovascular and all-cause mortality (202-204).

Subclinical measures of atherosclerosis have helped shape our understanding of the pathologic processes leading to CVDs including PAD (91, 103). These measures allow for the assessment of subclinical atherosclerosis in different stages of progression and for the identification of individuals at risk of cardiovascular events or complications. The detection of arterial plaque by B-mode ultrasound is an relatively inexpensive and non-invasive method of

directly identifying atherosclerosis, specifically, lesions present in the superficial arteries such as the carotid and femoral arteries (101). Plaque identified in the femoral artery is associated with reduced functional performance, plaque in the carotid and coronary arteries, and an increased risk of cardiovascular events and coronary death (90, 116, 205).

In addition to the detection of plaque, plaque burden and characteristics may also be assessed via B-mode ultrasound. Increased femoral plaque burden, defined as total plaque area, is predictive of symptomatic peripheral artery disease (PAD) and lower functional performance (116, 206). Grey-scale median (GSM), a continuous measure of plaque echogenicity, can be utilized as a surrogate marker of plaque composition. In histologic studies, low GSM has been associated with lipid content, necrotic core presence, and composite measures of plaque instability (134-136). Conversely, higher GSM is correlated with greater fibrous content (134). In a prospective study of individuals with PAD, higher GSM of femoral artery plaque was found to be protective against the risk of myocardial infarction (MI) and ischemic stroke (149). Additionally, calcification, which can provide insight into the complexity and potential stability of plaque, may be measured via B-mode ultrasound (147, 207).

Research investigating the inflammatory mechanisms contributing to atherosclerosis has led to the identification of circulating biomarkers of inflammation and coagulation, including C-reactive protein (CRP), intracellular adhesion molecule (ICAM)-1, interleukin (IL)-6, fibrinogen, D-dimer, Lipoprotein-a (LP(a)), and pentraxin-3 (PTX3), as independent predictors of cardiovascular risk (39, 43, 58, 64, 67, 208-214). Moreover, circulating levels of CRP, IL-6, and ICAM-1 have specifically been associated with PAD in longitudinal studies (41, 215, 216). However, less is known about the potential associations or involvement of these biomarkers in

the various stages of the development and progression of atherosclerotic disease, particularly in the lower extremity arteries.

In the present study, we used an exploratory factor analysis (EFA) in order to determine if there is an underlying structure of seven circulating markers of inflammation and coagulation (CRP, IL-6, ICAM-1, fibrinogen, D-Dimer, PTX-3, and Lp(a)) in a population-based sample of health older adults. Next, we measured the associations of the biomarker groupings (factors) with various measures of plaque presence, burden, and characteristics in the common and superficial femoral arteries of healthy older adults.

3.3 MATERIALS AND METHODS

3.3.1 Participants

The San Diego Study Population (SDPS) is a prospective, population-based, multi-ethnic cohort study of lower extremity PAD and venous disease in men and women averaged age 60 at baseline that has been described in detail elsewhere (217-219). Briefly, current and former employees of the University of California, San Diego and the significant others of these employees, all of whom resided in San Diego County, were recruited to participate in the study. Participants were selected randomly within strata of age, sex, and race/ethnicity. Women and racial/ethnic minorities (African-American, Hispanic, Asian) were over-sampled. The present study includes 1068 SDPS participants who had a femoral artery ultrasound completed during a follow-up exam between 2007-2011, on average 11 years after the baseline clinical examinations that took place between 1994-1998 (220). At the follow-up exam, participants ranged in age

from 48-94 years with a mean age of 70 years. The cohort was approximately 65% women, and 60% non-Hispanic White, 15% Hispanic, 13% African American, and 12% Asian. All participants provided signed informed consent at both the baseline and follow-up examinations, and the University of California-San Diego Institutional Review Board Committee on Investigations Involving Human Subjects approved the study.

3.3.2 Ultrasound Measurements

Doppler ultrasound scans were conducted using an Acuson Aspen (Seimens, Inc) in order to obtain clips of three 10 mm segments of the right and left common and superficial femoral arteries: one at the common femoral artery (CFA) as it emerged from under the inguinal ligament proximal to the bifurcation, one at the bifurcation of the CFA and the superficial femoral artery (SFA) and one at the SFA distal to the bifurcation. Five-second image clips were obtained for these segments at an angle of isonation of 90 degrees. Regular quality control monitoring was performed throughout the study using images from 10-20 participants.

Plaque presence and total number of plaques in each segment were determined by trained ultrasound readers at the University of Pittsburgh Ultrasound Research Laboratory (URL). Presence of plaque was defined using the Mannheim consensus criteria as a focal structure that encroaches into the arterial lumen at least 0.50 mm or 50% greater thickness than the surrounding IMT or that demonstrates a thickness > 1.5 mm as measured from the media-adventitia border to the intima-lumen border (117). In some femoral segments, visualization of plaque presence was limited due to the presence of artifact. Focal structures fitting the Mannheim consensus identified in these images were classified as “probable plaques” by the readers. Plaque presence was defined as a participant having plaque in at least one arterial

segment (left SFA, right SFA, left CFA, or right CFA) and total number of plaques was defined as the sum of all plaques across the four arterial segments.

Plaque area, GSM, and presence of calcification were determined by one trained ultrasound reader using semi-automated Carotid Analyzer software from the Vascular Research Tools 5 Suite (Medical Imaging Applications LLC, Coralville, IA). Plaque area for each plaque was calculated by the software once the plaque had been traced by the reader and automatically outlined by the software. Total plaque area was defined as the sum of plaque area in each of the four arterial segments. GSM, a continuous measure of plaque echogenicity, was calculated by the software for each plaque following grey level normalization. Normalization, which aims to minimize the effect of different ultrasound machine gain settings during image acquisition, was performed manually by the reader selecting a dark area of blood from the lumen and the brightest area of the adventitial layer along the anterior or posterior wall. Mean GSM was calculated by averaging the GSM values for all plaques in the four arterial segments. In a 2016 reliability study conducted by the URL, the within-reader intraclass correlation coefficients (ICC) for plaque area and GSM were .95 and .99, respectively. Calcification was subjectively identified by the reader after assessing each plaque for highly echogenic areas with or without acoustic shadowing. Presence of calcification was defined as a participant having a calcified plaque in at least one of the four arterial segments.

3.3.3 Inflammatory and Coagulation Biomarkers

Nephelometric assays on a BMI System (Siemens Heallthcare, Erlange, Germany) were used to measure C-reactive protein (CRP) and fibrinogen from EDTA plasma, and lipoprotein-a(Lp(a)) in serum. Inter-assay coefficients of variation (CVs) were 4.1-5.1%, 3.2-4.7%, and 5.2-

8.2% for CRP, fibrinogen, and Lp(a), respectively. Intercellular adhesion molecule (ICAM)-1 was measured in EDTA plasma using a non-allele specific enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) with a CV range of 10.3-11.0%. ELISA was also used to measure pentraxin-3 (PTX3) in EDTA plasma and Interleukin(IL)-6 in serum with CVs of 9.3-14.9% and 4.2-6.3%, respectively. Finally, D-dimer was measured in EDTA plasma using an Evolution Coagulation Analyzer (Diagnostica Stago, Parsippany, NJ) with a CV range of 2.7-24.7%.

3.3.4 Covariates

Age, sex, race/ethnicity, and current and former cigarette smoking habits were determined via self-report. Smoking habits were categorized as current, former, or never use of cigarettes. Height (centimeters) and weight (kilograms) was obtained in order to calculate body mass index (BMI) as kg/m^2. CVD (previous MI, stroke, angioplasty, or revascularization) was also determined via self-report. Diabetes was ascertained via self-report, or the use of anti-diabetic medications or insulin. Participants' seated blood pressure was obtained from the right arm using a standard manual sphygmomanometer after five minutes of rest. Hypertension was defined as SBP ≥140 mmHg, diastolic blood pressure (DBP) ≥90, or the use of anti-hypertensive medications. Total and high density lipoprotein (HDL) cholesterol were measured from non-fasting blood samples using a Roche Cobas 6000 analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Low density lipoprotein (LDL) was calculated using the Friedewald equation (221). The CKD-Epi equation was used to calculate estimated glomerular filtration rate (eGFR), and a Roche Cobas 6000 analyzer (Roche Diagnostics Corporation, Indianapolis, IN) was utilized to measure serum creatinine levels via isotope dilution mass spectrometry (222).

Physical activity level was assessed via self-report by asking participants to rate their perceived activity level compared to other persons of their age.

3.3.5 Statistical Analysis

An exploratory factor analysis was performed in order to estimate the underlying structure of the seven measured inflammatory and coagulation biomarkers. Log transformed biomarker concentrations were calculated then extracted using maximum likelihood methods with direct oblimin rotation on Pearson correlation matrix using PROC Factor in SAS 9.3 (SAS Institute, Cary, NC). A scree plot and parallel analysis suggested that two factors should be retained. We compared the model fit and interpretability of models with one- through four-factor solutions, and retained the two-factor solution. Both the standardized root mean-square residual (SRMR) and the root mean square error approximation (RMSEA) indicate good fit of the two-factor solution: SRMR = .028 and RMSEA = .032. Estimated factor scores were obtained via the SCORE option in PROC Factor based on the five biomarkers with loadings $\geq|0.3|$ in the two-factor solution. ICAM-1 and LP(a) were excluded from the final analysis since they did not load highly on any factor. Factor scores were categorized based on tertiles of the distribution.

Chi-square and Kruskal-Wallis tests were used to compare differences in categorical and continuous participant characteristics, respectively, among those with and without femoral plaque and across tertiles of the factor scores. Chi-square and Kruiskal-Wallis tests were also used to measure the univariate associations between tertiles of factor scores and subclinical outcomes (plaque presence, total number of plaques, total plaque area, average GSM, and calcification). Logistic regression was used to assess the associations between the tertiles of factor scores with plaque presence and the presence of calcification. Linear regression was used

to determine associations between the tertiles of factor scores total plaque area and average GSM, and zero-inflated Poisson regression was used to determine associations between the tertiles of factor scores and total number of plaques. Minimally adjusted models included age, sex, and race/ethnicity, while fully adjusted models also included BMI, SBP, total cholesterol, eGFR, diabetes, smoking status, statin use, and self-reported activity level. All plaque variables include both the definite and "probable" plaques identified by ultrasound image readers. Sensitivity analyses were conducted excluding the 75 "probable" plaques (22%), but the exclusion of these plaques did not change the results. Similarly, sensitivity analyses were performed excluding those with diagnosed CVD (9%), CKD (14%), diabetes (10%), and PAD (3%).

3.4 RESULTS

Table 3.1 describes participant characteristics among those with and without plaque identified in at least one femoral segment. Participants with femoral plaque identified were older, more likely to be male, had higher SBP, and lower BMI compared to those without plaque. Those with plaque were also more likely to have hypertension, chronic kidney disease, PAD, and CVD, and on average had lower ABI compared to those without plaque. Additionally, circulating levels of IL-6, ICAM-1, D-dimer, and PTX-3 were significantly higher in those with plaque compared to those without plaque. A total of 1001 participants were included in the EFA based on the complete availability of biomarker data. The 67 participants who were excluded did not differ from those included in the final analysis (data not shown). Two factors were derived from the EFA Table 3.2. Factor 1 was composed of CRP, IL-6, and fibrinogen with factor loadings

ranging from .49-.83. Factor 2 was composed of D-dimer and PTX-3 with factor loadings ranging from .43-.48. ICAM-1 and LP(a) did not load on either factor.

Age, systolic blood pressure, BMI, pulse pressure, and levels of all of the circulating biomarkers increased across tertiles of both Factor 1 and Factor 2 (Supplemental Table TO BE ADDED IN APPENDIX). Additionally, participants were more likely to be female, hypertensive, suffer from chronic kidney disease, and be less physically active across the increasing tertiles of both factors. HDL cholesterol decreased across the tertiles of Factor 1 only, and participants were more likely to use statins, or have PAD, CVD, or diabetes as the tertiles of Factor 2 increased.

Approximately 25% (246) of the participants in the final analysis had at least one plaque present among the examined femoral artery segments. The maximum total number of plaques observed for a given participant was four, and among those with plaque, 66 participants (27%) had at least one calcified plaque. In the univariate analyses, plaque presence, burden, and characteristics did not differ significantly across the tertiles of Factor 1 (Table 3.3). However, the prevalence of plaque presence, and the prevalence of >2 plaques present differed significantly across the tertiles of Factor 2 such that individuals in the higher tertiles of Factor 2 were more likely to have plaque present and a greater number of plaques. There were no significant differences in total plaque area, average GSM, or presence of calcification among the tertiles of Factor 2 in the univariate analysis.

Table 3.4 displays the results of the multiple regression analysis measuring the associations between the biomarker factor scores and measures of plaque presence and plaque burden. There were no statistically significant associations between Factor 1 and plaque presence or plaque burden in unadjusted models (Model 1) or in models adjusted for confounding

variables (Models 2-3). In unadjusted models, the highest tertile of Factor 2 was associated with significantly increased odds of femoral plaque presence (OR=2.19 95% CI=1.48-3.25) compared to the lowest tertile. Factor 2 was also associated with an increased total number of plaques such that participants in the highest tertile of Factor 2 had a mean number of femoral plaques that was 2.36 (95% CI=1.66-3.36) times higher than those in the lowest tertile. However, neither of these associations remained statistically significant following adjustment for covariates. There were no statistically significant associations between either Factor 1 or Factor 2 with the measures of plaque characteristics before or after adjustment for confounders (Table 3.5). The results of the various sensitivity analysis excluding participants diagnosed with probable plaques or CVD, CKD, diabetes, and PAD did not differ from the results described above.

3.5 DISCUSSION

In this multi-ethnic, population-based cohort of predominately healthy aging men and women, we utilized an EFA in order to identify two latent constructs underlying seven circulating biomarkers of inflammation and coagulation. Factor 1, composed of CRP, IL-6, and fibrinogen, was not associated with measures of femoral plaque presence, burden, or characteristics in our sample. In unadjusted models, Factor 2, composed of PTX-3 and D-dimer, was associated with increased likelihood of femoral plaque presence (OR=2.19 CI=1.47-3.25) and a greater number of plaques (OR=2.36 CI=1.64-3.32); however, these associations did not remain statistically significant after adjustment for demographics and comorbidities. Similar to Factor 1, Factor 2 was not significantly associated with femoral plaque characteristics in the SDPS.

We chose to utilize an EFA approach because due to the complex nature of atherosclerosis, many biomarkers of inflammation and coagulation are interdependent. These markers may in fact jointly contribute to the initiation or progression of pathologic processes involved in atherosclerosis. EFA is a technique that allows for the identification of underlying constructs, or factors, among a group of items with no a priori hypothesis (223, 224). The identification of groupings of circulating inflammatory and coagulation markers, or latent processes to which these markers contribute may not only provide insight into the relationships among these biomarkers, but may also help us to characterize patients who are at greatest risk (225). This approach differs from a principal components analysis, in that it is not a data reduction technique; therefore, EFA can help address concerns regarding multicollinearity without sacrificing information. Factor analyses, originally developed for social science research, have become increasingly common in epidemiologic research involving inflammation, nutrition, and metabolism due to their ability to simultaneously evaluate associations between many biomarkers with health outcomes (226-229). These types of techniques can shed light on the underlying biological relationships among biomarkers, and therefore, may prove particularly useful for the study of complex, multifaceted processes like atherosclerosis.

Our study identified two factors whose components were somewhat unanticipated but are plausible biologically. Factor 1, which seems likely to represent a non-specific inflammatory process, is composed of CRP, IL-6, and fibrinogen. The grouping of CRP and IL-6 was expected considering that CRP is synthesized by hepatocytes in response to IL-6 secretion by macrophages and T cells, and both are non-specific markers of inflammation with prognostic value in regards to CVD (39, 43, 208, 209). Fibrinogen is a glycoprotein that contributes to platelet aggregation and is converted to fibrin in order to form blood clots (214, 230). Similar to

CRP, fibrinogen is an acute phase reactant synthesized by hepatocytes in response to inflammation, and has been associated with risk of CHD and stroke (210, 230). The composition of Factor 2 (D-dimer and PTX3) is seemingly less straight-forward. PTX3 is an inflammatory protein and an activator of the classical complement pathway that is associated with numerous CVD outcomes including incident coronary heart disease (CHD), coronary artery calcium (CAC), PAD, and components of coronary plaques (67, 211-213). While both CRP and PTX3 are members of the pentraxin superfamily, the results of our EFA suggest that PTX3 is more strongly correlated with D-dimer in this predominately healthy sample of older adults (64). Unlike CRP, PTX3 is a long pentraxin whose relationship with CVD is beyond the scope of inflammatory processes (63). High levels of PTX3 have been observed within coronary arterial thrombi, and PTX3 increases the expression of Tissue Factor (TF), an activator of the coagulation cascade, in endothelial cells and lymphocytes (63, 64). D-dimer, a fibrin degradation product, is a marker of thrombogenesis that is present in the blood following the activation of the coagulation cascade (231, 232). Like PTX3, D-dimer is associated with increased risk of CHD in prospective studies (58, 233). Thus, the grouping of these two biomarkers within Factor 2 may be indicative of a thrombogenic process.

The association of Factor 2 with plaque presence and burden in unadjusted models suggests that coagulation burden may be more important in regards to femoral plaque development than inflammatory burden in this older population. Although we did not find any statistically significant associations between our factors and plaque measures, each biomarker included in our analysis has been previously associated with plaque (45, 213, 234-236). The majority of these prior studies examine samples consisting of participants who are already clinically diagnosed with CVD. Studies examining femoral plaque presence and burden report

strong associations between plaque presence with low ABI and symptomatic PAD (116, 206, 237). Our cross-sectional study included active, healthy, adults averaged age 70 among whom only 25% had femoral plaque, 9% had CVD, and 3% had PAD. The limited burden of femoral artery atherosclerosis in our study sample may have contributed to our null findings due to the fact that there may not have been sufficient plaque or heterogeneity of plaque characteristics in order to see associations with our factors. The specific inflammatory and coagulation markers measured in this study may be better indicators of later atherosclerotic disease processes than what was present in our sample. Additionally, the relationships between these biomarker factors and measures of plaque characteristics may be different if examined in another arterial bed, like the carotid artery, where atherosclerotic plaque is more likely to be high-risk (238).

Strengths of our study include the large, multi-ethnic, population-based sample of participants who underwent standardized clinical examinations and ultrasound examination conducted by trained technicians. Our statistical methodology allowed us to simultaneously model the effects of several biomarkers of inflammation and coagulation, and we performed sensitivity analysis excluding femoral plaques that were difficult to visualize via ultrasound. While the use of B-mode ultrasound is a non-invasive and efficient method of measuring plaque in the general population, B-mode ultrasound is limited in its ability to characterize plaque. GSM is a surrogate marker of plaque components, and calcification is subjectively assessed by an ultrasound reader. These measures may not have been sufficiently sensitive to identify associations between the biomarker factors and plaque characteristics in this population with relatively little femoral plaque. Additionally, the cross-sectional nature of this investigation limits our ability to draw conclusions regarding the causal relationships, or lack thereof, between the biomarker factors and plaque measures. Finally, only seven biomarkers were included in our

analysis, two of which did not load on either of the identified factors. The consideration of additional biomarkers in the EFA may have yielded additional factors incorporating ICAM-1 and Lp(a), although these markers were not individually associated with any of the outcomes in this study.

In conclusion, two factors emerged following an EFA of seven circulating biomarkers of inflammation and coagulation; however, neither factor was associated with measures of femoral plaque presence, burden, or characteristics in the SDPS after adjustment for covariates. EFA is a useful method that can help shed light on the interdependencies of inflammatory and coagulation biomarkers in relation to the development of CVD. Further studies are needed in populations with more advanced CVD in order to elucidate the relationship between circulating biomarker groups with specific atherosclerotic processes contributing to CVD.

3.6 TABLES

Table 3.1 Characteristics of San Diego Study Participants by Plaque Presence

Characteristic	Plaque (N=262)	No Plaque (N=806)	p-value
Age (years)	77 (70-83)	68 (61-76)	<0.0001
Sex (male)	101 (39%)	250 (31%)	0.0274
Race/Ethnicity			0.0002
Non-Hispanic White	189 (72%)	451 (56%)	
African-American	22 (8%)	108 (13%)	
Hispanic	22 (8%)	128 (16%)	
Asian	28 (11%)	106 (13%)	
Systolic BP (mmHg)	132 (120-146)	130 (118-140)	0.0016
Diastolic BP (mmHg)	72 (65-80)	76 (70-82)	<0.0001
Body Mass Index (kg/m2)	25.53 (23.10-28.37)	26.56 (23.59-30.11)	0.0065
Estimated GFR (min/mL/1.73m2)	73.99 (58.40-85.95)	82.62 (69.28-92.85)	<0.0001
Total Cholesterol (mg/dL)	190 (164-213)	197 (170-226)	0.0029
HDL Cholesterol (mg/dL)	58 (44-71)	57 (45-72)	0.5884
LDL Cholesterol (mg/dL)	104 (80-125)	107 (87-132)	0.0079
Pulse Pressure	61.4 (50.0-70.0)	54.2 (42.0-62.5)	<0.0001
Smoking History			0.0312
Current	10 (4%)	29 (3%)	
Former	94 (36%)	222 (28%)	
Never	156 (60%)	550 (69%)	
Hypertension	194 (74%)	471 (58%)	<0.0001
Statin Use	101 (40%)	241 (31%)	0.0059
Cardiovascular Disease	40 (15%)	53 (7%)	<0.0001
Diabetes	38 (15%)	68 (8%)	0.0043
Chronic Kidney Disease	70 (28%)	90 (11%)	<0.0001
Peripheral Artery Disease	24 (9%)	12 (1%)	<0.0001
Ankle Brachial Index	1.12 (1.04-1.18)	1.14 (1.09-1.19)	0.0003
Activity Level	3.7 (3.5-3.8)	3.6 (3.4-3.7)	0.6735
CRP (mg/L)	1.17 (.58-2.34)	1.02 (.5-2.17)	0.1582
IL-6 (pg/mL)	2.07 (1.41-3.14)	1.72 (1.13-2.64)	<0.0001
ICAM-1 (ng/mL)	357.20 (297.96-432.33)	340.49 (285.16-412.04)	0.0459
Fibrinogen (mg/mL)	386 (335-433)	380.00 (333.00-429.00)	0.7365
D-dimer (ug/mL)	0.46 (.26-.77)	0.40 (.21-.65)	0.0014
LP(a) (g/L)	0.15 (.04-.34)	0.11 (.04-.35)	0.3068
PTX-3 (ng/mL)	1.34 (.90-1.96)	1.23 (.81-1.79)	0.0224

[a] Median (25^{th}-75^{th}) provided for all continuous variables, Kruskal-Wallis test used to compare groups.

[b] N (%) provided for categorical variables, Chi-square test used to compare group

Table 3.2 Rotated Factor Loadings based on Exploratory Factor Analysis

	Factor 1:	Factor 2:
CRP	0.83	-
IL-6	0.52	-
Fibrinogen	0.49	-
D-Dimer	-	0.43
PTX-3	-	0.48
ICAM-1	-	-
LP(a)	-	-

Table 3.3 Differences in Plaque Outcomes by Tertiles of Biomarker Factor Scores

	Factor 1: CRP, IL-6, Fibrinogen				Factor 2: PTX-3, D-dimer			
	T1	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**
Plaque Presence	68 (21%)	84 (25%)	94 (28%)	0.0921	62 (19%)	68 (21%)	116 (34%)	<.0001
Total # of Plaques				0.2481				0.0083
<=2	65 (96%)	74 (88%)	84 (89%)		59 (95%)	63 (93%)	101 (87%)	
>2	3 (4%)	10 (12%)	10 (11%)		3 (5%)	5 (7%)	15 (13%)	
Total Area	11.7 (8.2-36.7)	13.4 (9.3-24.6)	12.3 (8.4-21.3)	0.5148	14.2 (8.3-21.7)	12.0 (8.3-21.2)	12.6 (8.7-25.9)	0.6356
Average GSM	52.9 (39.8-59.8)	56.5 (42.7-73.3)	55.3 (44.2-68.1)	0.1956	49.6 (40.9-62.3)	56.5 (42.0-74.9)	55.5 (44.7-65.9)	0.1848
Calcification	19 (28%)	21 (25%)	26 (28%)	0.8963	13 (21%)	17 (25%)	36 (31)	0.3253

[a] Median (25^{th}-75^{th}) provided for all continuous variables, Kruskal-Wallis test used to compare groups.

[b] N (%) provided for categorical variables, Chi-square test used to compare groups

Table 3.4 Associations of Biomarker Factor Scores with Femoral Plaque Presence and Burden

	Outcome					
	Plaque Presence			**# of Plaques**		
Biomarker Factor	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
	Factor 1: CRP, IL-6, Fibrinogen					
Tertile 1 (ref)	-	-	-	-	-	-
Tertile 2	.13(.19)	-.03(.20)	-.01(.21)	.21(.17)	.17(.17)	.15(.17)
Tertile 3	.05(.21)	.12(.22)	.21(.23)	.04(.18)	.18(.17)	.25(.18)
	Factor 2: PTX-3, D-dimer					
Tertile 1 (ref)	-	-	-	-	-	-
Tertile 2	.10(.20)	-.26(.22)	-.25(.27)	.23(.19)	-.00(19)	.01(.19)
Tertile 3	.78(.20) ‡	.04(.24)	.08(.24)	.86(.18) ‡	.33(.20)	.28(.20)

[a] Beta estimates (se) provided. ‡ indicates significant at α=.05 level.

[b] Model 2 adjusts for age, sex, and race/ethnicity.

[c] Model 3 adjusts for Model 2 + BMI, SBP, total cholesterol, eGFR, diabetes, smoking status, statin use, and activity level.

Table 3.5 Associations of Biomarker Factor Scores with Plaque Presence

	Outcome								
Biomarker Factor	**Plaque Area**			**Average GSM**			**Calcification**		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
	Factor 1: CRP, IL-6, Fibrinogen								
Tertile 1 (ref)	-	-	-	-	-	-	-	-	-
Tertile 2	.01 (2.69)	-.90 (2.60)	-.81 (2.68)	.34 (5.32)	.72 (5.29)	.90 (5.44)	-.37 (.40)	-.40 (.40)	-.37 (.43)
Tertile 3	-.94 (2.87)	-1.43 (2.73)	-.68 (2.87)	-1.06 (5.45)	-2.11 (5.45)	-2.60 (5.77)	-.33 (.41)	-.27 (.41)	-.14 (.44)
	Factor 2: PTX-3, D-dimer								
Tertile 1 (ref)	-	-	-	-	-	-	-	-	-
Tertile 2	-.16 (2.89)	1.86 (2.92)	1.55 (2.93)	4.10 (5.78)	4.38 (5.95)	4.76 (6.02)	.38 (.45)	.29 (.49)	.16 (.50)
Tertile 3	3.55 (2.76)	5.53 (2.96)	4.58 (3.00)	2.05 (5.35)	4.34 (5.88)	4.76 (6.04)	.70 (.42)	.52 (.29)	.26 (.51)

[a] Beta estimates (se) provided. ‡ indicates significant at α=.05 level.

[b] Model 2 adjusts for age, sex, and race/ethnicity.

[c] Model 3 adjusts for Model 2 + BMI, SBP, total cholesterol, eGFR, diabetes, smoking status, statin use, and activity level

4.0 MANUSCRIPT 2: ASSOCIATIONS OF INFLAMMATORY AND COAGULATION BIOMARKERS WITH CAROTID PLAQUE PRESENCE, BURDEN, AND CHARACTERISTICS

Natalie C. Suder[1], Emma Barinas-Mitchell, Maria M. Brooks, Suresh R. Mulukutla, Trevor J. Orchard, Christina L. Wassel, Rebecca C. Thurston

*Authorship Order to be Determined

Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA

Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

Division of Cardiology, University of Pittsburgh, Pittsburgh, PA

Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT

4.1 ABSTRACT

Introduction: Inflammation is one of the primary mechanisms driving atherosclerosis, a process which has been postulated to accelerate among women in midlife. Questions remain regarding the role that circulating inflammatory and coagulation burden may play in progression of atherosclerosis in midlife women. Better understanding of the relationships between inflammatory and coagulation burden with measures of subclinical atherosclerotic plaque may help shed light on the mechanisms contributing to the dramatic increase in CVD risk observed in women at midlife.

Methods: A sample of 277 late peri- and postmenopausal women free of clinical CVD underwent a blood draw and bilateral carotid ultrasound for the measurement of carotid plaque. We conducted an exploratory factor analyses (EFA) in order to identify distinct groupings of circulating inflammatory and coagulation biomarkers including CRP, IL-6, fibrinogen, D-dimer, Factor VII, and vwAntigen. Factor scores were calculated based on the results of the EFA, and multiple regression was used to measure the associations between the biomarker factors and measures of plaque presence, burden (total area, total number of plaques), and characteristics (maximum height, minimum grey scale median (GSM), and calcification). Interactions were tested between the factors derived from the EFA with race/ethnicity, hot flashes, sleep, years since final menstrual period (FMP), and BMI.

Results: No significant associations were observed between either factor or the various subclinical measures among the complete MS Heart sample. Significant interactions were observed between continuously modeled BMI and the factors when predicting total number of plaques ($p=0.02$ and $p<0.01$ for =Factors 1 and 2, respectively) and total plaque area ($p=0.03$ for Factor 2). Among women with normal BMI ($<25 kg/m^2$, n=98), Factor 1 (composed of CRP, IL-

6, and fibrinogen) and Factor 2 (composed of D-dimer and vwAntigen) were associated with a greater total number of plaques (OR=2.75 95% CI=1.56-4.85, and OR=1.81 95% CI=1.09-3.02 for Factors 1 and 2, respectively). The association between Factor 2 and total number of plaques remained significant after adjustment for demographics, traditional CVD risk factors and medication use. Among women of normal BMI, Factor 1 was also associated with greater total plaque area (B=24.70, p=0.03) in minimally adjusted models, but not after adjustment for traditional CVD risk factors.

Similarly, interactions were observed between continuously modeled time since final menstrual period (FMP) and the factors when predicting minimum GSM (p=0.07 and p=0.03 for Factors 1 and 2, respectively) and maximum plaque height (p=0.04 and p=0.01 for Factors 1 and 2, respectively). Among women with <1 years since their FMP (n=56), Factor 1 was significantly associated with greater maximum plaque height after adjustment for demographics and medication use (B=2.05 p=0.01) and borderline associated after adjustment for traditional CVD risk factors (B=1.76 p=0.05). Among women with ≥ 1 year since their FMP (n=248), Factor 1 was associated with lower minimum GSM (-28.29 p=0.01) after adjustment for demographics and medication use, but not after adjustment for traditional risk factors.

Discussion: Higher circulating inflammatory and coagulation burden is associated with greater plaque burden in the carotid artery of midlife women with normal BMI. Higher inflammatory burden is also associated with greater maximum plaque height among perimenopausal women. These findings highlight important associations between circulating inflammatory and coagulation burden with measures of carotid plaque in midlife women, and suggest that the inflammatory and coagulation processes represented by these EFA-derived factors may serve as mechanisms contributing to the progression of atherosclerotic plaque in midlife women.

4.2 INTRODUCTION

Midlife is a period marked by dramatic changes in women's cardiovascular health (239). Atherosclerotic CVD, the leading cause of death among women, manifests on average 10 years later in women compared to men (240, 241). Evidence suggests that during the menopausal transition, atherosclerosis is accelerated, and CVD risk increases to reach that of men (240, 242).

Inflammation is one of the driving forces of CVD development and the progression of atherosclerosis (25, 26). The study of inflammatory mechanisms contributing to atherosclerosis has led to the identification of circulating biomarkers of inflammation and coagulation such as CRP, IL-6, fibrinogen, D-dimer, and Factor VII as independent predictors of cardiovascular risk (58, 208-210, 243). Furthermore, higher circulating CRP, IL-6, fibrinogen, and D-dimer levels have been associated with unstable and symptomatic atherosclerotic plaques in the carotid artery (56, 244). Still, relatively little is known about how these inflammatory and coagulation markers may jointly contribute to atherosclerotic plaque progression, erosion, and rupture, or how circulating inflammatory and coagulation burden may contribute to the increase in CVD risk observed among women in midlife.

Measures of subclinical atherosclerosis, such as B-mode carotid ultrasound, allow for the assessment of atherosclerotic plaque in different stages of progression (103, 245). B-mode ultrasound is an inexpensive and noninvasive method that allows for the direct identification of plaque in the carotid artery (101). Carotid plaque is associated longitudinally with increased risk of stroke (100, 246), MI (247) , MACE (128, 248), and cardiovascular death (127, 249, 250). In addition to identifying prevalent atherosclerotic plaque, B-mode ultrasound can be used to assess plaque burden and to characterize plaques found in the carotid artery. Greater plaque burden, defined by plaque area, is associated with increased risk of stroke (124), MI (125, 126), and

MACE (120). Grey-scale median (GSM), a continuous measure of plaque echogenicity that may be obtained via B-mode ultrasound, is a surrogate marker of plaque components. Low GSM has been associated with increased lipid content, necrotic core presence, and composite measures of plaque instability in histologic studies (134-136). Conversely, higher GSM is correlated with greater fibrous content among plaques assessed via histology (134). Lower GSM plaque in the carotid artery is prospectively associated with increased hazard of MACE among asymptomatic individuals with carotid artery disease (148). Other characteristics of plaque that may be obtained via B-mode ultrasound, such as plaque height and calcification, which are both susceptible to changes following the initiation of statin therapy, may provide further insight into the complexity and potential stability of carotid plaques (251, 252). Greater plaque height is longitudinally associated with greater risk of cardiovascular events (253, 254). Additionally, individuals with calcified carotid plaque have a higher risk of mortality and cardiovascular events compared to those who do not have plaque (150, 151). However, studies documenting an increase in calcification of plaque following the initiation of statin treatment suggest that calcification may be a beneficial characteristic of plaque among those with high plaque burden (252, 255).

The purpose of the present study is to identify distinct factors following an EFA of six circulating markers of inflammation and coagulation (CRP, IL-6, fibrinogen, D-Dimer, Factor VII, and vwAntigen) in a sample of midlife women, then determine whether the identified factors are associated with plaque presence, burden, and characteristics in the carotid arteries of women at midlife. We hypothesize that higher inflammatory and coagulation burden are associated with greater plaque burden, and that higher inflammatory burden is associated with B-mode ultrasound-identified characteristics of plaque that are indicative of instability including

lower GSM, greater maximum height, and reduced calcification among those with prevalent carotid atherosclerosis.

4.3 MATERIALS AND METHODS

4.3.1 Participants

The MS Heart Study comprises of 304 late perimenopausal (2-12 months amenorrhea) and postmenopausal (≥12 months amenorrhea) nonsmoking women aged 40-60 years. Participants were recruited through advertisements, mailings, fliers, referral from local clinics, and online message boards for a study investigating the relationship between vasomotor symptoms and CVD risk. By design, half of them women reported daily hot flashes or night sweats within the past three months, and half of the women reported no hot flashes or night sweats in the past three months. Women were excluded from the study if they had previously had a hysterectomy and/or bilateral oophorectomy, history of heart disease, stroke, arrhythmia, ovarian/gynecological cancer, pheochromocytoma, pancreatic tumor, kidney failure, seizures, Parkinson's disease, Raynaud phenomenon, endarterectomy, endometrial ablation, were currently pregnant, or had used the following medications in the past three months: oral/transdermal estrogen or progesterone, selective estrogen receptor modulators, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, gabapentin, insulin, beta blockers, calcium channel blockers, alpha-2 adrenergic agonists, or other antiarrhythmic agents. Additionally, women currently undergoing dialysis or chemotherapy were excluded.

Upon inclusion in the study, participants underwent physical measurements, questionnaire completion, blood draw, ambulatory physiologic hot flash monitoring for three days, electrocardiogram, actigraphic sleep monitoring, and a carotid artery ultrasound. Procedures were approved by the University of Pittsburgh Institutional Review Board, and all participants provided written informed consent.

The present analysis includes 277 women from the MS Heart Study sample for whom all biomarker data was available. Four women were excluded from the analysis because their circulating il-6 levels were extreme outliers (>12pg/mL), and 23 women were excluded due to missing values for at least one of the biomarkers assessed in the exploratory factor analysis. The 27 excluded women were significantly more likely to be non-white, and had higher BMI, insulin, and HOMA compared to those who were included in the analysis (data not shown).

4.3.2 Ultrasound Measurements and Plaque Characterization

Bilateral carotid images were obtained via B-mode ultrasound using a Sonoline Antares (Siemens, Malvern, PA) high-resolution duplex scanner with a VF10-5 transducer by trained sonographers at the University of Pittsburgh Ultrasound Research Laboratory (URL) according to a standardized protocol. Five carotid segments within both the left and right carotid arteries were assessed for plaque: the proximal common carotid artery (CCA), the distal common carotid artery (CCA one centimeter distal to the carotid bulb), the carotid bulb (where the near and far walls of the common carotid are no longer parallel, extending to the flow divider), the internal carotid artery (ICA), and the external carotid artery (ECA).

Consistent with the Mannheim Consensus Statement, presence of plaque was defined as a focal structure that encroaches into the arterial lumen with 50% greater thickness than the

surrounding intima-media thickness (117). Plaque presence and total number of plaques in each segment were determined by trained ultrasound readers. Plaque presence was defined as a participant having plaque in at least one arterial segment and total number of plaques was defined as the sum of all plaques across all of the visualized arterial segments. One trained ultrasound reader determined plaque area, height, GSM, and presence of calcification by one ultrasound reader using semi-automated Carotid Analyzer software from the Vascular Research Tools 5 Suite (Medical Imaging Applications LLC, Coralville, IA). Plaque area for each plaque was calculated by the software once the plaque had been traced by the reader and automatically outlined by the software. Total plaque area was defined as the sum of plaque area in each of the arterial segments. GSM was calculated by the software for each plaque following grey level normalization, which aims to minimize the effect of different ultrasound machine gain settings during image acquisition. Normalization was performed manually by the ultrasound reader selecting the darkest area of blood from the lumen and the brightest portion of the adventitial layer along the anterior or posterior wall. Maximum height was defined as greatest plaque height value for each participant with plaque present. Minimum GSM was defined as the GSM value of the plaque with the lowest GSM for each participant with plaque present. The reader subjectively identified calcification after assessing each plaque for highly echogenic areas with or without acoustic shadowing. Presence of calcification was defined as a participant having a calcified plaque in at least one of the visualized arterial segments.

4.3.3 Inflammatory and Coagulation Biomarkers

A high-sensitivity reagent set (Beckman Coulter, Brea, CA, USA) was used to measure hsCRP, and IL-6 was measured using an R&D Systems high sensitivity enzyme-linked

immunosorbant assay (Minneapolis, MN, USA). Fibrinogen was measured using a modified Clauss method and Siemens Multifibren U reagent (Newark, DE, USA), and quantitative D-dimer was measured using a Diagnostica Stago's Asserachrom assay (Asnieres sur Seine, France). Factor VII clotting activity was measured using standard clotting methods via a Siemens Innovin thromboplastin reagent with Factor VII-deficient plasma (HRF, Raleigh, NC, USA), and vWF Antigen was assessed using Diagnostica Stago's STA-Liatest reagent (Asnieres sure Seine, France).

4.3.4 Covariates and Additional Measures

Participants self-reported their race/ethnicity, education level, shift work, medical and psychiatric history, health behaviors, and medication use (BP lowering, lipid lowering, diabetes, depression, anxiety, sleep) via questionnaire. Height and weight were measured using a fixed stadiometer and a balance beam scale in order to calculate BMI. Resting BP was measured using a Dinamap device after a seated 10-minute rest period, and the final BP was defined as the average of the second and third BP reading. Menopausal status was categorized as perimenopausal (>2-<12 months amenorrhea) or postmenopausal (≥12 months amenorrhea) (256). Participants also completed the Pittsburgh Sleep Quality Index (PSQI) in order assess sleep quality (257) and the Berlin Sleep Questionnaire in order to assess sleep apnea symptoms such as snoring (258). Glucose, total cholesterol, triglycerides, and HDL, were assessed enzymatically (Vital Diagnostics, Lincoln, RI) following a blood draw that took place after a 12-hour fast, and LDL was calculated using the Friedewald equation (221). Insulin level was measured by radioimmunoassay, and HOMA was calculated. Sex hormone binding globulin (SHBG) was measured using chemiluminescent assay.

Measurement of physiologic hot flashes in the MS Heart sample has been described in detail elsewhere (259). Briefly, physiologic hot flashes were monitored over a 24-hour period using sternal skin conductance monitoring (260, 261). A VU-AMS monitor (VU University Amsterdam, Netherlands) was used to sample skin conductance from the sternum at 1 Hz from two Ag-Ag Cl electrodes. For the present analysis, participants were categorized into groups consisting of those who experienced physiologic hot flashes over the 24-hour monitoring period and those who did not.

4.3.5 Statistical Analysis

An exploratory factor analysis was performed in order to estimate the underlying structure of the six measured inflammatory and coagulation biomarkers. Biomarker concentrations were log transformed and then extracted using maximum likelihood methods with direct oblimin rotation on Pearson correlation matrix using PROC Factor in SAS 9.3 (SAS Institute, Cary, NC). A scree plot and parallel analysis suggested that two factors should be retained. We compared the model fit and interpretability of models with one- through three-factor solutions, and retained the two-factor solution based on model fit statistics. Estimated factor scores were obtained via the SCORE option in PROC Factor based on the five biomarkers with loadings $\geq|0.3|$ in the two-factor solution. Factor VII did not load on any factor and was therefore excluded from the remainder of the analysis. Factor scores were then categorized based on tertiles of the distribution.

Chi-square and Kruskal-Wallis tests were used to compare differences in participant characteristics across tertiles of the factor scores. Similarly, in order to measure the univariate associations between tertiles of factor scores and plaque outcomes (plaque presence, total

number of plaques, total plaque area, maximum height, minimum GSM, and calcification) Chi-square and Kruskal Wallis tests were used. Logistic regression was used to assess the associations between the tertiles of factor scores with plaque presence and the presence of calcification. Linear regression was used to determine associations between the tertiles of factor scores total plaque area, maximum height, and minimum GSM. Finally zero-inflated Poisson regression was used to determine associations between the tertiles of factor scores and total number of plaques. Models were initially unadjusted, then adjusted for age, race, and self-rated health (Model 2). Separate models were used to adjust for CVD risk factors including BMI, SBP, HDL-C, HOMA, PSQI, physiologic hot flashes and time since FMP (Model 3), as well as medication use including lipid lowering, blood pressure, anti-diabetics, and sleep medications (Model 4). Interactions were tested for using cross product terms in models. When significant interactions were identified among continuous variables, PROC PLM in SAS 9.3 (SAS Institute, Cary, NC) was used to compare effects at various levels of the continuous moderators. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

4.4 RESULTS

Table 4.1 details the characteristics of participants included in the EFA. Briefly, participants were on average 54 years old, overweight, predominately white, and approximately 46% of study participants had plaque present in at least one segment of the carotid artery. Two factors emerged from the EFA (Table 4.2). Factor 1 was composed of CRP, IL-6, and fibrinogen, with factor loadings ranging from 0.38-0.99. Factor 2 was composed of D-dimer and vWF antigen with factor loadings ranging from 0.39-0.63. Factor VII did not load on any factor. BMI, SBP, DBP,

insulin, HOMA, estrone, estradiol, and each of the circulating inflammatory and coagulation markers increased over the increasing tertiles of both Factor 1 and Factor 2 (Table 4.3). Additionally, across the increasing tertiles of Factor 1 and Factor 2 individuals were more likely to have lower self-rated health, lower HDL-C, utilize medication for hypertension, and be considered high risk based on the Berlin Sleep Questionnaire. Across the increasing tertiles of Factor 1, participants were significantly more likely to have difficulty paying for basics, have higher blood glucose levels, higher triglycerides, and lower SHBG, while across the increasing tertiles of Factor 2 participants were significantly more likely to be on medication for diabetes, have higher LDL-C, and more likely to report snoring. There were no differences in plaque presence, total number of plaques, minimum plaque GSM, maximum plaque height, and presence of calcification across the tertiles of Factor 1 and Factor 2 in univariate models (Table 4.4).

In the regression analyses, there were no statistically significant associations observed between either Factor 1 or Factor 2 with measures of plaque presence and burden in the total MS Heart sample (Table 4.5). However, significant interactions were observed between Factor 1 and BMI when predicting plaque presence (B=0.20, p=0.016) and total plaque area (B=0.14, p=0.01), and also between Factor 2 and BMI when predicting plaque presence (B=-0.24, p<0.01), total number of plaques (B=1.08, p=0.03), and total plaque area (B=-0.18, p<0.01). After stratification by BMI (<25 kg/m^2 vs. ≥25 kg/m^2), a significant association was observed between Factor 1 and a greater total number of plaques (OR=1.81 95% CI=1.09, 3.02) among women with BMI <25 kg/m^2 (n=98); however, this association did not remain significant after adjustment for covariates. Additionally, the highest tertile of Factor 1 was associated with greater plaque area in unadjusted models (B=24.70, p=0.03) and after adjustment for medication use (B=33.45,

p=0.04), but not after adjustment for CVD risk factors (Figure 4.2). Similarly, a significant association was observed between Factor 2 and total number of plaques such that among women with BMI<25 kg/m^2, such that those in the highest tertile of Factor 2 had a mean number of plaques that was 2.75 (95% CI=1.56, 4.85) times higher than those in the lowest tertile (Figure 4.1). This relationship remained significant after adjustment for CVD risk factors (OR=2.10 95% CI=1.15, 3.82) as well as after adjustment for medication use (OR=2.64 95% CI=1.45, 4.81). Among those with BMI ≥25 kg/m^2 (n=206) there were no significant associations between either of the biomarker factors and the various measures of plaque presence or burden. Scatter plots depicting the relationship between Factor 1 and BMI among the BMI-stratified groups (<25 kg/m^2 and ≥25kg/m^2) can be found in Appendix A.

Similarly, there were no significant associations between either of the biomarker factors with the measures of plaque characteristics in the entire sample (Table 4.6). However, significant interactions were observed between both Factors 1 and 2 with time since final menstrual period (FMP) when predicting maximum height (B= -0.03 p=0.04 and B=0.04 p=0.01 for Factors 1 and 2, respectively), and between Factor 2 with time since FMP when predicting minimum plaque GSM (B=-1.29 p=0.03). After stratifying the sample based on years since FMP (<1 year vs. ≥1 year), a significant association was observed between Factor 1 and maximum plaque height among women with <1 years since their FMP (n=56) such that those in the highest tertile of Factor 1 had a maximum plaque height an average of 1.51 mm greater than those in the lowest tertile (p=0.04). This association remained significant (B=1.76 p=0.05) after adjustment for CVD risk factors (Figure 4.3). In unadjusted models the highest tertile of Factor 2 was associated with a maximum plaque height 1.13 mm lower than those in the lowest tertile; however this relationship was not significant after adjustment for CVD risk factors. There were no

associations between either factor and maximum plaque height among women with ≥1 year since their FMP.

Among women with ≥1 year since their FMP (n=248), the highest tertile of Factor 1 was significantly associated with lower minimum GSM (B=-28.29 p=0.01), but this association was attenuated after adjustment for CVD risk factors (Figure 4.3). Conversely, among this same group, Factor 2 trended toward an association with higher minimum GSM. There were no significant associations between either factor and minimum GSM among women with <1 year since their FMP. There were no significant interactions between the factors and age, menopausal stage, race/ethnicity, sleep, or hot flashes when predicting the plaque outcomes.

4.5 DISCUSSION

In a sample of 277 midlife women, two factors emerged following an EFA of circulating biomarkers of inflammation and coagulation: Factor 1 composed of CRP, IL-6, and fibrinogen, and Factor 2 composed of D-dimer and vwAntigen. Significant interactions were observed between both factors and BMI when predicting plaque burden. Among women with BMI ($<25 kg/m^2$), both factors were associated with an increased total number of plaques in the carotid artery. The association between Factor 2 and total number of plaques was independent of traditional CVD risk factors and medication use. Factor 1 was also associated with greater total plaque area after adjustment for demographics and medication use, but not traditional CVD risk factors.

Similarly, significant interactions were observed between both factors and time since FMP when predicting plaque height and GSM. Among women with <1 year since FMP, Factor 1

was significantly associated with greater maximum plaque height independent of CVD risk factors and medication use. Among women with ≥1 year since their FMP, Factor 1 was associated with lower minimum GSM. This association was only borderline statistically significant after adjustment for traditional CVD risk factors.

We chose to utilize an EFA approach due to the fact that many of the inflammatory and coagulation biomarkers associated with atherosclerotic CVD are interdependent, and this method addresses concerns of multicollinearity that arise when conducting regression analysis involving multiple biomarkers. EFA allows for the identification of underlying construct (factors) among a group of items with no *a priori* hypothesis (i.e. biologically naïve). Therefore, the results of our EFA provide insight into the underlying relationships among the circulating markers included in this analysis, and suggest that these markers may contribute jointly to the pathologic processes involved in carotid plaque development in midlife women. The factors that emerged from the present EFA are similar to those identified via the EFA described in the previous chapter (Section 3.4). In both analyses, Factor 1 was composed of CRP, IL-6, and fibrinogen, likely representing a non-specific inflammatory process. CRP is synthesized by hepatocytes in response to IL-6 secretion by macrophages and T cells, and both are generalized markers of inflammation with prognostic value in regards to CVD (39, 43, 208, 209). Similar to CRP, fibrinogen is an acute phase reactant synthesized by hepatocytes in response to inflammation that contributes to platelet aggregation and is converted to fibrin in order to form blood clots (214, 230). Elevated fibrinogen has been associated with risk of CHD and stroke (210, 230). As was the case in the previously described EFA, the second factor identified in this analysis is a factor likely representing a thrombogenic process within the vasculature. In the present sample, Factor 2 was composed of D-dimer and vwAntigen. D-dimer is a fibrin degradation product that is present in

the blood once the coagulation cascade has been activated (231, 232). It has been associated with increased risk of CHD in prospective studies (58, 233). vwAntigen is a marker of VWF, a protein produced by endothelial cells and megakaryocytes that facilitates clotting by binding to platelets and various proteins involved in the coagulation cascade (80).

When considering the composition of the factors in combination with our regression analyses, these results suggest that increased inflammatory and coagulation burden are predictive of greater atherosclerotic plaque burden among women within the normal BMI range, but not among women overweight or obese. Adipose tissue is pro-inflammatory and secretes cytokines, such as IL-6, that have atherogenic effects (262, 263), and the relationship between BMI and CRP in women is well-established (264, 265). Therefore, the existing low-grade inflammation inflammatory burden affecting women with greater adiposity may have contributed to the lack of effect seen among women who were overweight or obese.

We also found that inflammatory burden was related to greater plaque height among women with <1 year since their FMP, and lower GSM among women with ≥1 years since their FMP. These results support our hypothesis that circulating inflammatory burden is associated with B-mode ultrasound-derived carotid plaque characteristics that are indicative of plaques that may be more prone to rupture; however, suggest that these relationships are moderated by time since FMP. One possible explanation of the relationship between inflammatory burden and plaque height in perimenopausal women (<1 years since FMP) is that one hallmark of the menopausal transition is a dramatic drop in estrogen levels, and evidence suggests that some estrogens have anti-inflammatory effects within the vasculature (266, 267). Plaque height is a dynamic characteristic of plaque that capable of experiencing changes over a relatively short period of time. Rollefstad et al. have previously reported significant regression in plaque height

following the initiation of statin therapy, and found this effect was not dependent upon a reduction in LDL-cholesterol (252). An abrupt decrease in levels of anti-inflammatory hormones following the onset of the menopausal transition may influence remodeling or expansion of atherosclerotic plaque; however, this is entirely speculation. The observed relationship between Factor 1 and minimum GSM among postmenopausal women is also important in that a woman's risk of experiencing a cardiovascular event increases following menopause (241) and plaques with low echogenicity have been associated with increased risk of both stroke and coronary events in longitudinal studies (137, 138, 148). Low GSM plaques are more likely to contain plaque components associated with instability such as lipid rich necrotic core (LRNC), vascularity, and intraplaque hemorrhage (133-136). These results highlight the importance of inflammatory burden as a marker of cardiovascular risk in midlife women, and suggest that the inflammatory burden may increase a woman's cardiovascular risk by negatively influencing characteristics and components of atherosclerotic plaque.

Our study has several limitations worth noting. First, this cross-sectional analysis does not allow us to make conclusions regarding the causal influence of inflammatory and coagulation on plaque burden and characteristics. Since we used B-mode ultrasound to characterize plaques, we cannot directly identify specific components of plaque that are associated with inflammatory and coagulation burden, but utilized surrogate markers of these components. Additionally, our sample excluded smokers and those with any clinical CVD. The relationships found in our study may be different among smokers and those with CVD, who likely have higher inflammatory burden compared to our sample. Similarly, our participants were predominately white (76%), with very few Asian and Hispanic participants; therefore, these results are not generalizable across racial and ethnic groups.

In summary, we found that a higher circulating inflammatory and coagulation burden was associated with greater burden of atherosclerotic plaque in the carotid arteries of midlife women within the normal range of BMI who were clinically free of CVD. Additionally, we found that higher circulating inflammatory burden was associated with greater maximum plaque height among perimenopausal women and lower minimum GSM among postmenopausal women. These findings highlight important associations between circulating inflammatory and coagulation burden with measures of atherosclerotic plaque burden and characteristics, and suggest that increased inflammatory and coagulation burden may serve as mechanisms contributing to the progression and complication of atherosclerotic plaque in midlife women.

4.6 TABLES AND FIGURES

Table 4.1 Characteristics of the 277 MS Heart Participants in Sample

Characteristic	MS Heart Sample
Age	55 (52-57)
Race (non-white)	67 (24%)
Education	
High School/Some College	112 (40%)
College Graduate	80 (29%)
Post-Graduate	85 (31%)
Hard to Pay for Basics	
Not Hard	194 (71%)
Somewhat Hard	64 (23%)
Very Hard	17 (6%)
Self-Rated Health	
Excellent or Very Good	200 (72%)
Good or Fair	77 (28%)
Menopausal Status	
Perimenopausal	44 (16%)
Post-Menopausal	233 (84%)
Physiologic Hot Flashes	137 (49%)
Years Since FMP	3.3 (1.7-6.3)
BMI (kg/m^2)	27 (24-32)
SBP (mmHg)	118 (110-128)
DBP (mmHg)	69 (63-76)
Diabetes	7 (3%)
HOMA-IR	2.1 (1.6-3.0)
HDL (mg/dL)	63 (53-72)
LDL (mg/dL)	129 (108-152)
Triglycerides (mg/dL)	94 (71-128)
SHBG (nmol/L)	83.1 (53.9-131.2)
PSQI	5 (4-7)
Berlin Sleep High Risk	76 (27%)
Average HF-HRV	199.3 (108.0-358.7)
CRP (mg/L)	1.28 (0.64-2.90)
IL-6 (pg/mL)	1.44 (0.95-2.23)
Fibrinogen (mg/mL)	337.70 (292.40-381.70)
D-dimer (ug/mL)	233.87 (171.71-367.47)
vWF Antigen (IU/dL)	1.35 (1.01-1.68)
FVII (mg/dL)	1.26 (1.09-1.45)
Medication Use	
Anti-hypertensive	43 (16%)
Anti-Diabetic	8 (3%)
Lipid-Lowering	38 (14%)

Table 4-1 Continued

Sleep	14 (5%)
Anti-coagulation	28 (10%)
Beta Agonists	13 (5%)
Anti-Inflammatory	52 (19%)
Outcomes	
Plaque Present	127 (46%)
Total Number of Plaques	0 (0-1)
Among Those with Plaque:	
Total Plaque Area (mm^2)	16.8 (9.8-26.2)
Maximum Height (mm)	2.0 (1.7-2.5)
Minimum GSM	49.3 (39.8-67.4)
Presence of Calcification	40 (32%)

[a] Median (25^{th}-75^{th} percentile) provided for all continuous variables and
[b] N (%) provided for categorical variables

Table 4.2 Biomarker FActor Loadings based on Exploratory Factor Analysis

Biomarker	Factor 1	Factor 2
CRP (mg/L)	**0.99**	**-**
IL-6 (pg/mL)	**0.47**	**-**
Fibrinogen (mg/mL)	**0.38**	**-**
D-dimer (ug/mL)	**-**	**0.39**
vWF Antigen (IU/dL)	**-**	**0.63**
FVII (mg/dL)	**-**	**-**

Table 4.3 Characteristics of the Study Sample by Tertiles of Biomarker Factors

Characteristic	Factor 1 (CRP, IL-6, Fibrinogen)				Factor 2 (D-Dimer, VW Factor Antigen)			
	T1	T2	T3	*p*	T1	T2	T3	*p*
Age	54 (50-57)	55 (53-58)	55 (52-57)	0.08	54 (50-56)	55 (52-58)	55 (52-57)	0.07
Race (non-white)	20 (22%)	17 (18%)	30 (33%)	0.08	20 (22%)	17 (18%)	30 (33%)	0.08
Education				0.34				0.17
HS/Some College	30 (33%)	38 (41%)	44 (47%)		31 (34%)	35 (38%)	46 (49%)	
College Graduate	32 (35%)	26 (28%)	22 (23%)		25 (27%)	30 (33%)	25 (27%)	
Post-Graduate	29 (32%)	28 (31%)	28 (30%)		35 (38%)	27 (29%)	23 (24%)	
Hard to Pay for Basics				0.01				0.42
Not Hard	69 (76%)	70 (77%)	55 (58%)		65 (72%)	67 (74%)	62 (67%)	
Somewhat/Very Hard	22 (24%)	20 (23%)	39 (42%)		26 (28%)	24 (26%)	31 (34%)	
Self-Rated Health				<0.01				0.01
Excellent/Very Good	16 (18%)	20 (22%)	41 (44%)		15 (16%)	28 (30%)	34 (36%)	
Good or Fair	75 (82%)	72 (78%)	53 (56%)		76 (84%)	64 (70%)	60 (64%)	
Post-menopausal	72 (79%)	78 (85%)	83 (88%)	0.23	72 (79%)	81 (88%)	80 (85%)	0.24
Years Since FMP	3.0 (1.5-6.0)	3.4 (1.7-6.5)	3.3 (1.4-6.8)	0.63	3.2 (1.4-6.0)	3.1 (1.7-7.7)	3.4 (1.7-5.8)	0.40
BMI (kg/m^2)	24 (21-27)	27 (24-29)	33 (29-36)	<0.01	25 (22-28)	28 (25-32)	31 (25-35)	<0.01
SBP (mmHg)	113 (105-122)	118 (109-125)	125 (115-113)	<0.01	113 (106-122)	120 (110-131)	120 (113-131)	<0.01
DBP (mmHg)	68 (62-76)	68 (62-74)	72 (65-79)	0.03	67 (61-75)	70 (64-77)	69.8 (64.0-78.0)	0.03
Diabetes	1 (1%)	1 (1%)	5 (5%)	0.11	1 (1%)	1 (1%)	5 (5%)	0.11
HOMA	1.9 (1.5-2.3)	2.0 (1.6-2.7)	2.8 (1.9-4.2)	<0.01	1.8 (1.5-2.3)	2.3 (1.7-3.1)	2.4 (1.8-3.6)	<0.01
HDL-c (mg/dL)	67 (56-76)	63 (54-73)	58 (47-69)	<0.01	64 (55-76)	61 (55-70)	61 (49-71)	0.04
LDL-c (mg/dL)	123 (102-148)	129 (109-148)	134 (111-158)	0.13	122 (100-144)	137 (111-155)	126 (107-152)	0.04
Triglycerides(mg/dL)	82 (68-109)	98 (71-129)	102 (76-150)	<0.01	86 (67-120)	93 (75-130)	100 (76-135)	0.17

SHBG (nmol/L)	107.8 (65.4-155.1)	89.9 (58.7-132.4)	62.7 (45.6-93.0)	<0.01	90.0 (53.1-138.0)	85.2 (52.2.-126.3)	78.4 (54.3-131.2)	0.79
PSQI	5 (3-7)	5 (3-7)	5 (4-7)	0.70	5 (3-7)	5 (4-7)	5 (4-7)	0.69
Berlin High Risk	17 (19%)	27 (29%)	46 (49%)	<0.01	17 (19%)	26 (28%)	47 (50%)	<0.01
Average HF-HRV	219.1 (132.9-367.2)	191.1 (112.9-346.0)	171.6 (88.0-361.2)	0.41	193.6 (122.0-351.9)	191.1 (99.6-388.4)	210.5 (99.6-354.9)	0.99
Medication Use								
Anti-hypertensive	8 (9%)	13 (14%)	22 (23%)	0.02	9 (9%)	11 (12%)	23 (23%)	0.01
Anti-diabetic	2 (2%)	1 (1%)	5 (5%)	0.20	1 (1%)	1 (1%)	6 (6%)	0.05
Lipid-lowering	10 (11%)	12 (13%)	16 (17%)	0.43	10 (11%)	11 (12%)	17 (18%)	0.31
Sleep	7 (8%)	5 (5%)	2 (2%)	0.22	8 (9%)	4 (4%)	2 (2%)	0.11
Beta-agonist	3 (3%)	4 (4%)	6 (6%)	0.60	4 (4%)	6 (7%)	3 (3%)	0.55
Anti-coagulation	11 (12%)	5 (5%)	12 (13%)	0.19	10 (11%)	8 (8%)	10 (11%)	0.86
Any Anti-inflammatory	13 (14%)	22 (24%)	17 (18%)	0.24	15 (16%)	21 (23%)	16 (17%)	0.47
CRP (mg/L)	0.5 (0.3-0.6)	1.3 (1.1-1.5)	4.2 (2.9-6.2)	<0.01	0.8 (0.4-1.4)	1.4 (0.6-2.8)	2.5 (1.2-4.6)	<0.01
IL6 (pg/mL)	0.9 (0.7-1.2)	1.3 (0.9-1.8)	2.0 (1.5-2.7)	<0.01	0.9 (0.7-1.3)	1.3 (1.1-1.8)	2.1 (1.6-2.8)	<0.01
Fibrinogen (mg/mL)	300.5 (260.9-333.7)	330.2 (286.2-358.0)	374.4 (334.8-421.7)	<0.01	288.0 (251.5-328.5)	334.6 (299.6-372.2)	373.8 (335.9-429.6)	<0.01
D-dimer (ng/mL)	204.2 (145.3-331.6)	225.7 (162.8-305.4)	293.3 (217.5-466.7)	<0.01	172.4 (128.8-246.7)	224.4 (173.6-311.5)	367.1 (232.4-510.7)	<0.01
Factor VII (IU/dL)	1.2 (1.0-1.4)	1.2 (1.1-1.4)	1.3 (1.1-1.5)	<0.01	1.2 (1.1-1.4)	1.3 (1.1-1.4)	1.3 (1.1-1.5)	<0.01
vWF Antigen(mg/dL)	1.2 (1.0-1.5)	1.2 (1.0-1.5)	1.4 (1.1-1.8)	0.01	0.9 (0.8-1.1)	1.4 (1.2-1.5)	1.7 (1.4-2.1)	<0.01

[a] Median (IQR) provided for all continuous variables, Kruskal Wallis test used to compare groups.

[b] N (%) provided for categorical variables, Chi-square test used to compare groups

Table 4.4 Characteristics of the Study Sample by Tertiles of Factor Scores

	Factor 1 (CRP, IL-6, Fibrinogen)				Factor 2 (D-Dimer, VW Factor Antigen)			
	T1	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**
Plaque Present	37 (40%)	46 (36%)	44 (35%)	0.44	39 (31%)	40 (32%)	48 (38%)	0.46
Total # of Plaques	0 (0-1)	0 (0-1)	0 (0-2)	0.49	0 (0-1)	0 (0-1)	0 (0-2)	0.22
Total Plaque Area (mm^2)	14.4 (8.3-24.2)	16.8 (9.8-26.9)	19.7 (11.1-30.3)	0.42	14.6 (8.8-22.5)	16.8 (9.9-24.8)	20.6 (11.1-30.8)	0.27
Minimum GSM	56.5 (36.9-77.6)	47.8 (37.7-65.2)	53.6 (39.8-67.4)	0.83	49.3 (42.7-66.7)	49.3 (34.8-73.9)	50.0 (39.8-63.8)	0.94
Maximum Height (mm)	1.9 (1.7-2.3)	2.0 (1.6-2.6)	2.1 (1.8-2.4)	0.66	1.8 (1.7-2.4)	2.0 (1.7-2.5)	2.1 (1.6-2.6)	0.84
Calcification	9 (10%)	15 (16%)	16 (17%)	0.32	7 (8%)	18 (20%)	15 (16%)	0.06

[a] Median (IQR) provided for all continuous variables, Kruskal-Wallis test used to compare groups.

[b] N (%) provided for categorical variables, Chi-square test used to compare groups

Table 4.5 Associations of Biomarker Factor Tertiles with Measures of Plaque Presence and Burden

Biomarker Factor Tertile	PLAQUE PRESENCE			TOTAL # OF PLAQUES			TOTAL PLAQUE AREA		
	Model 2	Model 3	Model 4	Model 2	Model 3	Model 4	Model 2	Model 3	Model 4
				Factor 1: CRP, IL-6, Fibrinogen					
Tertile 1 (ref)									
Tertile 2	1.28 (0.70,2.33)	1.56 (0.81,3.03)	1.26 (0.68,2.33)	1.12 (0.78,1.62)	1.03 (0.69,1.56)	1.02 (0.70,1.49)	-2.20 (4.47)	-4.27 (4.38)	-0.67 (4.49)
Tertile 3	1.10 (0.57,2.13)	1.85 (0.81,4.30)	0.94 (0.47,1.90)	1.23 (0.84,1.81)	1.16 (0.68,1.86)	1.32 (0.88,1.99)	1.18 (4.79)	-4.70 (5.30)	1.03 (4.80)
				Factor 2: D-dimer, vWF Antigen					
Tertile 1 (ref)									
Tertile 2	0.97 (0.53,1.78)	0.82 (0.42,1.60)	0.94 (0.50,1.76)	1.23 (0.84,1.80)	1.07 (0.73,1.56)	1.20 (0.81,1.77)	-1.15 (4.63)	-1.34 (4.39)	-1.24 (4.57)
Tertile 3	1.27 (0.66,2.43)	1.14 (0.56,2.34)	1.08 (0.54,2.12)	1.37 (0.94,2.00)	1.16 (0.78,1.74)	1.07 (0.72,1.77)	1.93 (4.63)	-0.32 (4.49)	0.05 (4.71)

[a] Beta estimates (se) and odds ratios (95% CI) provided where appropriate.

[b] Model 2 adjusts for age, race, and self-rated health

[c] Model 3 adjusts for Model 2 + BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health

[d] Model 4 adjusts for Model 2 + medication use (antihypertensive, lipid-lowering, diabetes, sleep)

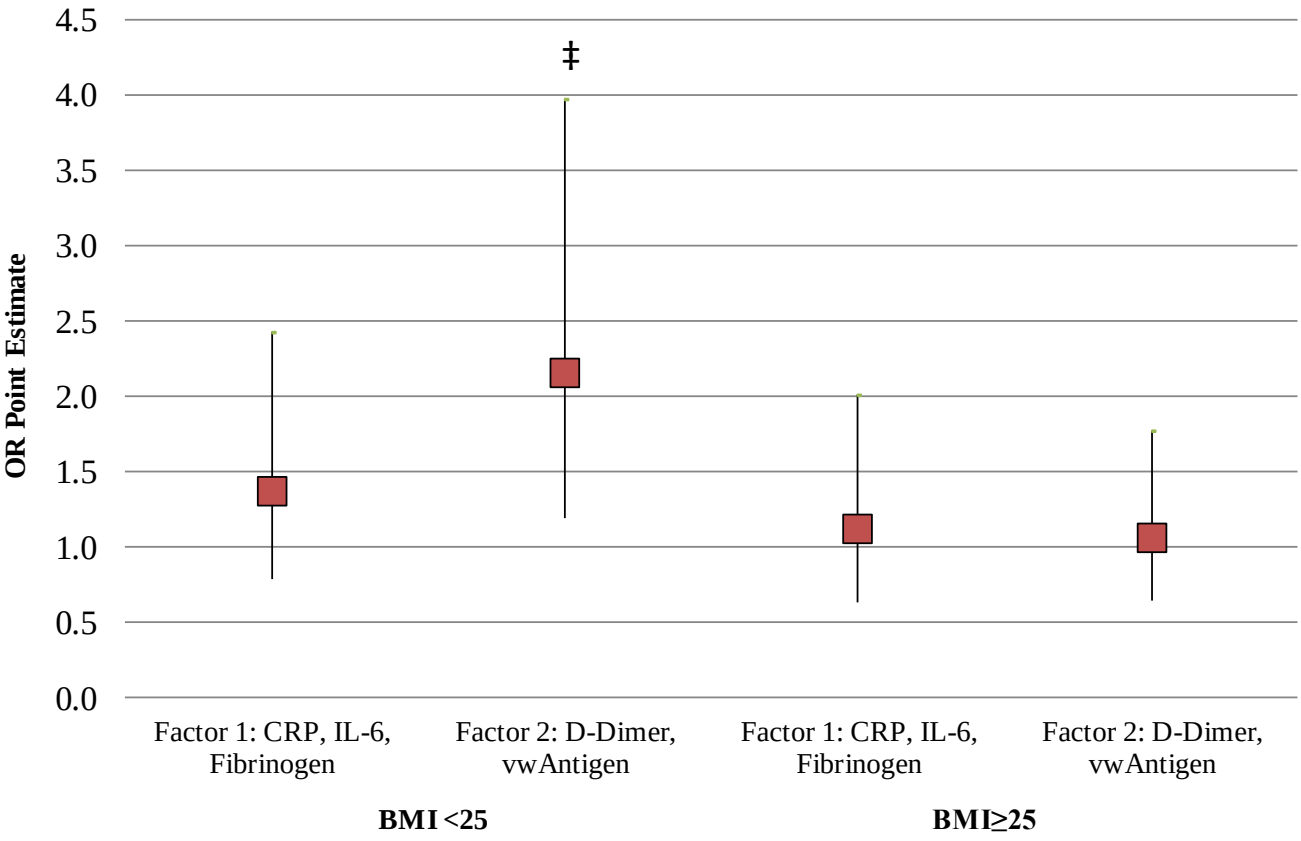


Figure 4.1 Associations of the Highest Biomarker Factor Tertiles with Total Number of Carotid Plaques

[a] OR point estimates with corresponding 95% CIs depicted

[b] Model adjusts for age, race, self-rated health, BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health

[c] ‡ Indicates significance at the α=0.05 level

[d] BMI<25 n=98; BMI ≥25 n=206

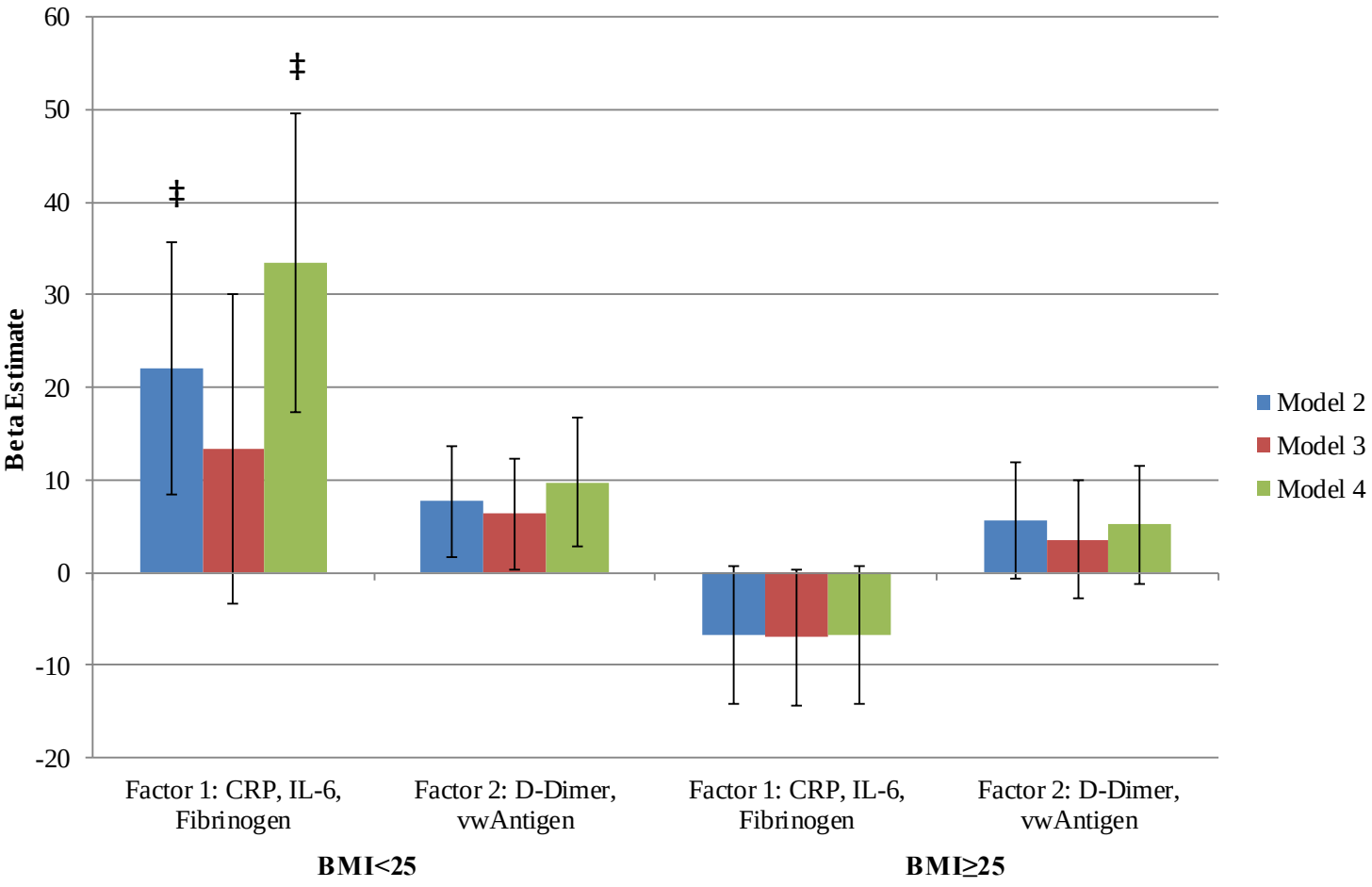


Figure 4.2 Associations of the Highest Biomarker Factor Tertiles with Total Plaque Area

[a] Beta estimates with corresponding SE estimates depicted
[b] Model 2 adjusts for age, race, and self-rated health
[c] Model 3 adjusts for Model 2 + BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health
[d] Model 4 adjusts for Model 2 + medication use (antihypertensive, lipid-lowering, diabetes, sleep)
[e] ‡ Indicates significance at the α=0.05 level
[f] BMI<25 n=98; BMI ≥25 n=206

Table 4.6 Associations of Biomarker Factor Tertiles with Carotid Plaque Characteristics

Biomarker Factor Tertile	MINIMUM GSM			MAXIMUM HEIGHT			PRESENCE OF CALCIFICATION		
	Model 2	Model 3	Model 4	Model 2	Model 3	Model 4	Model 2	Model 3	Model 4
				Factor 1: CRP, IL-6, Fibrinogen					
Tertile 1 (ref)									
Tertile 2	-2.51 (5.38)	-4.26 (5.46)	-2.74 (5.48)	0.07 (0.15)	-0.00 (0.16)	0.09 (0.15)	1.31 (0.46,3.76)	0.80 (0.23,2.81)	1.10 (0.35,3.44)
Tertile 3	-5.48 (5.75)	-4.90 (6.61)	-5.63 (5.87)	0.07 (0.16)	0.06 (0.19)	0.07 (0.17)	1.84 (0.60,5.58)	0.77 (0.15,3.97)	2.17 (0.63,7.49)
				Factor 2: D-dimer, vWF Antigen					
Tertile 1 (ref)									
Tertile 2	-0.67 (5.56)	-1.07 (5.48)	-1.05 (5.58)	-0.03 (0.16)	-0.03 (0.16)	-0.04 (0.16)	2.77 (0.9,8.30)	2.97 (0.89-9.91)	3.02 (0.94,9.68)
Tertile 3	1.70 (5.57)	0.86 (5.61)	2.69 (5.75)	0.00 (0.16)	-0.09 (-0.16)	-0.03 (0.16)	1.49 (0.49,4.57)	1.11 (0.31,4.03)	1.26 (0.35,4.51)

[a] Beta estimates (se) and odds ratios (95% CI) provided where appropriate.

[b] Model 2 adjusts for age, race, and self-rated health

[c] Model 3 adjusts for Model 2 + BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health

[d] Model 4 adjusts for Model 2 + medication use (antihypertensive, lipid-lowering, diabetes, sleep)

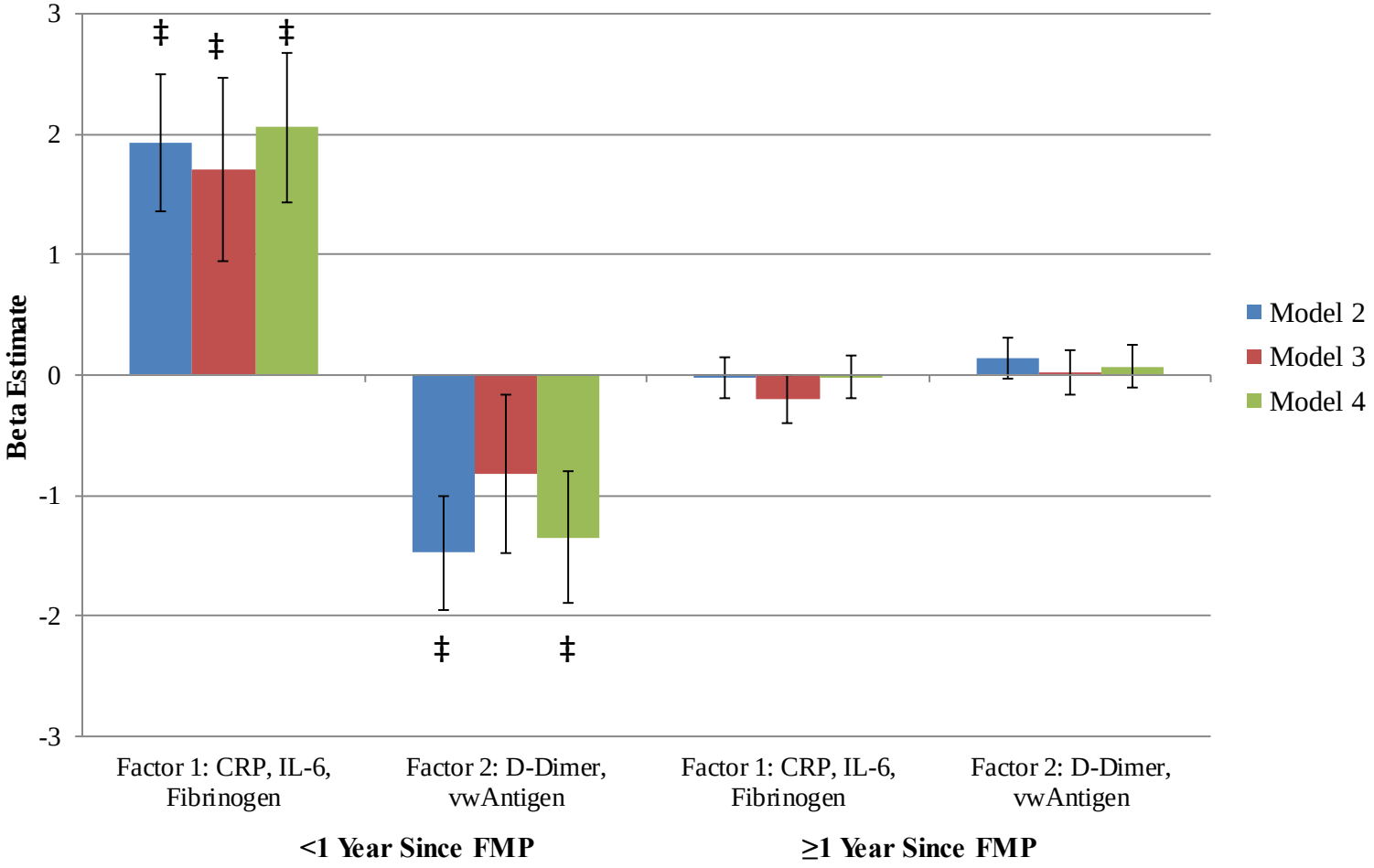


Figure 4.3 Associations of Biomarker Factor Tertiles with Maximum Plaque Height

[a] Beta estimates with corresponding SE estimates depicted
[b] Model 2 adjusts for age, race, and self-rated health
[c] Model 3 adjusts for Model 2 + BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health
[d] Model 4 adjusts for Model 2 + medication use (antihypertensive, lipid-lowering, diabetes, sleep)
[e] ‡ Indicates significance at the α=0.05
[f] <1 Year since FMP n=56, ≥1Years since FMP n=248

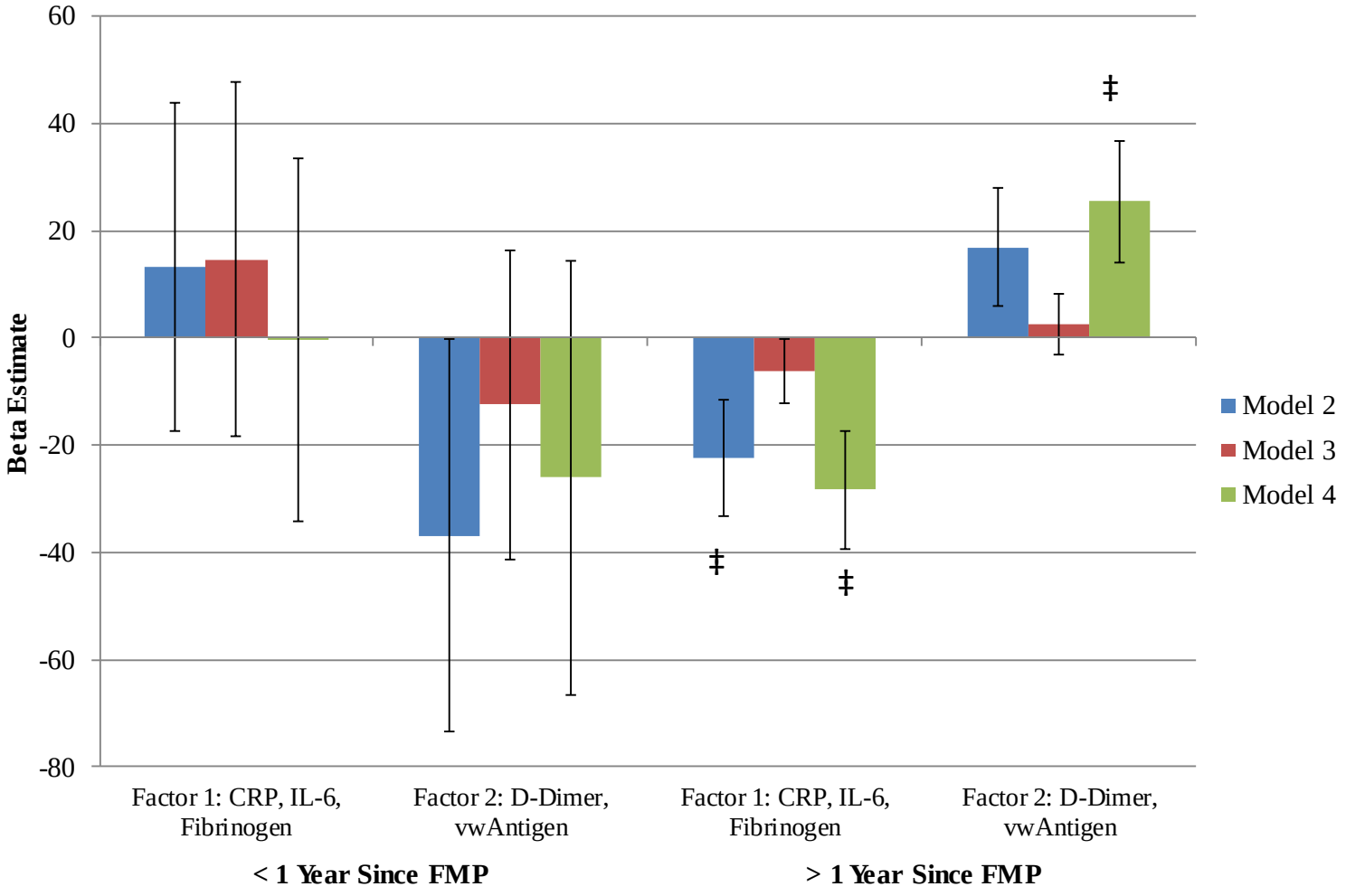


Figure 4.4 Associations of Biomarker Factor Tertiles with Minimum Plaque GSM

[a] Beta estimates with corresponding SE estimates depicted

[b] Model 2 adjusts for age, race, and self-rated health

[c] Model 3 adjusts for Model 2 + BMI, SBP, HDL, SHBG, HOMA, PSQI, physiologic hot flashes, and self-rated health

[d] Model 4 adjusts for Model 2 + medication use (antihypertensive, lipid-lowering, diabetes, sleep)

[e] ‡ Indicates significance at the $\alpha=0.05$

[f] <1 Year since FMP n=56, ≥1Years since FMP n=248

5.0 MANUSCRIPT 3: ASSOCIATIONS BETWEEN PERCEVED EVERDAY DISCRIMINATION AND MEASURES OF PLAQUE PRESENCE, BURDEN, AND CHARACTERISTICS IN MIDLIFE WOMEN

Natalie C. Suder[1], Emma Barinas-Mitchell, Maria M. Brooks, Suresh R. Mulukutla, Trevor J. Orchard, Christina L. Wassel, Rebecca C. Thurston

*Authorship Order to be Determined

Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA

Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

Division of Cardiology, University of Pittsburgh, Pittsburgh, PA

Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT

5.1 ABSTRACT

Introduction: Perceived discrimination is positively associated with cardiovascular risk factors and events. However, questions remain regarding the potential mechanisms through which perceived discrimination may influence cardiovascular risk and how experiences of discrimination may be associated with markers of subclinical CVD including atherosclerotic plaque presence, burden, and characteristics.

Methods: 300 late peri- and postmenopausal women who were free of clinical CVD completed the Everyday Discrimination scale and underwent B-mode carotid ultrasound. Associations between everyday discrimination (frequency of day-to-day interpersonal mistreatment) and measures of carotid plaque presence, burden (total number of plaques, total plaque area), and characteristics (maximum height, minimum GSM, and calcification) were evaluated using regression analyses adjusting for demographics, as well as CVD and psychosocial risk factors. Circulating inflammatory burden was evaluated as a potential mediator of the relationship between everyday discrimination and subclinical CVD.

Results: After adjustment for demographics, CVD and psychosocial risk factors, women who experienced high levels of everyday discrimination had maximum plaque height 0.29 mm greater (p=0.03) than those who did not experience high levels of discrimination. Circulating inflammatory burden was a partial mediator of this relationship (natural indirect effect through mediator=1.21 95%CI=1.17, 1.25). Everyday discrimination score and a modified high discrimination score were both associated with a greater total number of carotid plaques (OR=1.03 95%CI=1.00- 1.07 and OR=1.09 95%CI=1.01-1.07, respectively) in minimally adjusted models including age, race, and difficulty paying for basic items; however these

relationships were no longer statistically significant after adjustment for CVD and psychosocial risk factors.

Discussion: These results add to the existing evidence that experiences of everyday discrimination have negative health effects among women of various racial/ethnic groups, and suggest that increased inflammatory burden and greater carotid plaque height may be mechanisms through which experiences of discrimination increase women's cardiovascular risk at midlife.

5.2 INTRODUCTION

CVD is the leading cause of death among U.S. women, and self-reported experiences of discrimination have been associated with greater risk of incident cardiovascular events and all-cause mortality across racial and ethnic groups (185, 268). The associations between self-reported discrimination with lifestyle and risk factors implicated in CVD vary slightly based on the population of interest and measure of discrimination, but ultimately, perceived discrimination has been associated with smoking (177), preterm birth (269, 270), hypertension (192), poor sleep (176), and depression (184) among women. Understanding the relationship between experiences of discrimination and CVD in women is important because the evidence suggests that the physiologic mechanisms contributing to CVD differ among men and women, and that interpersonal stress may elicit a more robust stress response in women compared to men (155, 271).

Atherosclerotic plaque in the carotid artery is an important preclinical marker of CVD that is associated with increased risk of ischemic stroke (100, 246), CHD (127, 247), and cardiovascular mortality (249, 272). In addition to plaque presence, greater burden of plaque in the carotid artery is associated with increased risk of stroke (124), MI (125, 126), and MACE (120). One study has previously linked everyday discrimination with greater carotid plaque burden among African American women; however, this relationship was only marginally statistically significant (198).

B-mode ultrasound, one method of visualizing and quantifying carotid plaque, can also be utilized to identify characteristics of plaque that may provide insight regarding the vulnerability of plaque to rupture or erode. For example, grey-scale median (GSM), a continuous measure of plaque echogenicity, is a surrogate marker of plaque components. Low GSM has

been associated with increased lipid content, necrotic core presence, and composite measures of plaque instability in histologic studies (134-136), while higher GSM is correlated with greater fibrous content among plaques assessed via histology (134). Lower GSM plaque in the carotid artery is prospectively associated with increased hazard of MACE among asymptomatic patients with carotid artery disease (148). Additionally, the measurement of plaque height and calcification may provide further insight into the complexity and potential stability of carotid plaques (251, 252). Greater maximum plaque height, or thickness, in the carotid artery is longitudinally associated with greater risk of cardiovascular events (253, 254). Calcification of plaque in the carotid artery is associated with increased risk of mortality and cardiovascular events when comparing individuals with calcified plaque to those with no carotid plaque (150, 151). However, the increase in calcification observed following the initiation of statin treatment suggests that calcification may be a beneficial characteristic of plaque among those with high plaque burden (252, 255).

One of the mechanisms through which perceived discrimination is hypothesized to influence cardiovascular risk is increased inflammation (175). Findings from the Study of Women's Health Across the Nation indicate that discrimination is longitudinally associated with higher CRP levels in non-obese women of various racial/ethnic groups (273). CRP is a marker of systemic inflammation that has been well established as a marker of CVD risk among the general population (40), and is has been associated with carotid plaque presence and lower echogenicity of carotid plaques (39). Multiple cross-sectional analyses have reported associations between perceived discrimination and elevated circulating CRP levels (274, 275), and one study from the Multi-Ethnic Study of Atherosclerosis reported an association between perceived discrimination and higher levels of IL-6 among women (276).

In the present study, we investigate the relationship between perceived discrimination, measured by the Everyday Discrimination scale, with measures of carotid plaque presence, burden, and characteristics in a sample of midlife women. We hypothesize that greater everyday discrimination is associated with greater carotid plaque burden and ultrasound-measured characteristics indicative of instability. Furthermore, we hypothesize that the association between perceived discrimination and carotid plaque characteristics is mediated by circulating inflammatory burden.

5.3 MATERIALS AND METHODS

5.3.1 Participants

The MS Heart Study includes 304 late perimenopausal (2-12 months amenorrhea) and postmenopausal (≥12 months amenorrhea) nonsmoking women aged 40-60 years. Advertisements, mailings, fliers, referral from local clinics, and online message boards were used to recruit participants to the study, which was intended to investigate the relationship between menopausal symptoms and CVD risk. By design, half of the participants reported daily hot flashes or night sweats within the past three months, while the other half of the participants reported no hot flashes or night sweats in the past three months. Women who had previously undergone a hysterectomy and/or bilateral oophorectomy, had a history of heart disease, stroke, arrhythmia, ovarian/gynecological cancer, pheochromocytoma, pancreatic tumor, kidney failure, seizures, Parkinson's disease, Raynaud phenomenon, previous endarterectomy, endometrial ablation, or were currently pregnant, undergoing chemotherapy or dialysis were excluded from

the study. Women were also excluded if they had used the following medications in the past three months: oral/transdermal estrogen or progesterone, selective estrogen receptor modulators, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, gabapentin, insulin, beta blockers, calcium channel blockers, alpha-2 adrenergic agonists, or other antiarrhythmic agents.

Upon inclusion in the study, all participants underwent physical measurements, questionnaire completion, blood draw, ambulatory physiologic hot flash monitoring for three days, electrocardiogram, actigraphic sleep monitoring, and a carotid artery ultrasound. Four women were excluded from the present study due to missing data from the carotid ultrasound (poor image quality n=1 and equipment failure n=3). Procedures were approved by the University of Pittsburgh Institutional Review Board, and all participants provided written informed consent.

5.3.2 Ultrasound Measurements and Plaque Characterization

B-mode ultrasound using a Sonoline Antares (Siemens, Malvern, PA) high-resolution duplex scanner with a VF10-5 transducer was utilized in order to obtain bilateral carotid images Trained sonographers at the University of Pittsburgh Ultrasound Research Laboratory (URL) obtained the images according to a standardized protocol. Five carotid segments within both the left and right carotid arteries were assessed for plaque: the proximal common carotid artery (CCA), the distal common carotid artery (CCA one centimeter distal to the carotid bulb), the carotid bulb (where the near and far walls of the common carotid are no longer parallel, extending to the flow divider), the internal carotid artery (ICA), and the external carotid artery (ECA).

Presence of plaque was defined as a focal structure that encroaches into the arterial lumen with 50% greater thickness than the surrounding intima-media thickness consistent with the Mannheim Consensus Statement (117). Plaque presence and total number of plaques in each segment were determined by trained ultrasound readers. Plaque presence was defined as a participant having plaque in at least one arterial segment and total number of plaques was defined as the sum of all plaques across all of the visualized arterial segments. One trained ultrasound reader determined plaque area, height, GSM, and presence of calcification using semi-automated Carotid Analyzer software from the Vascular Research Tools 5 Suite (Medical Imaging Applications LLC, Coralville, IA). Plaque area for each plaque was calculated by the software once the plaque had been traced by the reader and automatically outlined by the software. Total plaque area was defined as the sum of plaque area in each of the arterial segments. GSM was calculated by the software for each plaque following grey level normalization, which aims to minimize the effect of different ultrasound machine gain settings during image acquisition. Normalization was performed manually by the ultrasound reader selecting a dark area of blood from the lumen and the brightest area of the adventitial layer along the anterior or posterior wall. Maximum height was defined as the greatest height value of all plaques among participants with plaque present. Minimum GSM was defined as the GSM value of the plaque with the lowest GSM for each participant with plaque present. The reader subjectively identified calcification after assessing each plaque for highly echogenic areas with or without acoustic shadowing. Presence of calcification was defined as a participant having a calcified plaque in at least one of the visualized arterial segments.

5.3.3 Perceived Discrimination

A modified version of the Detroit Area Study Everday Discrimination Scale was used to assess perceived discrimination among the participants (186). This 10-item instrument utilizes a 4-point scale (1=never, 2=rarely, 3=sometimes, 4=often) in order to determine the frequency of interpersonal maltreatment in the previous 12 months. Examples of items included in the Everday Discrimination scale include “You are treated with less respect than other people”, “People act as if they think you are not smart”, and “You receive poorer service than other people in restaurants or stores. The Everyday Discrimination scale has high levels of internal consistency (186, 277), and is validated across racial and ethnic groups (187).

Responses for each item were summed in order to determine the total Everyday Discrimination score (range 0-40) for each participant. We also calculated a high discrimination score (range 0-10) where responses of “never” and “rarely” were scored as a zero and responses of “sometimes” and “often” were scored as a one. In order to compare participants who reported high interpersonal mistreatment compared to those who did not, we categorized individuals who responded to at least one item on the Everyday Discrimination scale with “sometimes” or “often” into a high perceived discrimination group. This approach has been used previously (191) and was suggested by Williams et al (186). Women who reported that they experience high discrimination (answered “sometimes” or “often” to at least one item) were asked to identify the characteristic to which they attributed this interpersonal maltreatment. Options included race, ethnicity, gender, age, income level, language, physical appearance, sexual orientation, and other.

5.3.4 Inflammatory and Coagulation Biomarkers

High sensitivity CRP was measured using a high-sensitivity reagent set (Beckman Coulter, Brea, CA, USA), and IL-6 was measured using an R&D Systems high sensitivity enzyme-linked immunosorbant assay (Minneapolis, MN, USA). Fibrinogen was measured using a modified Clauss method and Siemens Multifibren U reagent (Newark, DE, USA), and quantitative D-dimer was measured using a Diagnostica Stago's Asserachrom assay (Asnieres sur Seine, France). Standard clotting methods via a Siemens Innovin thromboplastin reagent with Factor VII-deficient plasma (HRF, Raleigh, NC, USA) was used in order to measure Factor VII clotting activity, and vWF Antigen was measured using Diagnostica Stago's STA-Liatest reagent (Asnieres sure Seine, France).

5.3.5 Covariates and Additional Measures

Participants self-reported their race/ethnicity, education level, shift work, medical and psychiatric history, health behaviors, and medication use (BP lowering, lipid lowering, diabetes, depression, anxiety, sleep) via questionnaire. Height and weight were measured using a fixed stadiometer and a balance beam scale in order to calculate BMI. Resting BP was measured using a Dinamap device after a seated 10-minute rest period, and the final BP was defined as the average of the second and third BP reading. Menopausal status was categorized as perimenopausal (>2-<12 months amenorrhea) or postmenopausal (≥12 months amenorrhea) (256). Depressive symptoms were assessed by the Center for Epidemiologic Studies Depression scale (278) and anxiety symptoms were assessed via the State Trait Anxiety Inventory (279). Participants also completed the Pittsburgh Sleep Quality Index (PSQI) in order assess sleep

quality (257) and the Berlin Sleep Questionnaire in order to assess sleep apnea symptoms such as snoring (258). Glucose, total cholesterol, triglycerides, and HDL, were assessed enzymatically (Vital Diagnostics, Lincoln, RI) following a blood draw that took place after a 12-hour fast, and LDL was calculated using the Friedewald equation (221). Insulin level was measured by radioimmunoassay, and HOMA was calculated. Physiologic hot flashes were monitored over a 24-hour period using sternal skin conductance monitoring (260, 261). A VU-AMS monitor (VU University Amsterdam, Netherlands) was used to sample skin conductance from the sternum at 1 Hz from two Ag-Ag Cl electrodes. Participants were categorized into groups consisting of those who experienced physiologic hot flashes over the 24-hour monitoring period and those who did not.

5.3.6 Statistical Analysis

Chi-square and Kruskal-Wallis tests were used to compare differences in categorical and continuous participant characteristics, respectively, among those who indicated that they experienced high levels of discrimination (at least one “sometimes” or “often” response) compared to those who did not. Logistic regression was used to assess the associations between the various measures of perceived discrimination with plaque presence and presence of calcification. Zero-inflated Poisson regression was used to measure the associations between the measures of perceived discrimination with total number of plaques. Among those with plaque, linear regression was used to determine association between perceived discrimination with total plaque area, maximum plaque height, and minimum GSM. Minimally adjusted models (Model 2) included age, race, and difficulty paying for basics, and more fully adjusted models (Model 3) included age, race, difficulty paying for basics, BMI, SBP, PSQI, depressive symptoms,

menopausal status, physiologic hot flashes, social support, lipid-lowering medication use, and diabetes medication. Interactions were tested for using cross product terms in models and Wald tests were used to indicate statistical significance.

The potential mediating effect of circulating inflammatory burden on significant relationships between perceived discrimination and plaque characteristics was tested using a causal inference approach. This approach, which is based on inverse probability weighting, allows for the estimation of natural direct and natural indirect effects, and works best when both the exposure and outcome are dichotomous (280). Inflammatory burden was modeled as a score corresponding to an inflammatory factor consisting of CRP, IL-6, and fibrinogen that emerged from an EFA of circulating biomarkers previously conducted in the MS Heart sample, and outcomes were dichotomized. The proportion of the relationship explained by the mediator was calculated using the following formula: *Proportion Explained = (Total Effect – Direct Effect) / Total Effect*. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

5.4 RESULTS

Women who reported experiencing high levels of perceived discrimination (high everyday discrimination score ≥1) had poorer self-rated health, greater difficulty paying for basic items, higher PSQI scores, and were more likely to be considered high-risk by the Berlin Sleep Questionnaire (Table 5.1). They also had higher total CESD scores, state and trait anxiety scores, and less social support compared to those who did not indicate that they experienced high levels of discrimination. Among non-white women, the most common characteristic to which the women attributed their experiences of discrimination was race (42%) followed by “other’ (27%)

and physical appearance (12%) (Table 5.2). Among white women the most common attribution responses were “other” (53%), age (18%), and income level (12%).

Nearly half of the women had at least one carotid plaque (46%), and among those with plaque 33% had plaque that was calcified (Table 5.3). In univariate analyses, there were no differences in plaque presence, total number of plaques, minimum GSM, and presence of calcification between women who reported experiencing high levels of everyday discrimination compared to those who reported low levels of everyday discrimination. However, women who reported experiencing high levels of everyday discrimination had greater total plaque area (22.41 mm^2 vs. 15.50 mm^2, p=0.02) and greater maximum plaque height (2.24 mm vs. 1.87 mm, p=<0.01) compared to women who reportedly experienced low levels of discrimination.

In the multiple regression analysis, everyday discrimination score was associated with a greater total number of plaques such that a 1-point increase in the score was associated with a 1.03 times higher number of plaques after adjustment for age, race, and difficulty paying for basic items (95% CI = 1.00-1.07) (Table 5.4). Similarly, a 1-point greater high discrimination score was associated with a 1.09 times higher number of plaques after adjustment for age, race, and difficulty paying for basics (95% CI=1.01-1.07). These associations were no longer statistically significant after adjustment for traditional CVD risk factors and psychosocial measures. No associations were observed between any of the discrimination measures with plaque presence and total plaque area before or after adjustment for covariates.

Women who indicated that they experienced high discrimination had on average a maximum plaque height 0.30 mm greater (p=0.01) than those who indicated that they did not regularly experience discrimination (Table 5.5, Figure 5.1). This association was maintained even after adjustment for covariates. Greater high discrimination scores were similarly associated

with greater plaque height (B=0.07 p=0.02), but this relationship was attenuated to borderline significance after adjustment for CVD risk factors and psychosocial measures; particularly depression and social support. These results were not moderated by race, physiologic hot flashes, or menopausal statues.

The results of the mediation analysis indicated that women who experienced high Everyday Discrimination were 2.75 times more likely to have a plaque in the highest tertile of plaque height, and that inflammatory burden (factor composed of CRP, IL-6, and fibrinogen) explained 31% of the relationship between high discrimination and plaque height (Figure 5.2). The natural direct effect of high discrimination on plaque height was 1.52 (95% CI=1.49-1.58) while the natural indirect effect through the inflammatory mediator was 1.21 (95% CI=1.17-1.25).

5.5 DISCUSSION

In a sample of midlife women free of clinical CVD we found that high perceived everyday discrimination was independently associated with greater maximum plaque height in the carotid artery. Furthermore, the observed relationship between everyday discrimination and plaque height was mediated by greater circulating inflammatory burden. These associations were independent of demographics, as well as traditional CVD and psychosocial risk factors. In minimally adjusted models, we also observed a significant association between everyday discrimination and a greater total number of carotid plaques; however, this relationship was not statistically significant after adjustment for CVD and psychosocial risk factors. We did not find

any significant associations between everyday discrimination with carotid plaque presence, area, minimum GSM, or calcification.

To our knowledge, this is the first study to evaluate the associations between perceived discrimination and characteristics of atherosclerotic plaque. One prior study aimed at examining the associations between various forms of psychosocial stress and carotid IMT reported a marginal relationship between everyday discrimination attributed to race and higher plaque score; however, these analyses were exploratory and limited in sample size (198). The Everyday Discrimination Scale utilized in the present study is intended to measure relatively minor day-to-day experiences of interpersonal maltreatment. These experiences are considered to be ongoing, and more common in daily life than more major experiences of discrimination (e.g. being denied a job or loan) (183). Furthermore, evidence suggests that responses to the Everyday Discrimination Scale remain stable overtime (196). Our study adds to the growing literature supporting an association between these relatively minor, ongoing experiences of discrimination to negative effects on the cardiovascular health of women.

Additionally, maximum carotid plaque height (or thickness) has been identified as an important predictor of risk of a cardiovascular event in MESA, and the Northern Manhattan Study (253, 254). Plaque height is a dynamic characteristic that may experience significant changes over a relatively short period of time; for example, following initiation of statin therapy (252). In our analyses, the effect of high perceived everyday discrimination on maximum carotid plaque height was nearly 1.5 times that of lipid-lowering medication (B=0.30 $p<0.01$ vs. B=-0.18, p=0.13, respectively), and our results were consistent even after exclusion of women using lipid-lowering drugs. These findings highlight an important relationship between everyday discrimination and subclinical CVD, and add to the growing evidence of the negative impact that

day-to-day discriminatory experience may have on cardiovascular health of women across racial/ethnic groups.

The associations between everyday discrimination and carotid atherosclerosis in our sample did not differ by race. These findings are consistent with prior studies reporting no differences in the associations between everyday discrimination and CVD risk factors among women of various racial/ethnic groups. For example Lewis et al. have previously reported no differences in the associations between everyday discrimination and poor sleep (176) as well as greater visceral adiposity (189) among multi-ethnic SWAN participants. Furthermore, in their 2009 review of the health effects of perceived discrimination, Pascoe and Richman report that the evidence suggests perceived discrimination has similar negative health consequences regardless of racial/ethnic background (175).

We did not find any significant associations between everyday discrimination with plaque presence, total area, minimum GSM, and calcification. However, it is worth mentioning that while the results were not statistically significant, the effects of high everyday discrimination on total plaque area and minimum GSM among women with carotid plaque trended in the expected direction (greater total plaque area and lower minimum GSM). When considered in conjunction with the observed relationship between everyday discrimination and plaque height, these results suggest that everyday discrimination may be a better predictor of carotid plaque progression than initial lesion development midlife women.

We also conducted a mediation analysis in order to determine whether the association between high everyday discrimination and greater plaque height is mediated by circulating inflammatory burden. Inflammatory burden, operationalized as factors score (composed of CRP, IL-6, and fibrinogen) based on a factor derived from a previous EFA in our study sample, was

identified as partial mediator that accounts for an estimated 31% of the relationship between everyday discrimination and maximum plaque height. These results are consistent with past studies linking everyday discrimination to higher circulating inflammation (273), and our previous findings in the MS Heart sample linking higher inflammatory burden to greater carotid plaque thickness. However, to our knowledge, this study is the first to identify inflammation as a mediator of the relationship between everyday discrimination and plaque height. Our results suggest that inflammatory burden may be a mechanism through which everyday discrimination influences plaque height; however, additional longitudinal studies are required in order to confirm this relationship.

This study has several limitations. First, the cross-sectional nature of the study does not allow us to draw conclusions about temporality or causality of the identified relationships. Our sample includes few Asian and Hispanic women, and therefore, the results may not be generalizable to those groups. Approximately 40% (n=120) of participants in the sample reported experiencing high discrimination and were asked to elaborate regarding the suspected reason to which they attributed their experiences of discrimination, and the majority of women chose to respond “other” and fill in a written response. Therefore, there were not enough women who attributed their experience of discrimination to the provided reasons (e.g. race, age, physical appearance) for us to determine if specific types of discrimination were more important in terms of subclinical CVD compared to others. Lastly, minimum GSM of plaque is only a surrogate marker of plaque components, so we are unable to draw any conclusions regarding the potential associations between everyday discrimination and specific components of carotid plaque that may put women at risk of a cardiovascular event.

Strengths of our analysis include that participants were asked to complete questionnaires for a variety of psychosocial risk factors (e.g. depression, anxiety, and social support) that are strongly associated with discrimination; therefore we were able to adjust for psychosocial risk factors in our analyses. Our results remained significant after taking these measures, as well as other traditional CVD risk factors, into account. Additionally, we conducted sensitivity analyses excluding those on lipid-lowering medications since they are known to affect plaque height, and our results remained consistent after excluding those participants.

In sum, we report that high levels of everyday discrimination are associated with greater maximum carotid plaque height among midlife women without clinical CVD after adjustment for demographics, as well as traditional CVD and psychosocial risk factors. Furthermore, we found that circulating inflammatory burden is a mediator of the relationship between high everyday discrimination and maximum carotid plaque height. These results suggest that increased inflammatory burden and greater atherosclerotic plaque height may be mechanisms through which experiences of discrimination increase women's cardiovascular risk at midlife. Future studies are warranted to evaluate the temporality of these relationships, and identify strategies to that may be useful in combatting both experiences of everyday discrimination and the associated negative health consequences in women.

5.6 TABLES AND FIGURES

Table 5.1Characteristics of MS Heart Study Sample by Perceived Discrimination Severity

Characteristic	Low Perceived Discrimination (n=184)	High Perceived Discrimination (n=120)	p-value
Age	55 (52-57)	55 (51-57)	0.8146
Race (white)	130 (73%)	86 (72%)	0.8251
Education			0.4199
High School/Some College	79 (43%)	50 (42%)	
College Graduate	57 (31%)	31 (26%)	
Post-Graduate	48 (26%)	39 (33%)	
Hard to Pay for Basics			0.0001
Not Hard	139 (77%)	67 (56%)	
Somewhat Hard	38 (21%)	40 (33%)	
Very Hard	4 (2%)	13 (11%)	
BMI (kg/m^2)	27 (24-33)	28 (26-33)	0.3834
Self-Rated Health			0.0009
Excellent/Very Good	143 (78%)	72 (60%)	
Good/Fair	41 (22%)	48 (40%)	
Post-menopausal	154 (84%)	101 (84%)	0.9131
Physiologic Hot Flashes	90 (49%)	62 (52%)	0.6388
SBP (mmHg)	118 (110-128)	119 (111-128)	0.4364
DBP (mmHg)	69 (63-77)	71 (64-76)	0.4817
Diabetes	5 (3%)	3 (3%)	0.9694
HOMA	2.1 (1.7-3.2)	2.4 (1.7-3.1)	0.4647
HDL (mg/dL)	61 (53-71)	64 (51-73)	0.8293
LDL (mg/dL)	125 (106-151)	136 (109-156)	0.2144
Triglycerides (mg/dL**)**	96 (73-127)	97 (70-139)	0.7777
CESD Total	3 (1-8)	9 (5-15)	<0.0001
State Anxiety Score	28 (24-35)	34 (26-39)	<0.0001
Trait Anxiety Score	29 (25-36)	37 (31-44)	<0.0001
PTSD	2 (1%)	5 (4%)	0.1171
Overall Support	44 (38-46)	38 (33-44)	<0.0001
PSQI	5 (3-6)	6 (4-8)	0.0002
Berlin Sleep High Risk	40 (22%)	47 (40%)	0.0010
CRP (mg/L)	1.2 (0.6-2.8)	1.4 (0.7-3.1)	0.3029
IL6 (pg/L)	1.4 (1.0-2.2)	1.5 (0.9-2.2)	0.7598
Fibrinogen (mg/mL)	335.1 (290.1-382.3)	342.6 (294.2-379.5)	0.9771
D-dimer (ng/mL)	244.5 (171.2-380.4)	227.2 (173.5-331.8)	0.7136
Factor VII (mg/dL)	1.2 (1.1-1.4)	1.3 (1.1-1.5)	0.5164
vwAntigen (IU/dL)	1.4 (1.1-1.7)	1.2 (0.9-1.7)	0.0462
Medication Use:			
Anti-hypertensive	31 (17%)	17 (14%)	0.5309

Table 5.1 Continued

Lipid-lowering	29 (16%)	10 (8%)	0.0584
Anti-coagulant	19 (10%)	11 (9%)	0.7404
Anti-diabetic	7 (4%)	3 (3%)	0.7451
Anti-depressant	4 (2%)	2 (2%)	1.0000
Anti-anxiety	4 (2%)	1 (1%)	0.6515
Any Anti-inflammatory	30 (16%)	24 (20%)	0.4099
Sleep	8 (4%)	6 (5%)	0.7909

[a] Median (IQR) provided for all continuous variables, Kruskal-Wallis test used to compare groups.

[b] N (%) provided for categorical variables, Chi-square test used to compare groups

Table 5.2Attributions of Discrimination Among Those Who Perceive that They are Frequently Discriminated Against

Discrimination Attribution	Overall (n=120)	Non-White (n=34)	White (n=86)
Race	14 (12%)	14 (42%)	0
Ethnicity	0	0	0
Gender	7 (6%)	1 (3%)	6 (7%)
Age	17 (15%)	2 (6%)	15 (18%)
Income Level	13 (12%)	3 (9%)	10 (12%)
Language	0	0	0
Physical Appearance	12 (10%)	4 (12%)	8 (10%)
Sexual Orientation	0	0	0
Other	53 (46%)	9 (27%)	44 (53%)
No Response	4 (3%)	1 (2%)	3 (3%)

Table 5.3 Plaque Outcomes Among all MS Heart Participant and Comparison of Plaque Outcomes between Those with Low and High Perceived Discrimination

Outcome	All Participants (n=304)	Low Perceived Discrimination (n=184)	High Perceived Discrimination (n=120)	p-value
Any Plaque	139 (46%)	88 (48%)	51 (43%)	0.3181
Total # of Plaques	0 (0-1)	0 (0-1)	0 (0-1)	0.5598
Total Plaque Area (mm^3)	17.49 (9.87-36.51)	15.50 (9.18-24.70)	22.41 (14.05-31.32)	0.0249
Minimum GSM	53. 61 (39.82-67.39)	53.61 (39.82-67.40)	53.61 (41.29-67.38)	0.9712
Max Height (mm)	2.02 (1.71-2.46)	1.87 (1.63-2.26)	2.24 (1.90-2.78)	0.0016
Calcification	46 (33%)	31 (25%)	15 (29%)	0.4825

[a] Median (IQR) provided for all continuous variables, Kruskal-Wallis test used to compare those with and without high levels of perceived discrimination.
[b] N (%) provided for categorical variables, Chi-square test used to compare those with and without high levels of perceived discrimination

Table 5.4 Associations of Perceived Discrimination with Measures of Plaque Presence and Burden

Measure of Discrimination	Plaque Presence			Total Number of Plaques			Total Plaque Area		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Everyday Discrimination Score	0.98 (0.93,1.03)	0.98 (0.93,1.03)	0.98 (0.92,1.04)	1.03 (1.00,1.06)*	**1.03 (1.00,1.07)‡**	1.02 (0.99,1.06)	0.40 (0.41)	0.47 (0.45)	0.19 (0.44)
High Discrimination Score	0.97 (0.85,1.09)	0.97 (0.85,1.10)	0.96 (0.82,1.12)	1.06 (0.99,1.14)	**1.09 (1.01,1.18)‡**	1.08 (0.99,1.17)	1.69 (1.02)	2.19 (1.10)	1.74 (1.08)
High Discrimination (yes/no)	1.27 (0.80,2.01)	0.80 (0.49,1.30)	0.75 (0.44,1.28)	1.17 (0.87,1.57)	1.17 (0.87,1.58)	1.08 (0.79,1.49)	5.34 (3.73)	5.58 (3.96)	3.56 (3.94)

[a] Beta estimates (se) and odds ratios (95% CI) provided where appropriate.

[b] ‡ indicates significant at α=.05 level. * indicates borderline significance ($0.05 < p < 0.06$)

[a] Model 1: Unadjusted

[b] Model 2 Covariates: Age, race, difficulty paying for basics

[c] Model 3 Covariates: Age, race, difficulty paying for basics, SBP, BMI, diabetes medication, lipid-lowering medication, physiologic hot flashes, menopausal status, depressive symptoms, social support, and PSQI

Table 5.5 Associations of Perceived Discrimination with Plaque Characteristics

Measure of Discrimination	Minimum GSM			Maximum Height			Presence of Calcification		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Everyday Discrimination Score	-0.49 (0.45)	-0.67 (0.50)	-0.58 (0.51)	0.01 (0.01)	0.00 (0.01)	0.00 (0.01)	0.92 (0.85,1.01)	0.92 (0.84,1.12)	0.91 (0.81,1.01)
High Discrimination Score	-1.27 (1.12)	-1.79 (1.25)	-2.02 (1.27)	**0.07 (0.03)‡**	**0.08 (0.03)‡**	0.07 (0.04)*	0.80 (0.62,1.02)	0.83 (0.63,1.09)	0.81 (0.60,1.10)
High Discrimination (yes/no)	-0.85 (4.14)	-2.39 (4.49)	-4.40 (0.35)	**0.31 (0.11)‡**	**0.30 (0.12)‡**	**0.29 (0.14)‡**	1.31 (0.62,2.75)	0.85 (0.36,2.01)	0.87 (0.34,2.24)

[a] Beta estimates (se) and odds ratios (95% CI) provided where appropriate.

[b] ‡ indicates significant at α=.05 level. * indicates borderline significance ($0.05 < p < 0.06$)

[c] Model 1: Unadjusted

[d] Model 2 Covariates: Age, race, difficulty paying for basics

[e] Model 3 Covariates: Age, race, difficulty paying for basics, SBP, BMI, diabetes medication, lipid-lowering medication, physiologic hot flashes, menopausal status, depressive symptoms, social support, and PSQI

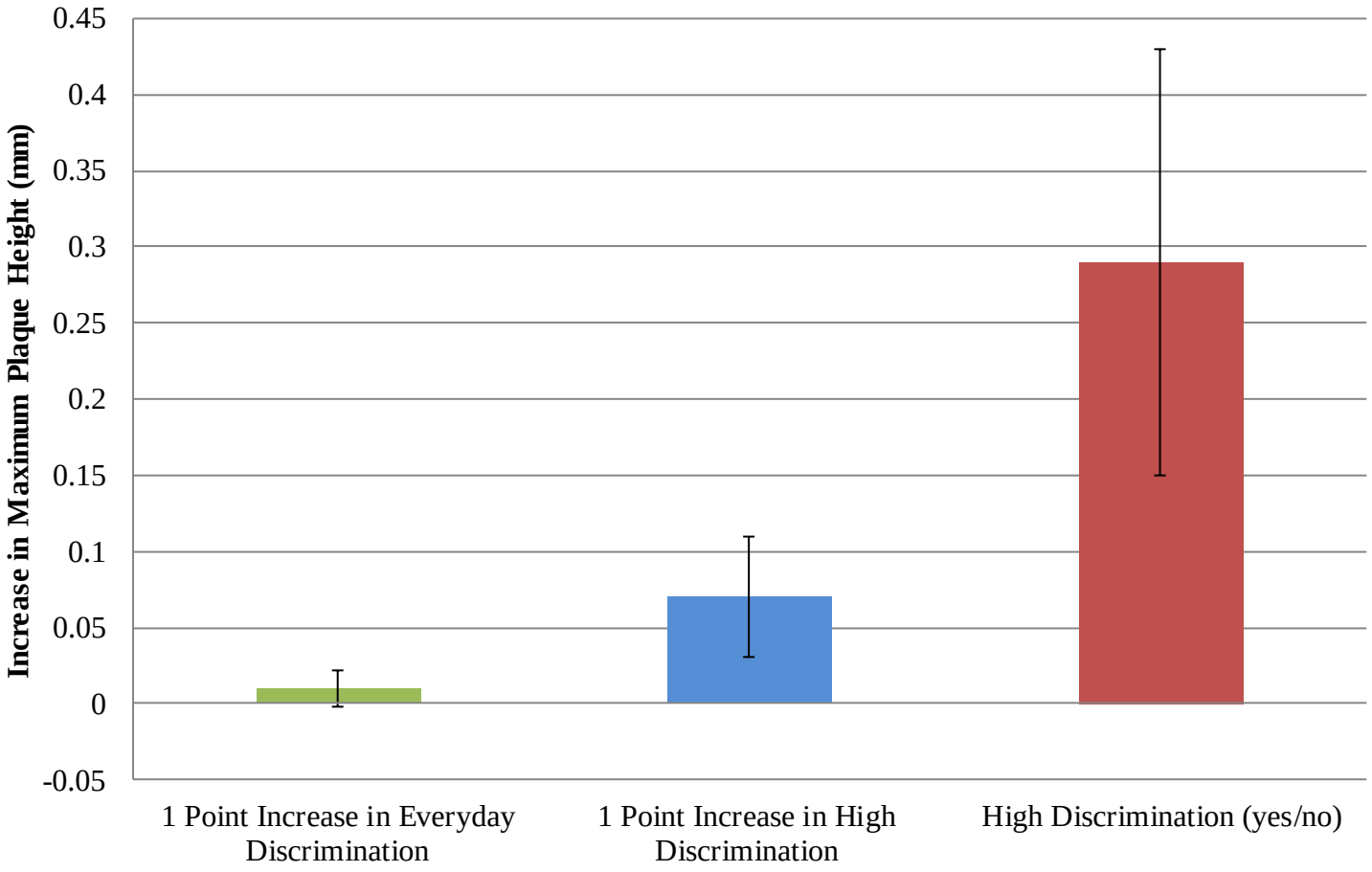


Figure 5.1Associations of Perceived Discrimination with Maximum Plaque Height

[a] Beta estimates (se) provided

[b] ‡ indicates significant at α=.05 level. * indicates borderline significance (0.05< *p* <0.06)

[c] Model 2 Covariates: Age, race, difficulty paying for basics

[d] Model 3 Covariates: Age, race, difficulty paying for basics, SBP, BMI, diabetes medication, lipid-lowering medication, physiologic hot flashes, menopausal status, depressive symptoms, social support, and PSQI

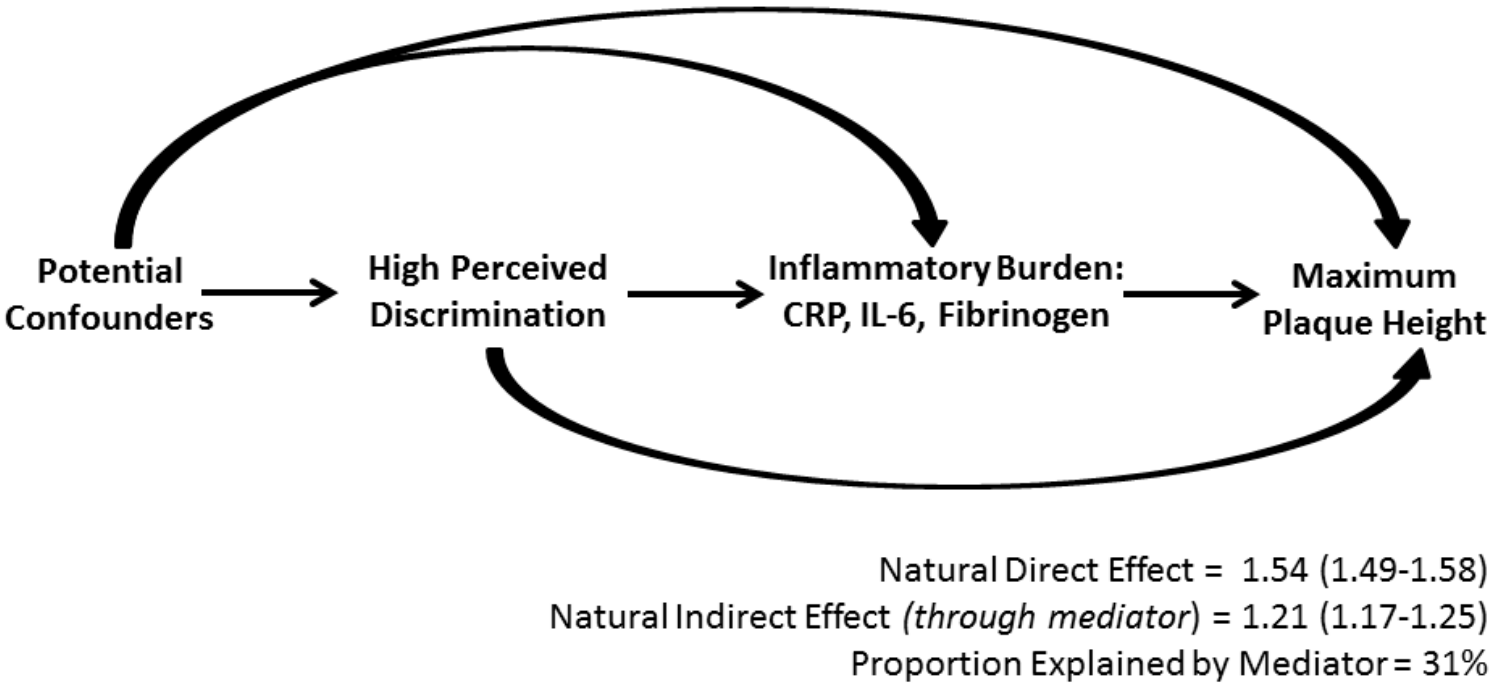


Figure 5.2 Inflammatory Burden as a Mediator of the Relationship between Perceived Discrimination and Maximum Plaque Height

6.0 DISSERTATION CONCLUSIONS

6.1 MAJOR FINDINGS

The analyses presented in this dissertation contribute significantly to the existing body of knowledge regarding the associations of markers of inflammatory and coagulation burden with subclinical atherosclerosis in the carotid and femoral arteries. Using exploratory factor analyses, this work consistently identified two distinct factors, one factor representing chronic low-grade inflammation and another factor representing coagulation in two different populations: community-living older adults and midlife women. The identified factors were differentially associated with B-mode ultrasound-derived measures of plaque presence, burden, and characteristics within the carotid and femoral arteries of the aforementioned populations.

In the community-living, healthy older adults of the San Diego Population Study, we found that the factor representing coagulation burden, comprised of PTX-3 and D-dimer, was initially associated with measures of femoral plaque presence and burden in unadjusted regression models, while the inflammatory factor, comprised of CRP, IL-6, and fibrinogen, was not significantly associated with any measures of plaque presence burden or characteristics before or after adjustment for covariates. The association of the factor representing coagulation with plaque presence and burden in these unadjusted models suggests that coagulation burden may be more important in regards to femoral plaque development than inflammatory burden.

This conclusion is supported by the evidence documenting a hypercoagulable state among those with PAD (281), and that higher coagulation burden is a predictor of PAD severity (43). It is also possible that the coagulation markers measured in this analysis were superior markers of atherosclerotic processes in this population compared to the inflammatory markers measured in this study.

Among midlife women in the MS Heart Study, we again identified two distinct factors following an exploratory factor analysis: an inflammatory factor comprised of CRP, IL-6, and fibrinogen as well as a coagulation factor comprised of D-dimer and vwAntigen. There were no significant associations between either factor with the measures of plaque presence, burden, or characteristics among the entire MS Heart sample. However, we identified relationships between the inflammatory and coagulation factors with measures of carotid plaque burden that were moderated by BMI. Among women with BMI < 25kg/m^2, higher levels of the factor representing chronic low-grade inflammation were associated with greater total plaque area after adjustment for covariates, and the factor representing coagulation burden was associated with a greater total number of plaques after adjustment for covariates. There were no significant associations between either factor with the measures of plaque burden among women with BMI ≥25kg/m^2. The lack of association between either factor and plaque burden among women with BMI > 25 kg/m^2 may be due to a combination of factors including that women with higher BMI may have been already exposed to some levels of chronic low-grade inflammation due to greater adiposity Additionally, the strict exclusionary criteria of the MS Heart study did not allow for the inclusion of women with numerous chronic diseases including history of heart disease, stroke, arrhythmia, and several cancers. Therefore, it is possible that the MS Heart participants considered

overweight or obese may be uniquely healthier compared to overweight or obese women in the general population.

Additionally, we identified several relationships between the inflammatory and coagulation factors with carotid plaque characteristics that were moderated by time since FMP in this sample. Among women with less than one year since their FMP, higher inflammatory burden was associated with greater maximum plaque height and higher coagulation burden was associated with lower maximum plaque height after adjustment for covariates. Among women with more than one year since their FMP, higher inflammatory burden was associated with lower minimum GSM after covariate adjustment. These results support our hypothesis that greater inflammatory burden is associated with carotid plaque characteristics indicative of potentially less stable plaque; however, they also indicate that the relationship between circulating inflammatory burden and plaque characteristics may differ throughout the menopausal transition. A previous analysis conducted in the Study of Women's Health Across the Nation (SWAN), found that lipid changes are differentially related to subclinical measures of atherosclerosis in the years leading up to, immediately preceding, and immediately following the FMP (282). Similar to the results of our analysis, SWAN investigators found that greater increases in low-density lipoprotein cholesterol were related to greater carotid plaque burden within the year following the FMP, but not at other time periods throughout the menopausal transition. Some evidence suggests that estrogens exert anti-inflammatory effects throughout the vasculature, and one possible explanation for these findings is that a dramatic drop in estrogen levels following the FMP may result in greater vascular inflammation leading to changes in plaque (266, 267); however, further longitudinal studies are necessary to investigate these hypothesized relationships.

In the third analysis of this dissertation, we found a significant association between high levels of perceived everyday discrimination and greater maximum carotid plaque height among midlife women in the MS Heart study. As we hypothesized, this relationship was partially mediated by inflammatory burden, specifically the inflammatory factor representing chronic low-grade inflammatory burden that we identified via exploratory factor analysis. These findings are consistent with the growing evidence that chronic low-grade inflammation is a mechanism through which psychosocial stress may influence CVD (152, 153). The associations observed in our analyses were consistent across racial groups. And while no statistically significant associations were observed between the inflammatory and coagulation markers with the other plaque characteristics (i.e. minimum GSM and calcification) considered in this analysis following covariate adjustment, these relationships trended in the hypothesized direction.

When considering the findings of these three analyses together, our results suggest that that B-mode ultrasound may have more value in the characterization of plaques that are more likely to be considered complex or high risk such as those in the carotid arterial bed compared to the femoral arterial bed (89). However, it is important to note that one of the major limitations of the femoral artery plaque analysis is that the femoral images of the SDPS were not originally collected with the intention of identifying and characterizing plaque. Therefore, the quality of some femoral plaque images may have limited our ability to accurately determine plaque characteristics. Furthermore, the relatively low burden of femoral artery atherosclerosis in the older adults of the San Diego Population Study may have limited our ability to identify relationships between circulating inflammatory and coagulation burden with femoral plaque measures. It is possible that the results of this analysis may differ had these relationships been examined within a more high risk population with more advanced femoral atherosclerosis.

Overall, the results of these analyses also suggest that higher coagulation burden may be more important in terms of the development of femoral atherosclerosis compared to inflammatory burden, while inflammatory burden seems to be of greater importance in regards to carotid plaque development and progression. These conclusions are supported by previously mentioned evidence documenting a hypercoagulable state among those with PAD (281), and that higher coagulation burden is a predictor of PAD symptoms and severity (43). Similarly, these findings are supported by several studies reporting a consistent relationship between inflammatory markers and the initiation and progression of atherosclerotic plaque in the carotid artery (39, 44, 45, 56).

Due to the fact that these analyses examined several plaque outcomes (i.e. plaque presence, total number of plaques, total plaque area, minimum GSM, maximum plaque height, and calcification) but only found consistent relationships between our predictors and maximum plaque height, it is important to consider what differences may exist between maximum plaque height and the various other plaque measures. Maximum plaque height, sometimes referred to as plaque thickness, is a dynamic characteristic of plaque that is capable of experiencing changes over a relatively short period of time. Rollefstad et al. have previously reported significant regression of plaque height in the 18 months following the initiation of statin therapy (252). Furthermore, this effect was not dependent upon a reduction in LDL-cholesterol, and the authors suggest that it may be primarily driven by the anti-inflammatory effects of statins. It is possible that maximum plaque height is more susceptible to the influence of inflammatory factors compared to the other plaque measures and characteristics we collected. Furthermore, plaque height may simply have the capacity to change over a shorter period of time compared to the other measures we examined such as GSM and calcification. Additionally, when considering

these findings it is important to note that maximum plaque height has been identified as an important predictor of risk of a cardiovascular event in MESA, and the Northern Manhattan Study (253, 254), and shows additive value in the prediction of vascular events and CHD beyond cIMT and coronary artery calcium, respectively (283, 284).

This dissertation has several important limitations. As mentioned above, the samples studied in this dissertation are unique and this may have influenced the results of our analyses. The SDPS is a relatively healthy aging cohort with a low burden of femoral artery atherosclerosis. The MS Heart study is comprised of non-smoking women without existing CVD and had strict criteria excluding women with a history of heart disease, stroke, arrhythmia, and the use of numerous medications. Therefore, it is possible that our analyses are not generalizable, and may differ if conducted in individuals more representative of the general population. Neither of the studies utilized in this dissertation were designed with the intention of studying the effects of inflammation cardiovascular disease. Therefore, limited measures of inflammatory and coagulation biomarkers were available for these analysis. It is possible that there are other markers of inflammation and coagulation that are more important in terms of plaque development and progression than those that were included in these analyses. Additionally, circulating levels of inflammatory and coagulation markers may be difficult to accurately ascertain from a single time point as was done in this study due to the instability of biomarkers and their variability over time. Due to the cross-sectional nature of these analyses, our ability to draw conclusions about the temporality of the relationships that we observed is limited. Although B-mode ultrasound is a relatively inexpensive and feasible method of visualizing plaque in the superficial arteries, and is particularly useful in large epidemiologic studies, it is limited in regards to plaque characterization. The fact that this dissertation utilized B-mode ultrasound

images for plaque characterization does not allow us to draw conclusions regarding the potential relationships between inflammatory and coagulation burden with specific components of atherosclerotic plaque.

6.2 PUBLIC HEALTH SIGNIFICANCE & FUTURE DIRECTIONS

Globally, CVD is the leading cause of death, and the majority of cardiovascular morbidity and mortality is due to atherosclerotic CVD (5). The rupture or erosion of atherosclerotic plaque is the primary cause of MI and ischemic stroke, which affect an estimated 1.4 million U.S. adults annually. Inflammation is one of the driving forces contributing to atherosclerosis (26), and the associations between greater levels of circulating inflammatory markers with CVD are well-established (208, 216, 285). Recently, the results of a randomized controlled trial published in the *New England Journal of Medicine* reported that adults who received treatment with a monoclonal antibody aimed at reducing inflammation had a significantly lower hazard of myocardial infarction, nonfatal stroke, or cardiovascular death over 48 months of follow-up (286). However, the mechanisms through which inflammation may influence cardiovascular risk are not entirely understood. Studying the relationships between inflammation and measures of atherosclerotic plaque in subclinical stages can help shed light on the mechanisms through which inflammation may influence one's cardiovascular risk.

Midlife is a dynamic time for women's cardiovascular health during which atherosclerosis is accelerated (239, 240) and risk of a cardiovascular event increases (242). This dissertation reports that higher circulating inflammatory burden are associated with carotid plaque characteristics that are indicative of potentially less stable plaques in midlife women, and

suggests that inflammatory burden may contribute to CVD risk in midlife women by negatively influencing carotid plaque characteristics. However, additional longitudinal analyses in generalizable samples of midlife women are needed in order to ascertain whether chronic low-grade inflammation influences carotid plaque characteristics and subsequently increases cardiovascular risk. Moreover, we found that the associations between higher inflammatory burden and plaque characteristics indicative of instability were not consistent across the menopausal transition. These results suggest that inflammation may influence to atherosclerosis differently throughout menopause, and warrant further longitudinal analyses in order to determine how inflammation may contribute to changes in atherosclerotic plaque characteristics and components at different times throughout the menopausal transition.

Among women, psychosocial stress is a well-established risk factor associated with increased CVD risk. The influence of chronic psychosocial stress on atherosclerosis is particularly important to study among women because women manifest CVD different than men, and inflammation, which is hypothesized to be a mechanism through which stress influence CVD risk, is a better predictor of CHD among women (155). Additionally, psychosocial stress may contribute to the vast racial/ethnic disparities in CVD observed in the U.S. (183).

This dissertation examined the associations between one form of psychosocial stress, everyday discrimination, with measures of carotid plaque presence, burden, and characteristics among midlife women. As described in previous sections, the literature regarding the associations between everyday discrimination and subclinical CVD is limited to few subclinical cardiovascular outcomes, and the results are mixed. This dissertation is the first study to report on associations between everyday discrimination with carotid plaque burden and characteristics. We found that high levels of everyday discrimination were significantly associated with higher

maximum carotid plaque height among midlife women. Furthermore, we report that inflammatory burden is a partial mediator of this relationship. These results were consistent among women of different racial groups. Plaque height is a dynamic plaque characteristic that has the ability to change over a relatively short period of time (252), and has additive value to cIMT and traditional risk factors in regards to predicting risk of a CVD event (287). Our results support the hypothesis that inflammation is a mechanism linking everyday discrimination and cardiovascular disease among women; however, longitudinal analyses conducted among generalizable groups of women are needed in order to determine temporality and causality. Lastly, these results are consistent with the existing literature suggesting that perceived everyday discrimination is associated with subclinical CVD among women of various racial and ethnic groups, and demonstrate the need for additional studies aimed at mitigating the negative health effects of discrimination among women.

APPENDIX: SUPPLEMENTARY DATA

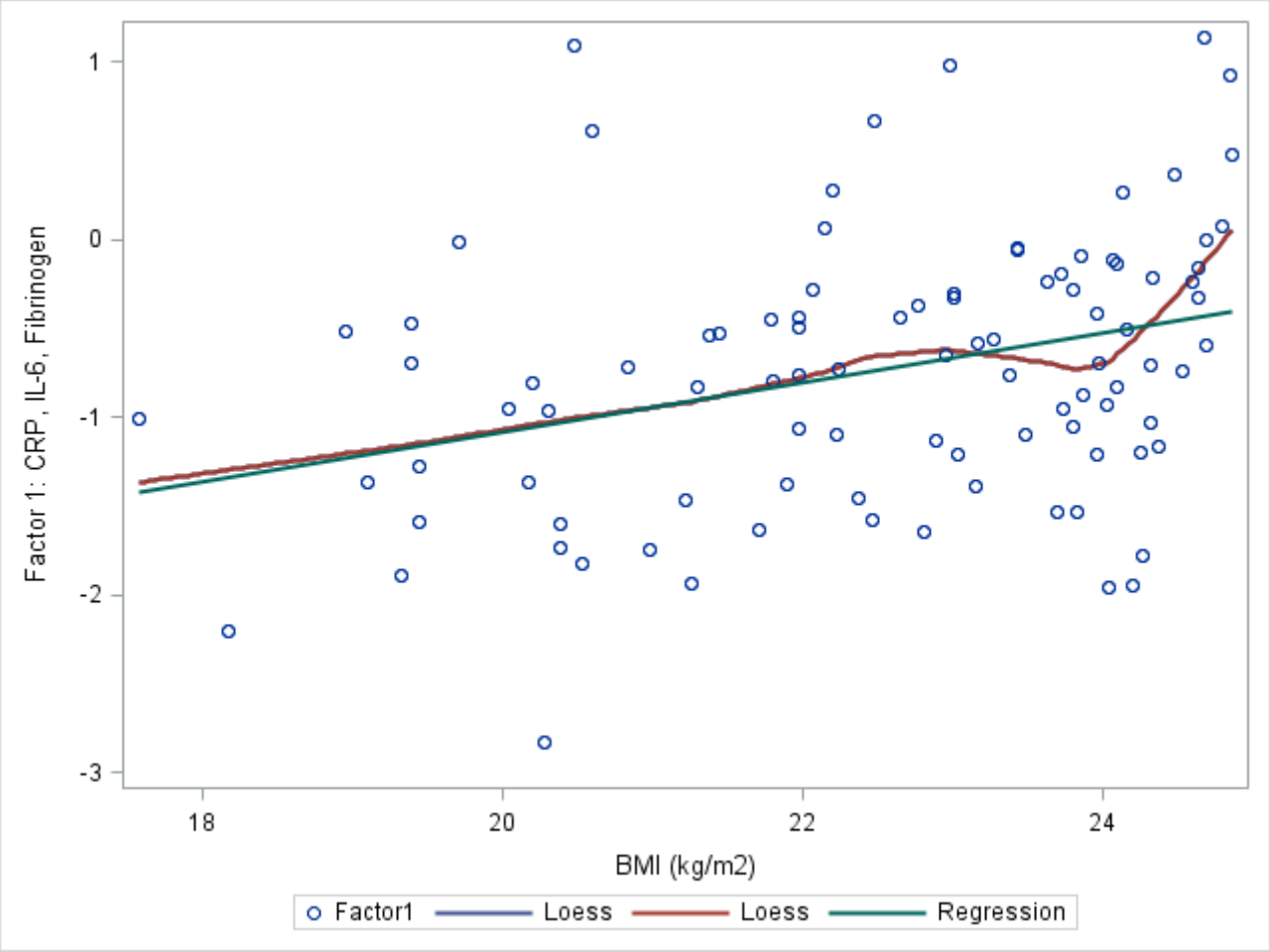


Figure 6.1 Relationship between Factor 1 and BMI among those with BMI <25 kg/m^2

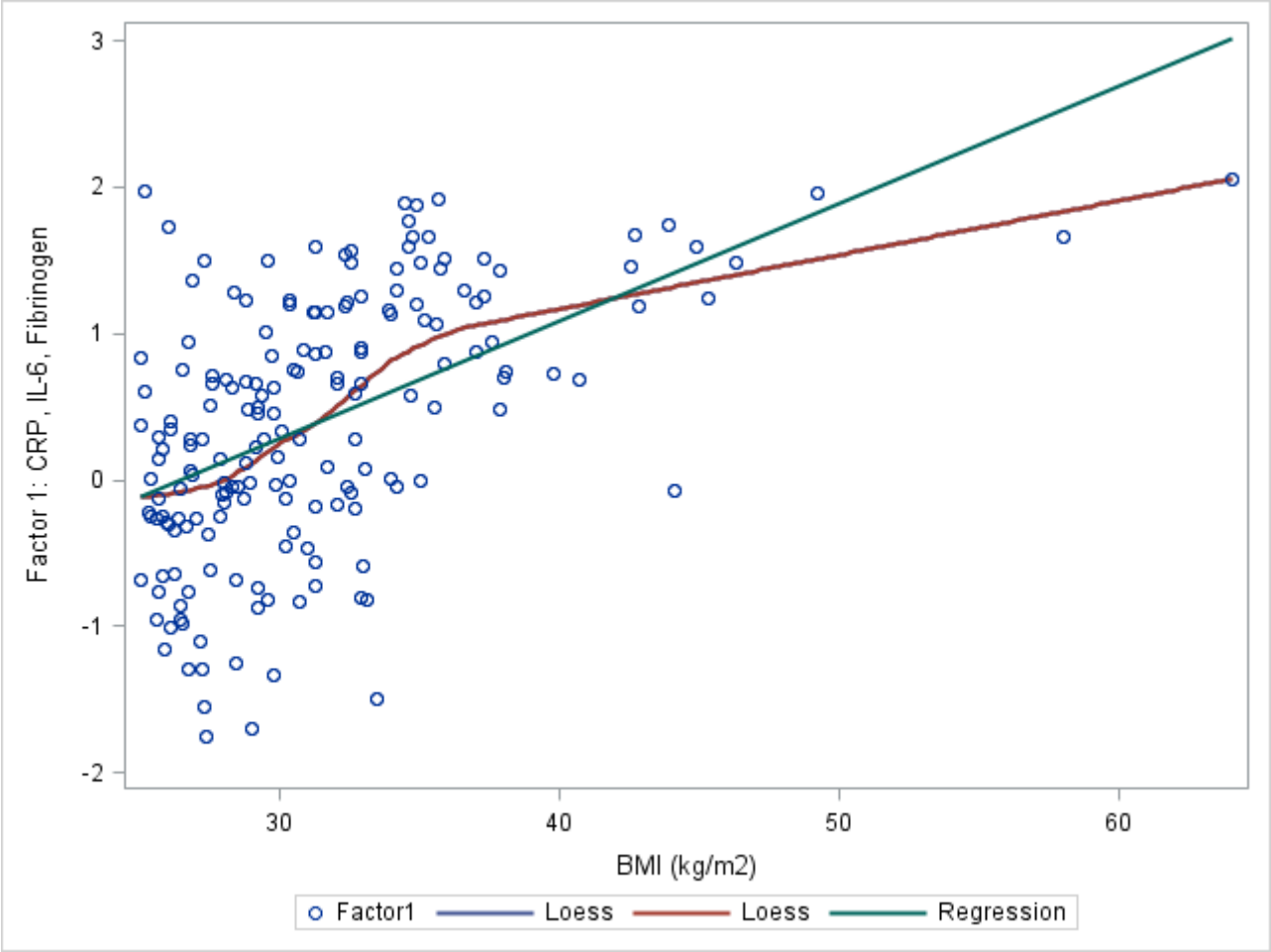


Figure 6.2 Relationship between Factor 1 and BMI among those with BMI ≥25 kg/m^2

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