INFLAMMATION IS ASSOCIATED WITH SUBCLINICAL ATHEROSCLEROSIS

by

Vinay Mehta

BA, Washington and Jefferson College, 2000

MS, SUNY at Albany, 2002

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2006

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This dissertation was presented

by

Vinay Mehta

It was defended on

April 3, 2006

and approved by

Sheryl Kelsey, PhD, Professor, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Anne B. Newman, MD, MPH, Associate Professor, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Jen Brach, PhD,PT, Assistant Professor, Department of Physical Therapy, School of Health and Rehabilitation Sciences, University of Pittsburgh

Howard Rockette, PhD, Professor and Chair, Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

Dissertation Advisor: Kim Sutton-Tyrrell, DrPH, MPH, Professor and Vice Chair for Academics, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

INFLAMMATION IS ASSOCIATED WITH SUBCLINICAL ATHEROSCLEROSIS

Vinay Mehta, PhD

University of Pittsburgh, 2006

Cardiovascular disease increases with age and menopause. Atherosclerosis directly contributes to cardiovascular disease and subclinical markers of atherosclerosis are noninvasive methods that help to detect early vascular changes. Thus, risk factors associated with markers of subclinical atherosclerosis may be targeted for interventions in individuals at high risk of developing cardiovascular disease. C-Reactive Protein (CRP), a marker of inflammation, has been found to be associated with cardiovascular events in a large number of populations. However, studies examining the association between CRP and markers of subclinical atherosclerosis have been limited.

The cross-sectional association between CRP and central arterial stiffness, assessed by carotid-femoral pulse wave velocity (PWV), was tested in a biracial (Caucasian and Black) cohort of 154 women transitioning through menopause within the Study of Women's Health Across the Nation (mean age 50.8 ± 2.6; 44.2% Black). After adjustment for age, systolic blood pressure, ethnicity, study site, waist circumference, diastolic blood pressure, and physical activity, the mean pulse wave velocity increased with increasing CRP tertiles (758.5 cm/s, 784.5 cm/s, 861.7 cm/s; p for trend = .01). Furthermore, the association was stronger in women later in their transition compared to women earlier in their transition (p for interaction = .02).

The cross-sectional association between CRP and systemic arterial stiffness, assessed by brachial artery distensibility, was tested in 1069 women of the same cohort (mean age 53.6 ± 2.6 years). After adjustment for confounders, the mean distensibility decreased with increasing

tertiles of CRP (p for trend = .001). This pattern was similar in women of different ethnic groups and stages of the menopausal transition.

The association between CRP and three year incident peripheral arterial disease (PAD), assessed by the ankle-brachial index (ABI), was tested in a biracial (Caucasian and Black) cohort of 1918 older adults within the Health, Aging, Body, and Composition Study (mean age 73.6±2.9; 40.3% Black). Participants in the top tertile of CRP had an increased odds of developing PAD compared to those in the bottom tertile (OR=1.87, 95% CI= 1.22 to 2.88).

Given the high prevalence of cardiovascular disease, finding risk factors associated with early vascular changes in high risk populations is of public health importance.

TABLE OF CONTENTS

PREFACE ..... **XI**

1.0 GENERAL INTRODUCTION **1**

1.1 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE **1**

1.2 ATHEROSCLEROSIS **2**

1.3 SUBCLINICAL ATHEROSCLEROSIS **3**

1.3.1 Arterial Stiffness **4**

1.3.2 Pulse Wave Velocity **4**

1.3.3 Brachial Artery Distensibility (Distensibility) **5**

1.3.4 Peripheral Arterial Disease **6**

1.3.5 Ankle-Brachial Index **6**

1.4 INFLAMMATION **7**

1.4.1 C-Reactive Protein **7**

1.5 SPECIFIC AIMS **9**

1.6 REFERENCES FOR CHAPTER 1 ..... **11**

2.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH CENTRAL ARTERIAL STIFFNESS IN A COHORT OF WOMEN TRANSITIONING THROUGH MENOPAUSE **19**

2.1 ABSTRACT **20**

2.2 INTRODUCTION **21**

2.3 METHODS **22**

2.3.1 Study Population **22**

2.3.2 Cardiovascular Risk Factors **23**

2.3.3 Hormones **24**

2.3.4 Physical Measures/Activity **24**

2.3.5 Menopausal Status **25**

2.3.6 Aortic Pulse Wave Velocity 25
2.3.7 Statistical Methods 26
2.4 RESULTS 27
2.5 DISCUSSION 35
2.6 ACKNOWLEDGEMENTS 38
2.7 REFERENCES FOR CHAPTER 2 39
3.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH SYSTEMIC ARTERIAL STIFFNESS IN A MIDDLE AGED COHORT OF WOMEN 45
3.1 ABSTRACT 46
3.2 INTRODUCTION 47
3.3 METHODS 48
3.3.1 Study Population 48
3.3.2 Brachial Artery Distensibility 49
3.3.3 Cardiovascular Risk Factors 51
3.3.4 Physical Measures 51
3.3.5 Menopausal Status 52
3.3.6 Medication Use/Disease: 52
3.3.7 Statistical Methods 52
3.4 RESULTS 53
3.5 DISCUSSION 63
3.6 REFERENCES FOR CHAPTER 3 66
4.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH INCIDENT PERIPHERAL ARTERIAL DISEASE IN AN ELDERLY COHORT 71
4.1 ABSTRACT 72
4.2 INTRODUCTION 73
4.3 METHODS 74
4.3.1 Study Population 74
4.3.2 Inflammatory Markers 75
4.3.3 Ankle-Brachial Index 76
4.3.4 Outcome Definition 76
4.3.5 Covariates 77

4.3.6 Statistical Methods 77
4.4 RESULTS 78
4.5 DISCUSSION 88
4.6 ACKNOWLEDGMENT 91
4.7 REFERENCES FOR CHAPTER 4 92
5.0 OVERVIEW OF DISCUSSION 98
5.1 INFLAMMATION AND ARTERIAL STIFFNESS 99
5.2 INFLAMMATION AND PERIPHERAL ARTERIAL DISEASE 103
5.3 LIMITATIONS/FUTURE RESEARCH--ANALYSES 1 AND 2 105
5.4 LIMITATIONS/FUTURE RESEARCH—ANALYSIS 3 108
5.5 SUMMARY 110
5.6 PUBLIC HEALTH SIGNIFICANCE 111
5.7 REFERENCES FOR CHAPTER 5 112
APPENDIX A: LITERATURE REVIEW OF STUDIES EXAMINING ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND ARTERIAL STIFFNESS 119
APPENDIX B: LITERATURE REVIEW OF STUDIES EXAMINING ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND PERIPHERAL ARTERIAL DISEASE 126
BIBLIOGRAPHY 132

LIST OF TABLES

Table 2-1. Baseline Characteristics of the SWAN Heart Study Sample 30
Table 2-2. Relation of Cardiovascular Risk Factors and Aortic Pulse Wave Velocity 31
Table 2-3. Percent Increase in Aortic Pulse Wave Velocity for a One Unit Increase in CRP ... 32
Table 3-1. Continuous Descriptive Statistics For Dynapulse Analysis 57
Table 3-2. Categorical Descriptive Statistics For Dynapulse Analysis 58
Table 3-3. Comparison of Dynapulse Excluded and Included Samples..... 59
Table 3-4. Relation of Cardiovascular Risk Factors and Brachial Artery Distensibility 60
Table 3-5. Linear Regression Models Testing the Association Between Log-Transformed CRP and Brachial Artery Distensibility 61
Table 4-1. Continuous Baseline Characteristics of the Health ABC Study Sample..... 83
Table 4-2. Categorical Baseline Characteristics of the Health ABC Study Sample..... 84
Table 4-3. Comparison of Incidence of PAD Between the Top and Bottom Tertiles of Risk Factors..... 85
Table 4-4. Comparison of Incidence of PAD Between Categorical Risk Factors..... 86
Table 4-5. Odds Ratios (95% C.I.) Describing the Associations Between Inflammatory Markers and Incident PAD..... 87

LIST OF FIGURES

Figure 2-1. Adjusted Mean Pulse Wave Velocity by CRP Tertiles 33

Figure 2-2. Adjusted Mean Pulse Wave Velocity by CRP Tertiles Stratified By Menopausal Status 34

Figure 3-1. Adjusted Mean Brachial Artery Distensibility by CRP Tertiles 62

Figure 4-1. Diagram of Participants In the Study 81

Figure 4-2. Percent of Incident PAD by CRP Tertile and Race 82

PREFACE

The past four years have provided numerous opportunities for me to learn from mistakes and grow as a public health researcher. Although I have an inherent ability (or perhaps misfortune) of thinking analytically, the study of epidemiology has also taught me to think outside the box. Throughout my short tenure as a graduate student at the University of Pittsburgh, I have met several people who have influenced me in more ways than I ever imagined possible. With that, I would like to acknowledge the following people for their support of my arduous journey that has finally met the light at the end of the tunnel.

I would like to especially acknowledge Dr. Kim Sutton-Tyrrell for constantly pushing me (and my fellow students) to grow as a researcher. I would also like to thank my entire doctoral committee for providing tremendous input to me every step of the way. Dr. Sarah Brockwell and Dr. Janet Johnston, both of whom have served as my GSR mentors, deserve recognition for allowing me the opportunity to gain expertise in various statistical techniques and methods. I have learned so much as a GSR and am truly grateful for their continual trust and support in my work.

I would like to thank my friends for influencing me in so many ways and enabling me to release myself from my introverted personality. First, I would like to thank the members of the "zoo" for providing a break from work to discuss lighter issues. I would also like to thank Alicia for being someone that I can trust and talk to about anything. Finally, I must thank Ami for

being the most influential and inspirational person in my life during the past four years. Without her, I would not be where I'm at right now.

Finally, I would not be here today without the love and support of my family. They are the source of my motivation to succeed in life.

1.0 GENERAL INTRODUCTION

1.1 EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

Cardiovascular disease is the leading cause of death in the United States for both men and women among all ethnic groups[1]. Nearly 2500 Americans die of cardiovascular disease each day, which equates to one death every thirty seconds. The magnitude of the problem is so great that it claims more lives each year than the next four leading causes of death combined. According to the Centers for Disease Control (CDC), if all forms of cardiovascular disease were eliminated, life expectancy in the United States would increase by approximately seven years[1]. There is evidence of an increase in risk of cardiovascular disease with age and the menopause. In 2002, 68% of the deaths attributed to cardiovascular disease occurred in individuals over the age of 75[1]. Although the number one cause of death for both men and women, cardiovascular events increase in women after the menopause[2]. Along with the public health implications of the disease, there are economic consequences as well. The estimated direct and indirect cost of cardiovascular disease for 2006 is approximately $400 billion[1].

1.2 ATHEROSCLEROSIS

Most forms of cardiovascular disease are caused by atherosclerosis[3], which involves the accumulation of deposits of fatty substances, cholesterol, body cellular waste products, calcium, and fibrin within the interior lining of the artery. This buildup (commonly referred to as a plaque) can partially or totally block the flow of blood through the artery and consequently lead to cardiovascular outcomes including heart attack or stroke.

The lesions of atherosclerosis occur in arteries of different size and composition and can result in ischemia of the heart, brain, or extremities, leading to infarction[4]. The process of atherosclerosis may occur early in a person's life and manifest itself later on[5].

Atherosclerosis has recently been considered an inflammatory disease[3,4]. Each characteristic lesion of the atherosclerotic process represents a different stage in a chronic inflammatory process of the artery. If the process is uncontrolled, it will continually progress into more advanced stages of atherosclerosis. The initial step in the atherosclerotic process is damage to the endothelium[4], the lining of the innermost layer of the arterial wall that acts as an interface between the blood and the artery. This initial damage to the endothelium activates cell adhesion molecules, which allow the attachment of leukocytes to the endothelial wall[3]. Next, monocyte chemoattractant protein-1 (MCP-1) triggers the leukocytes to enter the intima (the innermost layer of the arterial wall) where they begin to accumulate lipids. After recruitment to the intima, the leukocytes transform into a lipid laden macrophage called a foam cell[6]. This is considered the first major lesion of the atherosclerotic process--the characteristic "fatty streak". The fatty streak will eventually evolve into a more complex atheroma through multiplication of smooth muscle cells that accumulate in the plaque[3]. The growing lesion starts to form a fibrous cap that causes the arterial lumen, the open space through which blood flows, to narrow and

restrict blood flow[4]. Rupture of the fibrous cap, caused by a constant influx of macrophages and inflammatory cells into the plaque, can lead to thrombosis and occlusion of the arteries resulting in clinical events such as heart attack and stroke.

Several factors contribute to damage of the endothelium including homocysteine overload, oxidation of low-density lipoprotein (LDL) cholesterol, and inflammation. Homocysteine augments toxicity to the endothelium[7], is prothrombotic[8], increases the synthesis of collagen[9], and decreases the bioavailability of the smooth muscle relaxant nitric oxide[10]. Additionally, it is thought to contribute to the oxidation of LDL cholesterol[11]. All of these effects are related to the progression of atherosclerosis. Oxidized LDL can contribute to the development of atherosclerosis through a number of mechanisms[12]. First, it can have direct cytotoxic effects on endothelial cells. Second, it can increase stimuli involved in the migration of leukocytes into the intima. Finally, it may cause proliferation and migration of cells that lead to a thickening of the intima and eventual occlusion of the vessel. The effects of inflammation on the development of atherosclerosis will be covered in the section discussing inflammation.

1.3 SUBCLINICAL ATHEROSCLEROSIS

The following section introduces the methods used in this dissertation to assess subclinical atherosclerosis. These methods are clinically useful because they provide noninvasive measures to detect early vascular change that may predispose certain individuals to cardiovascular disease.

1.3.1 Arterial Stiffness

The artery acts as a buffer by converting pulsatile blood flow from the heart into continuous flow to the cells of the body[13]. In an elastic artery, a portion of each cardiac stroke volume is retained in the proximal arteries during systole. During diastole, the elastic recoil of the arterial wall pushes this "remnant" to the periphery maintaining a critical buffering action. In a stiff artery, the absence of the elastic recoil causes the full stroke volume to be delivered during systole leading to increased pulse pressure and workload for the heart.

Arterial stiffness, an indicator of vascular age, is an important determinant of systolic blood pressure and pulse pressure and precedes isolated systolic hypertension[14]. It is thought to contribute to the age associated increase in cardiovascular disease and is considered a root cause of left ventricular hypertrophy, aneurysm formation and rupture, atherosclerosis, stroke, myocardial infarction, and renal failure[14].

1.3.2 Pulse Wave Velocity

Carotid-femoral pulse wave velocity is a simple, noninvasive, and reproducible measure of central arterial stiffness[15]. A pulse wave is generated with each contraction of the heart and the speed in which it travels throughout the arterial tree is dependent on functional and structural properties of the arterial wall[16]. This pulse wave is composed of an incident wave, which travels away from the heart, and a reflective wave, which travels towards the heart. In elastic arteries, the speed of the wave (the pulse wave velocity) is slow and the reflection occurs in diastole resulting in increased diastolic blood pressure and coronary perfusion. In stiff arteries, the speed of the wave is fast and the reflected wave merges with the systolic portion of the incident wave

leading to increased systolic blood pressure and pulse pressure[14]. Thus, stiffer arteries are characterized by a higher pulse wave velocity than elastic arteries.

Prospective studies have shown an association between central arterial stiffness and increased cardiovascular and total mortality in clinical and healthy populations[17-21]. One study found central aortic stiffening to be a strong predictor of cardiovascular and overall mortality among patients with end stage renal disease undergoing hemodialysis[18]. Another study found aortic stiffness to be predictive of cardiovascular and overall mortality among hypertensive patients[20]. Shoji et al. showed that increased aortic stiffening of patients with end stage renal disease and diabetes contributes to an increase in cardiovascular and all-cause mortality[19]. A study of healthy older adults found elevated levels of aortic stiffness to be associated with increased cardiovascular mortality[21].

1.3.3 Brachial Artery Distensibility (Distensibility)

Distensibility, defined as the percent or relative change in arterial diameter or area for a given change in pressure, is inversely related to pulse wave velocity and is a useful measure for comparing the stiffness in arteries of different size[13]. The clinical advantage of distensibility over other methods that measure arterial stiffness is that it is less expensive and easier to perform[22]. While carotid-femoral pulse wave velocity provides an indicator of central artery stiffness, distensibility provides a measure of systemic or overall stiffness.

A few studies have found an association between brachial artery distensibility and cardiovascular risk factors. Researchers from the Bogulasa Heart Study found a strong negative association between blood pressure and distensibility and weaker associations between age and distensibility among healthy young adults[23]. Additionally, this study found that a clustering of

cardiovascular risk factors was more strongly associated with a reduction in distensibility than a single risk factor alone[24]. Budoff et al. found an association between decreased brachial distensibility and higher levels of coronary calcification—indicating the role of distensibility in identifying patients with atherosclerotic burden[25].

1.3.4 Peripheral Arterial Disease

In the United States, peripheral arterial disease (PAD) affects about eight million people and is associated with an increased cardiovascular morbidity and mortality[26,27]. The prevalence of the disease is higher in blacks and older adults[28] and its strongest risk factors appear to be cigarette smoking and diabetes[29]. PAD is considered the "silent killer" because only ten percent of persons with the disease have classic symptoms of intermittent claudication[1]. Thus, noninvasive methods are needed to assess PAD in asymptomatic individuals.

1.3.5 Ankle-Brachial Index

The ankle-brachial index (ABI), the ratio of the ankle to arm systolic blood pressure, is a noninvasive measure of assessing subclinical PAD. It is considered the most efficient and practical technique for determining the presence and severity of PAD. A major advantage of the ABI is that it can be used to assess prognosis in symptomatic as well as asymptomatic individuals. An ABI of less than 0.9 is indicative of PAD and an ABI of greater than 1.0 is generally considered normal[30].

Several studies have found an association between ABI and cardiovascular disease and mortality. Researchers from the Cardiovascular Health Study found participants with an ABI less than 0.8 to be more than twice as likely as those with an ABI between 1.0 and 1.5 to have a

history of myocardial infarction, angina, congestive heart failure, stroke, or transient ischemic attack[30]. Another study in this population found an ABI less than 0.9 to be associated with increased incidence and recurrence of cardiovascular disease and increased mortality[31]. The Study of Osteoporotic Fractures, a prospective study examining mortality in white women over the age of 65, found an increased four year mortality in women with an ABI less than 0.9 compared to women with an ABI greater than 0.9 (RR=3.1, 95% CI = 1.7 TO 5.5)[32]. A prospective study examining participants with systolic hypertension found an ABI less than 0.9 compared to an ABI greater than 0.9 to be predictive of total mortality (RR=3.8, 95% CI = 2.1 to 6.9) and cardiovascular mortality (RR=3.7, 95% CI = 1.8 to 7.7)[33]. The Edinburgh artery study, a population based study of men and women aged 55-74 years, found an ABI less than 0.9 compared to an ABI greater than 0.9 to be associated with an increased five year total (RR=1.58, 95% CI = 1.14 to 2.18) and cardiovascular mortality (RR=1.85, 95% CI = 1.15 to 2.97)[34].

1.4 INFLAMMATION

Inflammation is a feature in the onset, development, and evolution of atherosclerotic lesions[3,4]. Cardiovascular risk factors including hypertension, exposure to tobacco, lipoproteins, and elevated glucose promote the release of inflammatory cells into the growing lesion[3]. These processes result in plaque instability and rupture which leads to clinical cardiovascular outcomes.

1.4.1 C-Reactive Protein

C-Reactive Protein (CRP), the most widely directly measured acute phase protein, is hardly detectable in plasma but can increase up to a 1000-fold during an acute response. As a

member of the pentraxin family, this protein received its name because it binds to the C-polysaccharide of *Streptococcus pneumoniae*[35]. The ability to activate complement[36-38] and stimulate tissue-factor[39,40] production suggests that CRP may have pro-inflammatory effects.

CRP has a clinical advantage over other inflammatory markers because its assay is well-validated, inexpensive, and widely available[41]. Additionally, it shows little circadian or seasonal variability[42]. Because of these advantages, the CDC has implemented recommendations for the use of CRP as a clinical marker of cardiovascular risk[43].

CRP may initiate and promote atherosclerosis via a number of mechanisms. Growing evidence suggests that CRP acts on monocytes and macrophages, endothelial cells, and smooth muscle cells, all of which are associated with the progression of atherosclerosis[44]. CRP has been found to be associated with reduced production of the smooth muscle relaxant nitric oxide[45] and also act as a mediator for the chemotactic movement of leukocytes[46]. The rupture of the atherosclerotic plaque may also be accelerated by CRP[47]. Finally, studies have implicated CRP as an activator of complement[36-38] and stimulator of tissue factor production[39,40].

Several prospective studies in healthy and clinical populations have found a relationship between CRP and increased incidence of cardiovascular events[48-59]. The Women's Health Study found increased CRP to predict first cardiovascular event, including nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, and death from cardiovascular causes[52]. The Physicians Health Study, an investigation of apparent healthy men, found significantly elevated levels of CRP among individuals who developed vascular events compared to individuals who remained free of vascular events[48-51]. The Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Study examined the relationship between CRP and clinical cardiovascular outcomes among men who were free of clinical coronary artery

disease[58]. This group found an increased risk of future coronary events with increasing levels of CRP. Among individuals with established vascular disease, the European Concerted Action on Thrombosis and Disability (ECAT) study found CRP to be associated with increased risk of incident myocardial infarction or sudden cardiac death[56]. The Multiple Risk Factor Intervention Trial (MRFIT) Study found an association between CRP and coronary heart disease mortality among a cohort of men considered high risk based on traditional cardiovascular risk factors[59]. The Iowa Rural Health Study found higher levels of CRP to be predictive of overall mortality in a sample of healthy, non-disabled older adults[57]. Finally, the Healthy Aging, and Body Composition (HABC) study found CRP to be associated with incident congestive heart failure events[55].

1.5 SPECIFIC AIMS

Cardiovascular disease, the leading cause of death in the United States, creates a major burden on the nation's public health and economic sectors. Although it is considered a widespread problem that can afflict anyone, certain subgroups are at an increased risk. Two subgroups known to have a higher risk of cardiovascular disease than the general population are older adults and women transitioning through menopause.

Atherosclerosis is considered to be a major contributor to the development of cardiovascular disease. Inherent in its name, atherosclerosis is comprised of two components—atheroma (thickening of the arteries caused by plaque buildup) and sclerosis (hardening of the arteries)[60]. Subclinical markers of atherosclerosis including the ABI, which encompasses the atheroma component, and arterial stiffness, which encompasses the sclerotic component, are

important because they are inexpensive and noninvasive methods for measuring early vascular change that may predispose individuals to major cardiovascular disease.

CRP, the prototypic downstream marker of systemic inflammation, is a major player in the atherosclerotic process. Additionally, it has been shown to directly predict the incidence of cardiovascular events in several populations. Several studies have examined the relationship between CRP and subclinical atherosclerosis but limitations in the current research exist. One limitation is that the association between CRP and central arterial stiffness has not been extensively studied in women transitioning through menopause. Another limitation is that no study has examined the association between CRP and distensibility, a measure of systemic arterial stiffness. Finally, the association between CRP and incidence of subclinical PAD has not been thoroughly examined in an elderly cohort. Thus, the specific aims of this dissertation are as follows:

1. To investigate the cross-sectional association between CRP and carotid-femoral pulse wave velocity in a cohort of women transitioning through menopause
2. To investigate the cross-sectional association between CRP and brachial artery distensibility in the same cohort listed above
3. To investigate the association between CRP and incidence of subclinical PAD (assessed using the ABI) in an elderly cohort

1.6 REFERENCES FOR CHAPTER 1

1. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng ZJ, Flegal K, O'Donnell C, Kittner S, Lloyd-Jones D, Goff DC, Jr., Hong Y, Adams R, Friday G, Furie K, Gorelick P, Kissela B, Marler J, Meigs J, Roger V, Sidney S, Sorlie P, Steinberger J, Wasserthiel-Smoller S, Wilson M, Wolf P. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006;113:e85-151.

2. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801-1811.

3. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-874.

4. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999;138:S419-S420.

5. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 1997;100:2680-2690.

6. Gimbrone MA, Jr. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. *Am J Cardiol* 1995;75:67B-70B.

7. Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976;58:731-741.

8. Hajjar KA. Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. *J Clin Invest* 1993;91:2873-2879.

9. Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease. Enhanced collagen production and accumulation by smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1997;17:2074-2081.

10. Upchurch GR, Jr., Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF, Jr., Loscalzo J. Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 1997;272:17012-17017.

11. Nakano E, Taiwo FA, Nugent D, Griffiths HR, Aldred S, Paisi M, Kwok M, Bhatt P, Hill MH, Moat S, Powers HJ. Downstream effects on human low density lipoprotein of homocysteine exported from endothelial cells in an in vitro system. *J Lipid Res* 2005;46:484-493.

12. Lau BH. Suppression of LDL oxidation by garlic. *J Nutr* 2001;131:985S-988S.

13. Izzo JL, Jr., Shykoff BE. Arterial stiffness: clinical relevance, measurement, and treatment. *Rev Cardiovasc Med* 2001;2:29-40.

14. O'rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens* 2002;15:426-444.

15. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26:485-490.

16. Safar ME, Henry O, Meaume S. Aortic pulse wave velocity: an independent marker of cardiovascular risk. *Am J Geriatr Cardiol* 2002;11:295-298.

17. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int* 2003;63:1852-1860.

18. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998;32:570-574.

19. Shoji T, Emoto M, Shinohara K, Kakiya R, Tsujimoto Y, Kishimoto H, Ishimura E, Tabata T, Nishizawa Y. Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol* 2001;12:2117-2124.

20. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001;37:1236-1241.

21. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 2005;111:3384-3390.

22. Motiwala S, Brewster UC, Perazella MA, Peixoto AJ. Reliability of a noninvasive device to measure systemic hemodynamics in hemodialysis patients. *Blood Press Monit* 2006;11:33-36.

23. Urbina EM, Brinton TJ, Elkasabany A, Berenson GS. Brachial artery distensibility and relation to cardiovascular risk factors in healthy young adults (The Bogalusa Heart Study). *Am J Cardiol* 2002;89:946-951.

24. Urbina EM, Kieltkya L, Tsai J, Srinivasan SR, Berenson GS. Impact of multiple cardiovascular risk factors on brachial artery distensibility in young adults: the Bogalusa Heart Study. *Am J Hypertens* 2005;18:767-771.

25. Budoff MJ, Flores F, Tsai J, Frandsen T, Yamamoto H, Takasu J. Measures of brachial artery distensibility in relation to coronary calcification. *Am J Hypertens* 2003;16:350-355.

26. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, McDermott MM, Hiatt WR. Peripheral arterial disease detection, awareness, and treatment in primary care. *JAMA* 2001;286:1317-1324.

27. Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. *N Engl J Med* 1992;326:381-386.

28. Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000. *Circulation* 2004;110:738-743.

29. Criqui MH, Browner D, Fronek A, Klauber MR, Coughlin SS, Barrett-Connor E, Gabriel S. Peripheral arterial disease in large vessels is epidemiologically distinct from small vessel disease. An analysis of risk factors. *Am J Epidemiol* 1989;129:1110-1119.

30. Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, Wolfson SK. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. *Circulation* 1993;88:837-845.

31. Newman AB, Shemanski L, Manolio TA, Cushman M, Mittelmark M, Polak JF, Powe NR, Siscovick D. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group. *Arterioscler Thromb Vasc Biol* 1999;19:538-545.

32. Vogt MT, Cauley JA, Newman AB, Kuller LH, Hulley SB. Decreased ankle/arm blood pressure index and mortality in elderly women. *JAMA* 1993;270:465-469.

33. Newman AB, Sutton-Tyrrell K, Vogt MT, Kuller LH. Morbidity and mortality in hypertensive adults with a low ankle/arm blood pressure index. *JAMA* 1993;270:487-489.

34. Leng GC, Fowkes FG, Lee AJ, Dunbar J, Housley E, Ruckley CV. Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. *BMJ* 1996;313:1440-1444.

35. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure Fold Des* 1999;7:169-177.

36. Volanakis JE. Complement activation by C-reactive protein complexes. *Ann N Y Acad Sci* 1982;389:235-250.

37. Volanakis JE, Kaplan MH. Interaction of C-reactive protein complexes with the complement system. II. Consumption of guinea pig complement by CRP complexes: requirement for human C1q. *J Immunol* 1974;113:9-17.

38. Jiang H, Robey FA, Gewurz H. Localization of sites through which C-reactive protein binds and activates complement to residues 14-26 and 76-92 of the human C1q A chain. *J Exp Med* 1992;175:1373-1379.

39. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood* 1993;82:513-520.

40. Ramani M, Khechai F, Ollivier V, Ternisien C, Bridey F, Hakim J, de Prost D. Interleukin-10 and pentoxifylline inhibit C-reactive protein-induced tissue factor gene expression in peripheral human blood monocytes. *FEBS Lett* 1994;356:86-88.

41. Koenig W. Predicting risk and treatment benefit in atherosclerosis: the role of C-reactive protein. *Int J Cardiol* 2005;98:199-206.

42. Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clin Chem* 2001;47:426-430.

43. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.

44. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? *Hypertension* 2004;44:6-11.

45. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Dhillon B, Mickle DA. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 2002;105:1890-1896.

46. Han KH, Hong KH, Park JH, Ko J, Kang DH, Choi KJ, Hong MK, Park SW, Park SJ. C-reactive protein promotes monocyte chemoattractant protein-1--mediated chemotaxis through upregulating CC chemokine receptor 2 expression in human monocytes. *Circulation* 2004;109:2566-2571.

47. Williams TN, Zhang CX, Game BA, He L, Huang Y. C-reactive protein stimulates MMP-1 expression in U937 histiocytes through Fc[gamma]RII and extracellular signal-regulated kinase pathway:: an implication of CRP involvement in plaque destabilization. *Arterioscler Thromb Vasc Biol* 2004;24:61-66.

48. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973-979.

49. Ridker PM. C-reactive protein and risks of future myocardial infarction and thrombotic stroke. *Eur Heart J* 1998;19:1-3.

50. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-428.

51. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97:2007-2011.

52. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-843.

53. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557-1565.

54. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000;321:199-204.

55. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation* 2003;108:2317-2322.

56. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet* 1997;349:462-466.

57. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Jr., Heimovitz H, Cohen HJ, Wallace R. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med* 1999;106:506-512.

58. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999;99:237-242.

59. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. *Am J Epidemiol* 1996;144:537-547.

60. Kimoto E, Shoji T, Shinohara K, Inaba M, Okuno Y, Miki T, Koyama H, Emoto M, Nishizawa Y. Preferential stiffening of central over peripheral arteries in type 2 diabetes. *Diabetes* 2003;52:448-452.

2.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH CENTRAL ARTERIAL STIFFNESS IN A COHORT OF WOMEN TRANSITIONING THROUGH MENOPAUSE

To be submitted to American Journal of Hypertension

[1]Vinay Mehta, M.S., [1]Rachel Mackey, Ph.D., [1]Sarah Brockwell,Ph.D., [1]Sheryl Kelsey, Ph.D., [1]Anne B. Newman, M.D. M.P.H., [2]Steven Hollenberg, M.D., [1]Kim Sutton-Tyrrell, Dr.P.H

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA
[2]Cooper Heart Institute, Camden, NJ

2.1 ABSTRACT

Arterial stiffness is associated with stroke, myocardial infarction, left ventricular hypertrophy, renal failure, and a host of other complications. Increased levels of inflammatory markers, most notably C-Reactive Protein, have also been linked to cardiovascular outcomes. Increases in both vascular stiffness and inflammation are known to occur with menopause. Few studies have examined the relationship between C-reactive protein and arterial stiffness and these studies have not examined the association in women transitioning through menopause.

The cross-sectional association between C-reactive protein and pulse wave velocity was evaluated in 154 middle-aged women enrolled in an ancilliary study to the Study of Women's Health Across the Nation. After adjustment for confounders, a one mg/L unit increase in continuous C-reactive protein was associated with a 0.8% increase in pulse wave velocity (p=.02). C-reactive protein, analyzed as tertiles, was also associated with pulse wave velocity (p for trend=.014). Stratification by menopausal status showed a strong association between continuous C-reactive protein and pulse wave velocity among post/late perimenopausal women (1.9%, p<.0001) but not among pre/early perimenopausal women (-0.41%, p=.33). Categorical C-reactive protein was associated with pulse wave velocity in post/late perimenopausal women (p for trend=.0053) but not in pre/early perimenopausal women (p for trend=.76).

C-reactive protein, whether analyzed continuously or categorically, was associated with aortic pulse wave velocity in post/late perimenopausal women but not in pre/early perimenopausal women. This study provides a potential mechanism explaining the increased risk of cardiovascular outcomes in women transitioning through menopause.

Key Words: Arterial Stiffness, C-reactive protein, Menopause, Atherosclerosis, Women's Health

2.2 INTRODUCTION

Aging of the arterial system involves both structural and functional changes in the arterial wall which leads to stiffening of the artery[1]. Arterial stiffness is thought to contribute to the age associated increase in cardiovascular disease, morbidity, and mortality[2,3]. Aortic pulse wave velocity (aPWV), assessed by carotid-femoral pulse wave velocity, is a simple, noninvasive, and highly reproducible measure of central arterial stiffness[4]. In clinical populations, including patients with hypertension, diabetes, and end stage renal disease, aPWV is a predictor of cardiovascular and all-cause mortality[5-8]. In addition, an association between aPWV and cardiovascular and overall mortality has been found in healthy older adults[9].

Inflammation is a major feature in the initiation and progression of atherosclerosis and subsequent cardiovascular disease[10,11]. Several inflammatory markers exist but high sensitivity C-reactive protein (CRP) appears to be clinically advantageous because it is well-validated, inexpensive, and widely available[12]. Prospective studies in clinical and healthy populations have shown a relationship between CRP and incidence of cardiovascular disease[13-18]. In patients with unstable angina, CRP has been predictive of death, acute myocardial infarction, and rheumatoid arthritis[19]. In healthy populations, CRP has been found to be associated with increased incidence of cardiovascular disease events including death due to coronary heart disease, nonfatal myocardial infarction, peripheral vascular disease, and stroke[13-18]. On the basis of these results, the CDC has recommended the utility of CRP as a screening test for individuals at high risk of cardiovascular disease[12].

Menopause is a critical stage in a woman's life associated with an increased incidence of cardiovascular disease[20]. Reduced estrogen levels that accompany menopause may induce inflammation by increasing expression and secretion of proinflammatory cytokines[21]. In

addition, decreased levels of estrogen may increase arterial stiffening by altering the collagen/elastin ratio within the arterial wall or by inhibiting smooth muscle cell proliferation[22,23]. Both of these may present mechanisms for the increased risk of cardiovascular disease in women transitioning through menopause.

Several studies have examined the relationship between inflammation and arterial stiffness but none have examined the influence of the menopausal transition on this relationship in a population-based cohort[24-33]. Menopause may act as a trigger that strengthens the relationship between inflammation and arterial stiffness and thus provides a mechanism for the increase in cardiovascular disease seen after menopause. The purpose of this study is to examine the effect of the menopausal transition on the association between CRP and aPWV. This study will help to provide rationale for targeting middle-aged women to healthier lifestyle patterns aimed at reducing inflammation and arterial stiffening.

2.3 METHODS

2.3.1 Study Population

The Study of Women's Health Across the Nation (SWAN) is a multi-site, multi-ethnic longitudinal epidemiological study designed to examine the physical, psychological, and social changes that women experience during the menopausal transition. At baseline, SWAN recruited a total of 3,302 women between the ages of 42 and 52 from seven clinical sites: Boston, Chicago, Detroit, Los Angeles, Newark, Pittsburgh, and Oakland. In order to be eligible for the study, women were required to have an intact uterus, at least one menstrual period and no use of reproductive hormones in the previous 3 months. The institutional review boards of the

participating institutions approved this study, and all women signed informed consent forms before participation.

Data from 154 women (97 Caucasian, 57 African American) enrolled in SWAN Heart, an ancillary study to SWAN, were used for the current analyses. The ancillary project is a study of the natural history of subclinical atherosclerosis during the menopausal transition and involves 2 out of the 7 SWAN sites (Pittsburgh and Chicago). The study began at the time of the 4^{th} and 5^{th} annual SWAN exam and all participants were between 46 and 57 years of age. Eligibility required that participants had no evidence of clinical atherosclerosis (MI, angina, intermittent claudication, cerebral ischemia, or revascularization), had not undergone a hysterectomy, and were not currently taking medication for hypertension, diabetes, or heart arrhythmias. Additional exclusion criteria for these analyses included missing information on CRP, aPWV, or menopausal status. Among 222 women with a CRP measurement, 186 had an aPWV measurement. An additional 32 women were excluded because their menopausal status could not be determined—primarily because they were on hormone therapy. Among the 36 women without an aPWV measurement, 9 were due to technical complications and 27 were due to patient difficulty, unreadable files, or inability to measure the distance between the carotid and femoral recording sites.

2.3.2 Cardiovascular Risk Factors

The SWAN protocol required a fasting blood draw between days 2 and 5 of the menstrual cycle for measurements of reproductive hormones, lipids and lipoproteins, insulin, glucose, and clotting factors. Blood samples were maintained at 4^0 C until separated and then frozen at -80^0 C and shipped on ice to Medical Research Laboratories, which is certified by the National Heart,

Lung and Blood Institute of the Centers for Disease Control[34]. Total cholesterol and triglycerides were analyzed with the Hitachi 747 analyzer[35] whereas high-density lipoprotein (HDL) cholesterol was isolated using heparin-2M manganese chloride[36]. Low-density lipoprotein cholesterol (LDL) was calculated using the Friedwald equation[37]. Plasma glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim, Indianapolis, In) and plasma insulin was measured using solid-phase radioimmunoassay procedure (DPC Coat-A-Count, Los Angeles, CA). Participants who satisfied at least 3 of the following 5 criteria were considered to have the metabolic syndrome: abdominal obesity, hypertriglyceridemia, low HDL, hypertension, and impaired fasting glucose and diabetes. CRP was assessed using the Behring Nephelometer II (hs-CRP on BN 100, Dade-Behring, Marburg, Germany).

2.3.3 Hormones

Hormone assays were conducted at the University of Michigan SWAN Endocrine Laboratory. FSH and estradiol (E2) assays were conducted using the ACS-180 automated analyzer. Serum E2 concentrations were measured with a modified, off-line ACS-180 immunoassay. Serum FSH concentrations were measured with a two-site chemiluminometric immunoassay using constant amounts of two monoclonal antibodies. Testosterone concentrations were evaluated with the ACS-180 total testosterone assay modified to increase precision in the low ranges. SHBG was measured using a competitive chemiluminescent assay.

2.3.4 Physical Measures/Activity

Two blood pressure measurements were averaged on the right arm after five minutes of rest in a seated position. Height was measured using a stadiometer or portable stadiometer whereas

weight was measured using a balance beam, digital scale, or portable scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured over undergarments or light clothing. Leisure-time physical activity was measured with an adaptation of the questionnaire by Baecke[38].

2.3.5 Menopausal Status

Information on menopausal status was collected using an annual interviewer administered questionnaire. Premenopausal status was defined as having a menstrual period in the past three months with no change in cycle regularity in the past twelve months. Early perimenopausal status was defined as having a menstrual period in the past three months with some change in cycle regularity in the past twelve months. Late perimenopausal status was defined as not having a menstrual period in the past three months but having one in the past twelve months. Postmenopausal status was defined as not having a menstrual period in the past twelve months. Due to the small sample of premenopausal (n=8) and late perimenopausal (n=18) women, late perimenopausal women was combined with postmenopausal women and early perimenopausal women with premenopausal women to form a dichotomous menopausal status variable.

2.3.6 Aortic Pulse Wave Velocity

Aortic pulse wave velocity was measured as the distance between the carotid and femoral recording sites divided by the foot to foot time delay between the carotid and femoral waveforms. The methodology has previously been described in detail[39]. Briefly, aPWV was assessed by trained technicians using Doppler ultrasound of the right carotid and femoral arteries. Participants were required to have 30 minutes of supine rest before measurement. The

distance between the carotid and femoral recordings was measured using a standard tape measure over the surface of the body. The time delay was calculated as the foot to foot time differential between simultaneously collected carotid and femoral wave forms, using the R-wave of the EKG. Three runs were recorded for each participant and usable runs were averaged. A greater pulse wave velocity is associated with a greater stiffening of the aorta.

2.3.7 Statistical Methods

Descriptive statistics (N, means/medians for continuous variables and percentages for categorical variables) for sociodemographic variables, anthropometric measures, lipids, blood pressures, hormones, fasting glucose, fasting insulin, metabolic syndrome, aPWV, and CRP were calculated. Categorical variables were compared across the dichotomous menopausal status variable via Chi-square/Fishers exact test and continuous variables were compared via t-test/Wilcoxon rank sum test. Spearman correlations between risk factors and aPWV were calculated to understand basic associations and to determine candidate variables for multivariate models. Multivariable linear regression was used to test the association between log transformed aPWV and continuous CRP while adjusting for confounders. Stepwise regression was used to develop the most parsimonious model. In order to facilitate the interpretation of the model, regression coefficients were exponentiated, thereby showing the relative or percent increase in aPWV with a one unit increase in CRP. Due to the skewed nature of the variable, CRP was also analyzed as tertiles. ANCOVA was used to test a trend in log aPWV across CRP tertiles (bottom tertile=0 to 1.3 mg/L, middle tertile=1.4 to 4.1 mg/L, top tertile > 4.1 mg/L) while adjusting for confounders. Logarithmic mean aPWV values were backtransformed to the original scale to facilitate the interpretation of the results. After final multivariable models were obtained, an

interaction term between CRP and menopausal status was added to test whether or not the association between CRP and aPWV differed according to menopausal status. Statistical significance was assessed using a type one error rate of 0.05. Assumptions of linear regression (homoscedasticity, linearity, and normality of residuals) were tested to verify the validity of the model. All analyses were done using SAS version 8.2.

2.4 RESULTS

Characteristics of the population stratified by menopausal status are presented in Table 2-1. Among the 154 women, 37.0% (n=57) were African American and 44.2% (n=68) were post/late perimenopausal. The proportion of African Americans was similar according to menopausal status (p=0.40). The mean age of the total population was 50.8 ± 2.6 and post/late perimenopausal women were significantly older than pre/early perimenopausal women. In addition, total and LDL cholesterol, fasting glucose, and fasting insulin were all significantly greater in post/late perimenopausal women. Estradiol and SHBG values were lower in post/late perimenopausal women and FSH was greater in this subgroup. Although CRP and aPWV were higher in post/late perimenopausal women, the differences were not statistically significant.

Women who were excluded from the analyses (n=68) were similar to women included in the analyses (n=154) in regards to all risk factors except the anthropometric measures and age. Women excluded from analyses had significantly ($p<.05$) less girth, weight, and BMI, and were significantly older than those women who were included.

Table 2-2 displays the linear correlations between risk factors and aPWV within the full population and within each menopausal status group. In the combined sample, all of the risk

factors were significantly associated with aPWV with the exception of the lipids, hormones, fasting glucose, and height. Stratification by menopausal status showed that the majority of risk factors were more strongly associated with aPWV in post/late perimenopausal women than in pre/early perimenopausal women.

Table 2-3 shows the results of multivariate models involving continuous CRP. Notably, a one unit increase in CRP was significantly associated with a 0.8% increase in aPWV (p=.02). The interaction between CRP and menopausal status was significant (p<.0001) and the association between CRP and aPWV was stronger in post/late perimenopausal women (1.9%, p <.0001) than in pre/early perimenopausal women (-0.41%, p=.33).

As depicted in Figure 2-1, the adjusted mean aPWV increased across CRP category (p for trend = 0.014). The interaction between categorical CRP and menopausal status was significant (p=.02) and this trend was primarily found in post/late perimenopausal women (Figure 2-2).

Table 2-2 shows that certain risk factors were more strongly associated with aPWV in post/late perimenopausal women than in pre/early perimenopausal women. In order to test whether these risk factors may attenuate the influence that status had on the relationship between CRP and aPWV, interaction terms between status and age, total cholesterol, LDL cholesterol, triglycerides, glucose, insulin, estradiol, FSH, SHBG, and testosterone were separately added to the model. Among these, glucose and insulin were the only variables that attenuated the influence of menopausal status on the relationship between CRP and aPWV. After the addition of an interaction term between insulin and menopausal status, the interaction between CRP and menopausal status was no longer significant (p=.16). Likewise, after the addition of an interaction term between glucose and menopausal status, the interaction between CRP and menopausal status was no longer significant (p=.17).

When analyses were re-run excluding an additional 37 women with the metabolic syndrome, a one unit increase in CRP was associated with a 1.2% higher aPWV (p=.0071). However, the interaction between continuous CRP and status was no longer significant (p=.325). The association between categorical CRP and aPWV within this subgroup was only marginally significant (p for trend=.07) and the interaction between categorical CRP and menopausal status was not significant (p=.38).

Table 2-1. Baseline Characteristics of the SWAN Heart Study Sample (Mean ± Standard Deviation)

	Full Group (N=154)	Pre/E. Peri(N=68)	L. Peri/Post (N=86)	P-value for Status Difference
Age (years)	50.8±2.6	49.7±2.0	52.3±2.5	**<.0001**
Activity Index	2.47±0.68	2.49±0.61	2.44±0.77	0.72
African American (%)	37.0	33.7	41.2	0.40
Body Composition:				
Height (mm)	163.2±5.9	163.1±6.1	163.3±5.7	0.84
BMI kg/m^2 (kg/m^2)	30.3±6.6	30.1±6.4	30.5±6.8	0.65
Waist Circumference (cm)	91.1±15.1	90.3±15.7	92.1±14.4	0.46
Weight (kg)	80.8±18.8	80.3±19.4	81.3±18.2	0.73
Blood Pressure:				
Systolic Blood Pressure* (mmHg)	118.0 (22.0)	118.0 (21.0)	118.0 (22.0)	0.83
Diastolic Blood Pressure (mmHg)	76.0±9.5	75.6±10.4	76.6±8.3	0.52
Pulse Pressure (mmHg)	44.4±12.0	44.0±10.7	44.8±13.4	0.69
Lipids:				
Total Cholesterol (mg/dL)	202.7±40.7	195.2±35.8	212.2±44.5	**0.01**
LDL Cholesterol (mg/dL)	121.2±36.2	115.9±31.5	128.1±40.7	**0.05**
HDL Cholesterol (mg/dL)	56.6±13.3	55.8±13.5	57.5±13.0	0.41
Triglycerides* (mg/dL)	104.0 (62.0)	99.5 (62.0)	108.5 (71.0)	0.42
Glucose Metabolism:				
Fasting glucose* (mg/dL)	90.0 (14.0)	88.0 (11.0)	93.0 (15.5)	**0.04**
Fasting insulin* (uIU/mL)	10.3 (7.3)	9.0 (6.7)	11.8 (8.9)	**0.01**
PWV* (cm/sec)	776.2 (227.1)	769.6 (213.7)	777.6 (233.8)	0.32
C-Reactive Protein* (mg/L)	2.3 (4.7)	2.1 (4.2)	2.9 (6.5)	0.11

* Values are median (Interquartile Range)

Table 2-2. Relation of Cardiovascular Risk Factors and Aortic Pulse Wave Velocity (Unadjusted Spearman Correlation Coefficients)

	Full Group (n=154)		Pre/Early Peri (n=86)		Post/Late Peri (n=68)	
Risk Factors	**Correlation Coefficient**	**P-value**	**Correlation Coefficient**	**P-Value**	**Correlation Coefficient**	**P-value**
Age	0.27	**.0007**	0.24	**.02**	0.32	**.007**
Activity Index	-.23	**.006**	-0.13	.25	-0.30	**.02**
Body Composition:						
Weight	0.24	**.003**	0.23	**.04**	0.25	**.04**
Height	0.02	.78	0.06	.62	0.002	.99
BMI	0.23	**.004**	0.24	**.03**	0.22	.08
Waist Circumference	0.27	**.0009**	0.24	**.03**	0.30	**.01**
Blood Pressure:						
Systolic Blood Pressure	0.19	**.02**	0.16	.15	0.20	.09
Diastolic Blood Pressure	0.17	**.04**	0.12	.30	0.22	.07
Pulse Pressure	0.16	**.05**	0.14	.21	0.18	.15
Lipids:						
Cholesterol	0.15	.07	0.02	.84	0.24	**.05**
LDL	0.14	.09	0.04	.73	0.21	.08
HDL	-0.14	.08	-0.05	.62	-0.22	.07
Triglycerides	0.14	.08	0.01	.92	0.26	**.03**
Glucose Metabolism:						
Fasting Glucose	-0.05	.53	-0.19	.08	0.07	.60
Fasting Insulin	0.18	**.03**	-0.03	.81	0.38	**.002**
Inflammatory/ Hemostatic Markers:						
C-Reactive Protein	0.37	**<.0001**	0.25	**.02**	0.52	**<.0001**

Table 2-3. Percent Increase in Aortic Pulse Wave Velocity for a One Unit Increase in CRP (mg/L)

	Full Group		Pre/Early Peri		Post/Late Peri	
	Percent Increase	**P-value**	**Percent Increase**	**P-value**	**Percent Increase**	**P-value**
Unadjusted	1.2	.0004	0.26	.58	2.3	<.0001
Adjusted*	0.8	.02	-0.41	.33	1.9	<.0001

*Adjusted for age, systolic blood pressure, ethnicity, site, waist circumference, diastolic blood pressure, and physical activity; Status*CRP interaction P-value <.0001

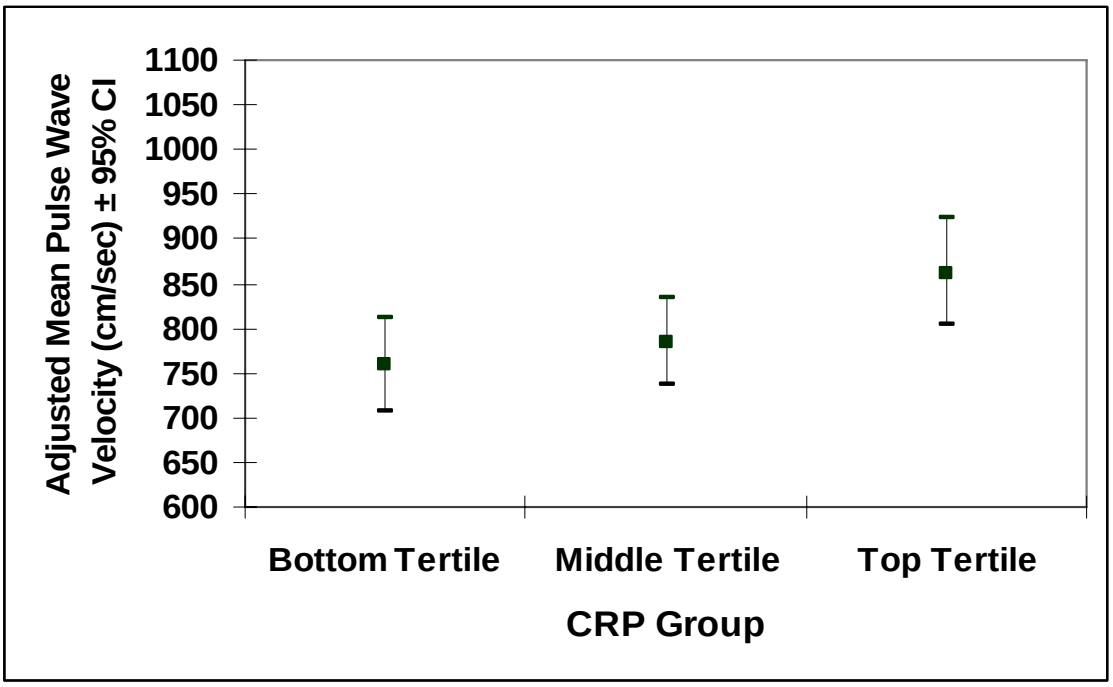

Figure 2-1. Adjusted* Mean Pulse Wave Velocity by CRP Tertiles†

*Adjusted for age, systolic blood pressure, ethnicity, site, waist circumference, diastolic blood pressure, and Physical Activity; p-value for trend=.014
†Bottom Tertile=0 to 1.3 mg/L; Middle Tertile=1.4 to 4.1 mg/L; Top Tertile > 4.1 mg/L

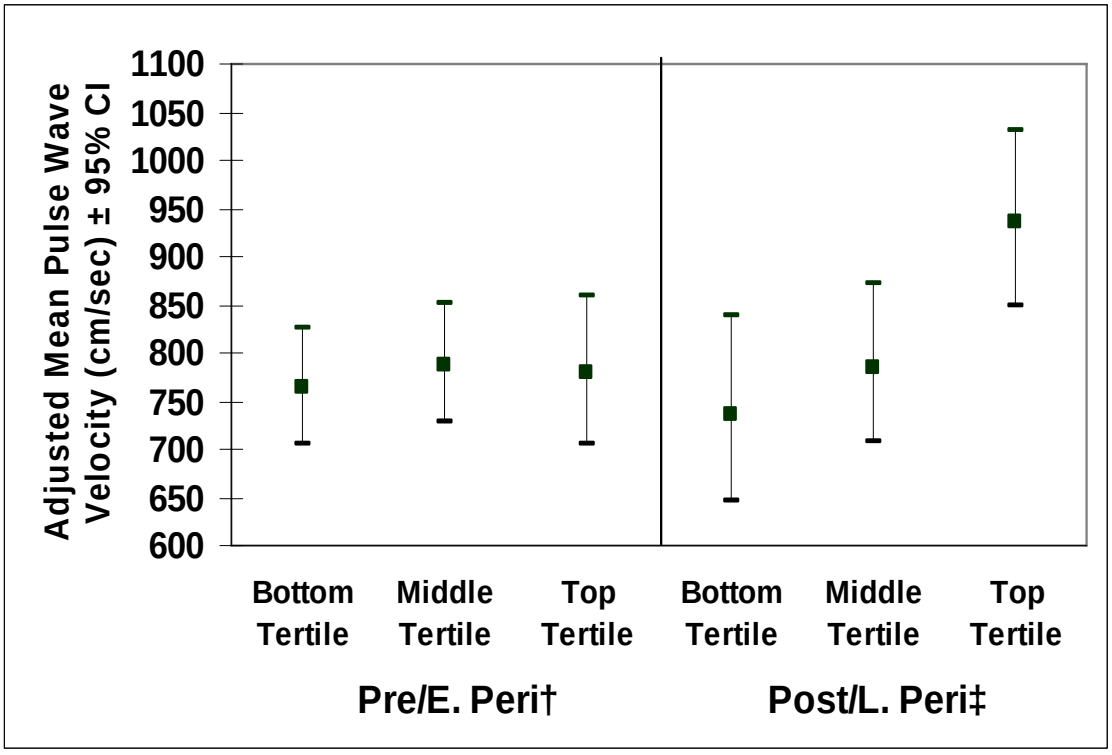

Figure 2-2. Adjusted* Mean Pulse Wave Velocity by CRP Tertiles Stratified By Menopausal Status

* Adjusted for age, systolic blood pressure, ethnicity, site, waist circumference, diastolic blood pressure, and Physical Activity; Status*CRP interaction P-value= .02
†P-Value for trend = 0.76
‡P-Value for trend = .0053

2.5 DISCUSSION

The primary finding of this study is that CRP is significantly associated with aPWV in a cohort of women transitioning through menopause and that this association is significantly stronger in women who are later in their transition than in women who are earlier in their transition.

Several studies have looked at the association between inflammation and arterial stiffness but only a few have used CRP and aPWV to measure inflammation and arterial stiffness respectively[24-27,29]. Among these studies, only one utilized a population based cohort but that study focused on older adults[24]. The other studies examined the association in healthy individuals free of cardiovascular related problems, individuals with newly diagnosed hypercholesterolaemia, and individuals with systemic vasculitis[25-27,29]. All of these studies found a strong positive association between CRP and aPWV. Our study supports these findings and extends them by characterizing a differential association between CRP and aPWV according to stage of the menopausal transition.

CRP was examined both as a continuous and categorical variable. In both cases, CRP was associated with aPWV and the relationship was significantly stronger in post/late perimenopausal women. Clinically defined cutpoints of CRP[12] were not used because a very small sample of the post/late perimenopausal women would be classified as low risk CRP (n=9). Thus, we would not expect to have the power to detect an interaction effect.

Hormone therapy users were excluded from the analyses because of the inconsistent literature on the relationship of arterial stiffness and inflammation with hormone replacement therapy use[40-43]. When hormone therapy users were added back into the analyses, the results were very similar to those obtained when excluding them.

Because CRP values greater than 10.0 mg/L can be indicative of acute inflammation, we re-ran analyses including women with CRP levels less than 10.0 mg/L (n=132). Both continuous and categorical CRP were more strongly associated with aPWV in post/late perimenopausal women than in pre/early perimenopausal women.

Several potential mechanisms could explain our findings. There is evidence of an increase in the expression and secretion of proinflammatory cytokines (IL-1, IL-6, and TNF- α) with a reduction in estradiol[21]. CRP is known to be stimulated by these proinflammatory cytokines. Increased inflammation due to estrogen deficiency could inhibit endothelium-dependent vasodilation and synthesis of nitric oxide, a smooth muscle relaxant. Increased inflammation could also adversely impact the collagen/elastin ratio of the vessel which could lead to increased stiffening[24,25]. Estradiol also has cardioprotective effects which may be mediated by activation of the estrogen receptor-α and estrogen receptor-β. These receptors could have direct vasodilatory effects on the vessels by activation of nitric oxide synthase and indirect effects on serum lipid concentrations, fibrinolytic, or inflammatory factors[20,22].

Another potential mechanism is that an increase in vessel diameter due to menopausal-induced stiffening could lead to an increase in tensile stress which would make the vessel more susceptible to risk factors for increased inflammation.

A third potential mechanism is that inflammation could indirectly impact arterial stiffening through its association with diabetes[24], which is more prevalent among postmenopausal women than premenopausal women[44]. Our results may lend some support to this theory because the addition of interaction terms between glucose and insulin with menopausal status diminished the interaction effect between CRP and menopausal status.

Additionally, exclusion of women with metabolic syndrome, which is known to be associated with diabetes, attenuated the interaction effect between CRP and menopausal status.

Several limitations of our study exist. Inherent in any cross-sectional design is the inability to determine causality. Thus, it cannot be ascertained whether increased inflammation results in increased arterial stiffening or vice versa. In addition, our relatively small sample size limited our capacity to further investigate the role of ethnicity on the findings. Finally, due to the sample size, tripartite comparisons between premenopausal, perimenopausal, and postmenopausal women could not be made.

This study is important because it explores a potential mechanism explaining the increased risk of cardiovascular outcomes in women who are later in their menopausal transition. It is likely that menopause is associated with an increase in inflammation, which in turn results in increased stiffening of the aorta and increased risk of cardiovascular outcomes. Both inflammation and arterial stiffness have been linked to cardiovascular outcomes in clinical and healthy populations[5-9,13-17]. In addition, there is evidence that inflammation and arterial stiffening can be tempered with certain medications, physical activity, and healthy lifestyles[45-49]. Therefore, it is likely that the menopause associated increase in cardiovascular risk can be alleviated or eliminated through lifestyle patterns aimed at reducing inflammation and arterial stiffening.

2.6 ACKNOWLEDGEMENTS

The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health, DHHS, through the National Institute on Aging, the National Institute of Nursing Research and the NIH Office of Research on Women's Health (Grants NR004061; AG012505, AG012535, AG012531, AG012539, AG012546, AG012553, AG012554, AG012495). SWAN Heart is funded through the NHLBI (Grants HL06559 and HL066558). Clinical Center: *University of Michigan, Ann Arbor - MaryFran Sowers, PI*; *Massachusetts General Hospital, Boston, MA - Robert Neer, PI 1995 - 1999; Joel Finkelstein, PI 1999-present*; *Rush University, Rush University Medical Center, Chicago, IL - Lynda Powell, PI*; *University of California, Davis/Kaiser - Ellen Gold, PI*; *University of California, Los Angeles - Gail Greendale, PI*; *University of Medicine and Dentistry - New Jersey Medical School, Newark –Gerson Weiss, PI 1995 – 2004; Nanette Santoro, PI 2004 – present*; and the *University of Pittsburgh, Pittsburgh, PA - Karen Matthews, PI.*

NIH Program Office: *National Institute on Aging, Bethesda, MD - Marcia Ory 1994 – 2001; Sherry Sherman 1994 – present; National Institute of Nursing Research, Bethesda, MD – Program Officers.*

Central Laboratory: *University of Michigan, Ann Arbor - Daniel McConnell;* (Central Ligand Assay Satellite Services).

Coordinating Center: *New England Research Institutes, Watertown, MA - Sonja McKinlay, PI 1995 – 2001; University of Pittsburgh, Pittsburgh, PA – Kim Sutton-Tyrrell, PI 2001 – present.*

Steering Committee: Chris Gallagher, Chair; Susan Johnson, Chair

We thank the study staff at each site and all the women who participated in SWAN.

2.7 REFERENCES FOR CHAPTER 2

1. Lakatta EG, Mitchell JH, Pomerance A, Rowe GG. Human aging: changes in structure and function. *J Am Coll Cardiol* 1987;10:42A-47A.

2. Arnett DK, Evans GW, Riley WA. Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol* 1994;140:669-682.

3. O'Rourke M. Mechanical principles in arterial disease. *Hypertension* 1995;26:2-9.

4. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26:485-490.

5. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int* 2003;63:1852-1860.

6. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998;32:570-574.

7. Shoji T, Emoto M, Shinohara K, Kakiya R, Tsujimoto Y, Kishimoto H, Ishimura E, Tabata T, Nishizawa Y. Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol* 2001;12:2117-2124.

8. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001;37:1236-1241.

9. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 2005;111:3384-3390.

10. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999;138:S419-S420.

11. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-874.

12. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.

13. Ridker PM. C-reactive protein and risks of future myocardial infarction and thrombotic stroke. *Eur Heart J* 1998;19:1-3.

14. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-428.

15. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97:2007-2011.

16. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-843.

17. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557-1565.

18. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiol Clin* 2003;21:315-325.

19. Ridker PM, Koenig W, Fuster V. C-reactive protein and coronary heart disease. *N Engl J Med* 2004;351:295-298.

20. Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol* 2002;89:12E-17E.

21. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 2002;23:90-119.

22. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801-1811.

23. Jonason T, Henriksen E, Kangro T, Vessby B, Ringqvist I. Menopause is associated with the stiffness of the common carotid artery in 50-year-old women. *Clin Physiol* 1998;18:149-155.

24. Mattace-Raso FU, van der Cammen TJ, van dM, I, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis* 2004;176:111-116.

25. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004;24:969-974.

26. Booth AD, Wallace S, McEniery CM, Yasmin, Brown J, Jayne DR, Wilkinson IB. Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. *Arthritis Rheum* 2004;50:581-588.

27. Selzer F, Sutton-Tyrrell K, Fitzgerald S, Tracy R, Kuller L, Manzi S. Vascular stiffness in women with systemic lupus erythematosus. *Hypertension* 2001;37:1075-1082.

28. Arroyo-Espliguero R, Mollichelli N, Avanzas P, Zouridakis E, Newey VR, Nassiri DK, Kaski JC. Chronic inflammation and increased arterial stiffness in patients with cardiac syndrome X. *Eur Heart J* 2003;24:2006-2011.

29. Pirro M, Schillaci G, Savarese G, Gemelli F, Vaudo G, Siepi D, Bagaglia F, Mannarino E. Low-grade systemic inflammation impairs arterial stiffness in newly diagnosed hypercholesterolaemia. *Eur J Clin Invest* 2004;34:335-341.

30. Wong M, Toh L, Wilson A, Rowley K, Karschimkus C, Prior D, Romas E, Clemens L, Dragicevic G, Harianto H, Wicks I, McColl G, Best J, Jenkins A. Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease risk factors and inflammation. *Arthritis Rheum* 2003;48:81-89.

31. Tomiyama H, Koji Y, Yambe M, Motobe K, Shiina K, Gulnisa Z, Yamamoto Y, Yamashina A. Elevated C-reactive protein augments increased arterial stiffness in subjects with the metabolic syndrome. *Hypertension* 2005;45:997-1003.

32. Tomiyama H, Arai T, Koji Y, Yambe M, Hirayama Y, Yamamoto Y, Yamashina A. The relationship between high-sensitive C-reactive protein and pulse wave velocity in healthy Japanese men. *Atherosclerosis* 2004;174:373-377.

33. Nagano M, Nakamura M, Sato K, Tanaka F, Segawa T, Hiramori K. Association between serum C-reactive protein levels and pulse wave velocity: a population-based cross-sectional study in a general population. *Atherosclerosis* 2005;180:189-195.

34. Myers GL, Cooper GR, Winn CL, Smith SJ. The Centers for Disease Control-National Heart, Lung and Blood Institute Lipid Standardization Program. An approach to accurate and precise lipid measurements. *Clin Lab Med* 1989;9:105-135.

35. Stein EA, Steiner PM, Gartside PS, Glueck CJ. Development and evaluation of a method for quantitation of plasma high-density-lipoprotein cholesterol. *Clin Chem* 1978;24:1112-1115.

36. Warnick GR, Albers JJ. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 1978;19:65-76.

37. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.

38. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 1982;36:936-942.

39. Sutton-Tyrrell K, Mackey RH, Holubkov R, Vaitkevicius PV, Spurgeon HA, Lakatta EG. Measurement variation of aortic pulse wave velocity in the elderly. *Am J Hypertens* 2001;14:463-468.

40. Maas AH, van der GY, van der Schouw YT, Grobbee DE. HRT and heart disease: problems and prospects. *Maturitas* 2004;47:255-258.

41. Stork S, van der Schouw YT, Grobbee DE, Bots ML. Estrogen, inflammation and cardiovascular risk in women: a critical appraisal. *Trends Endocrinol Metab* 2004;15:66-72.

42. Tanaka H, DeSouza CA, Seals DR. Arterial stiffness and hormone replacement use in healthy postmenopausal women. *J Gerontol A Biol Sci Med Sci* 1998;53:M344-M346.

43. Gorgulu S, Eren M, Celik S, Dagdeviren B, Uslu N, Suer N, Tezel T. The effects of hormonal therapy on aortic stiffness and left ventricular diastolic function. *Acta Cardiol* 2003;58:1-8.

44. Curtis J, Wilson C. Preventing type 2 diabetes mellitus. *J Am Board Fam Pract* 2005;18:37-43.

45. Ballantyne CM, Nambi V. Markers of inflammation and their clinical significance. *Atheroscler Suppl* 2005;6:21-29.

46. Mahmud A, Feely J. Antihypertensive drugs and arterial stiffness. *Expert Rev Cardiovasc Ther* 2003;1:65-78.

47. Boreham CA, Ferreira I, Twisk JW, Gallagher AM, Savage MJ, Murray LJ. Cardiorespiratory fitness, physical activity, and arterial stiffness: the Northern Ireland Young Hearts Project. *Hypertension* 2004;44:721-726.

48. Tanaka H, DeSouza CA, Seals DR. Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol* 1998;18:127-132.

49. Albert MA, Glynn RJ, Ridker PM. Effect of physical activity on serum C-reactive protein. *Am J Cardiol* 2004;93:221-225.

3.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH SYSTEMIC ARTERIAL STIFFNESS IN A MIDDLE AGED COHORT OF WOMEN

To Be Submitted to American Journal of Hypertension

3.1 ABSTRACT

The age associated increase in arterial stiffness is associated with a host of complications including stroke, myocardial infarction, diabetes, and atherosclerosis. C-reactive protein, a marker of systemic inflammation, is a major player in the initiation and progression of atherosclerotic plaques. Studies have found a positive relationship between C-reactive protein and arterial stiffness but these studies have focused on a localized region of the artery. The Dynapulse 5000A presents a simple, inexpensive and noninvasive technique to measure systemic arterial stiffness that adjusts for artery size. This study examined the association between C-reactive protein and systemic arterial stiffness in a middle-aged cohort of women.

The association between C-reactive protein and brachial artery distensibility, an indicator of systemic arterial stiffness, was evaluated in 1069 middle aged women enrolled in the Study of Women's Health Across the Nation. The mean age and distensibility of the population were 53.6 (s.d = 2.6) and 6.2 (s.d = 1.2) respectively. The ethnic distribution of the population was: African American (14.6%, n=156), Caucasian (54.0%, n=577), Chinese (11.6%, n=124), and Japanese (19.8%, n=212). After adjustment for confounders, log transformed C-reactive protein was significantly associated with lower distensibility (β=-0.13, p=.0011). The adjusted mean distensibility decreased with increasing tertiles of C-reactive protein (p for trend = .0031). The association between C-reactive protein and distensibility did not differ by ethnicity or stage of the menopausal transition.

This study found a strong association between C-reactive protein and systemic arterial stiffness, measured by the brachial artery distensibility. These results add to the existing literature by showing a relationship between inflammation and arterial stiffness independent of artery size.

3.2 INTRODUCTION

Central arterial stiffness is thought to contribute to the age associated increase in cardiovascular disease, morbidity, and mortality[1,2]. It has been found to be associated with cardiovascular and all cause mortality in patients with hypertension, diabetes, and end stage renal disease[3-6]. Additionally, an association between central arterial stiffness and cardiovascular and overall mortality has been found in healthy older adults[7]. Most of the studies have used aortic pulse wave velocity as the indicator of arterial stiffness. A system called the Dynapulse 5000A calculates the brachial artery distensibility, a measure of systemic arterial stiffness. The clinical advantage of distensibility over pulse wave velocity is that it is less expensive and easier to perform[8]. Furthermore, aortic pulse wave velocity is a measure of central arterial stiffness whereas distensibility measures whole body arterial stiffness.

Inflammation is a major feature in the initiation and progression of atherosclerosis[9,10]. Among the existing inflammatory markers, C-Reactive protein (CRP) appears to be clinically advantageous because it is well-validated, inexpensive, and widely available[11]. Several prospective studies in a variety of populations have found a relationship between CRP and cardiovascular and overall mortality[12-17].

Studies have found an association between CRP and aortic pulse wave velocity in healthy and clinical populations[18-32]. However, aortic pulse wave velocity only represents the properties of large arteries but not the arterial system as a whole[33]. Distensibility, on the other hand, is a measure of systemic arterial stiffness that is useful when comparing arteries of different size[34]. The Study of Women's Health Across the Nation (SWAN) is a multi-site, multi-ethnic longitudinal epidemiological study designed to examine how various risk factors change during the menopausal transition. The goal of the current study is to examine the cross sectional association between CRP and distensibility within this population. This study will expand upon the current literature by exploring the relationship between inflammation and arterial stiffness independent of artery size. Because structural and functional properties vary between arteries of different size, this study could identify a relationship between inflammation and arterial stiffness that is not restricted to the large central arteries.

3.3 METHODS

3.3.1 Study Population

SWAN is a multi-site, multi-ethnic longitudinal epidemiological study designed to examine the physical, psychological, and social changes that women experience during the menopausal transition. At baseline, SWAN recruited a total of 3,302 women between the ages of 42 and 52 from seven clinical sites: Boston, Chicago, Detroit, Los Angeles, Newark, Pittsburgh, and Oakland. Participants self identified themselves into five ethnic groups including Caucasian, African American, Hispanic, Japanese, and Chinese. Women who were Caucasian or a member of a designated ethnic group were enrolled at each site, including African American women at

Boston, Chicago, Detroit, and Pittsburgh, as well as Japanese, Chinese, and Hispanic women at Los Angeles, Oakland, and Newark respectively. In order to be eligible for the study, women were required to have an intact uterus, at least one menstrual period and no use of reproductive hormones in the previous 3 months. The institutional review boards of the participating institutions approved this study, and all women signed informed consent forms before participation.

In SWAN, the Dynapulse measure was conducted at the 7th follow-up visit (approximately 7 years after the baseline visit). Thus, all analyses in this study were conducted using data from this visit. At visit 7, there were 2,320 women remaining in SWAN.

3.3.2 Brachial Artery Distensibility

The Dynapulse derived distensibility, defined as the percent change in a section of the artery per one mmHg change in pressure, is a noninvasive measure of vascular stiffness. Lower values of distensibility represent stiffer arteries. The Dynapulse system used a pulse dynamic pattern recognition methodology to determine distensibility from the oscillometric signal of a standard cuff sphygmomanometer. Participants had a special blood pressure cuff placed around their upper arm after five minutes of rest to generate a pressure waveform. This study used three cuff sizes (pediatric, normal, and adult) based on the participant's arm circumference. The pressure waveform was calibrated and incorporated into a physical model of the cardiovascular system, assuming a straight tube brachial artery and a T-tube aortic system. Data from each clinic site was entered into a computer interfaced to the Dynapulse 5000A monitoring instrument (Pulse Metric, Inc., San Diego, CA). Three measurements were performed sequentially for each participant (with a 5 minute resting period between measurements) and uploaded to the

www.dynapulse.com website directly from the monitoring instrument. Analyses of the waveforms were conducted by Pulsemetric and hemodynamic parameters in addition to distensibility were calculated. The formulas for calculating the distensibility parameter have been published and validated[35]. Briefly, the distensibility is derived from a formula that incorporates the participant's effective cuff width and brachial artery diameter, which is estimated using an empirically derived model based on gender, height, weight, and mean arterial blood pressure.

In SWAN, the Dynapulse measure was completed on 1,347 women. Reasons for not completing the measure included mechanical problems with the equipment (n=403), participant's arm size exceeding the size of the largest cuff (n=228), participants being outside of the 3 month window for collection (n=98), participants being unable or unwilling to come to the clinic site (n=172), participants refusal (n=24), or other reasons (n=146). Because of mechanical/technical problems, no measurements were completed at Newark or Detroit. Since Hispanic women were only interviewed at Newark, this study only includes Caucasian, African American, Chinese, and Japanese women. Measurements with unreadable waveforms (determined by Pulsemetric) or outside of the 25-35 second range for cuff deflation time (part of SWAN's protocol) were considered unusable. Among the women who completed the Dynapulse measure, 1,135 had at least one usable measurement. An additional 66 women were excluded because they had a hysterectomy at some point during the study. Thus, a total of 1069 women were used in the current analyses. It should be noted that due to technical problems with obtaining the measurement on extremely heavy women (i.e. largest cuff size being too small for the woman's arm), this sample is an under representation of the heavier women transitioning through

menopause. Thus, any associations found with brachial artery distensibility would likely be an underestimate of the true association within this population.

3.3.3 Cardiovascular Risk Factors

The SWAN protocol required a fasting blood draw between days 2 and 5 of the menstrual cycle for measurements of reproductive hormones, lipids and lipoproteins, insulin, glucose, and clotting factors. Blood samples were maintained at 4^0 C until separated and then frozen at -80^0 C and shipped on ice to Medical Research Laboratories, which is certified by the National Heart, Lung and Blood Institute of the Centers for Disease Control. Total cholesterol and triglycerides were analyzed with the Hitachi 747 analyzer whereas high-density lipoprotein (HDL) cholesterol was isolated using heparin-2M manganese chloride. Low-density lipoprotein cholesterol (LDL) was calculated using the Friedwald equation. Plasma glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim, Indianapolis, In) and plasma insulin was measured using solid-phase radioimmunoassay procedure (DPC Coat-A-Count, Los Angeles, CA). CRP was assessed using the Behring Nephelometer II (hs-CRP on BN 100, Dade-Behring, Marburg, Germany). Smoking status (current vs. not current) was self-reported.

3.3.4 Physical Measures

Two blood pressure measurements were averaged on the right arm after five minutes of rest in a seated position. Height was measured using a stadiometer or portable stadiometer whereas weight was measured using a balance beam, digital scale, or portable scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured over undergarments or light clothing.

3.3.5 Menopausal Status

Information on menopausal status was collected using an annual interviewer administered questionnaire. Premenopausal status was defined as having a menstrual period in the past three months with no change in cycle regularity in the past twelve months. Early perimenopausal status was defined as having a menstrual period in the past three months with some change in cycle regularity in the past twelve months. Late perimenopausal status was defined as not having a menstrual period in the past three months but having one in the past twelve months. Postmenopausal status was defined as not having a menstrual period in the past twelve months. Hormone therapy use was identified as current, former, and never. Due to small sample sizes of premenopausal (n=29) and late perimenopausal (n=112) women, a dichotomous variable was created to combine early perimenopausal with premenopausal and late perimenopausal with postmenopausal women.

3.3.6 Medication Use/Disease:

Information on all medication use and diabetes status was based on the annual interviewer-administered questionnaire. Participants classified for medication use were required to be taking the respective medication twice a week for the past month at the time of the interview. Participants classified as having diabetes were told by a doctor that they had diabetes.

3.3.7 Statistical Methods

Descriptive statistics (N, means/medians for continuous variables and percentages for categorical variables) for sociodemographic variables, anthropometric measures, lipids, blood pressures,

fasting glucose, fasting insulin, distensibility and CRP were calculated. Spearman correlations between risk factors and distensibility were calculated to understand basic associations and to determine candidate variables for multivariate models. Stepwise multivariable linear regression was used to test the association between distensibility and log transformed CRP while adjusting for confounders. In addition to CRP, fasting glucose, fasting insulin, and triglycerides were all log transformed. Due to the skewed distribution of the variable, CRP was also analyzed as tertiles. ANCOVA was used to test a trend in distensibility across CRP tertiles (Bottom Tertile=0 to 0.5 mg/L, Middle Tertile=0.6 mg/L to 1.7 mg/L, Top Tertile > 1.7 mg/L) while adjusting for confounders. Interaction terms were added to the multivariate models to determine whether or not the association between CRP and distensibility differed by ethnicity or the stage of menopausal transition. A Bonferroni post hoc multiple comparisons test was used to examine pairwise differences in mean distensibility between the CRP tertile groups. Statistical significance was assessed using a type one error rate of 0.05. Assumptions of linear regression (homoscedasticity, linearity, and normality of residuals) were tested to verify the validity of the model. All analyses were done using SAS version 8.2.

3.4 RESULTS

Characteristics of the population are presented in Tables 3-1 and 3-2. Among the 1069 women in the study, 54.0% (n=577) were Caucasian, 14.6% (n=156) were Black, 11.6% (n=124) were Chinese, and 19.8% (n=212) were Japanese. The majority of women were postmenopausal (44.2%, n=473) while only 3% (n=29) were premenopausal. The sample consisted of 171 (16%)

hormone therapy users. The mean age of the population was 53.6 ± 2.6 years. The mean distensibility was 6.2 ± 1.2 %/mmHg with values ranging from 2.9 to 12.0.

Table 3-3 indicates that women who were excluded from the analyses (n=1251) had a worse cardiovascular profile than women included in the analyses (n=1069). The excluded sample had a higher BMI and waist circumference, were taller, had higher SBP, pulse pressure and mean arterial pressure, had higher fasting glucose, fasting insulin, triglycerides, and CRP. The women who were excluded had significantly lower total cholesterol, LDL cholesterol, and HDL cholesterol than the women who were included in the analyses. A smaller proportion of blacks were included in the analyses compared to all other ethnic groups combined (22% vs 57%, $p<.05$). A smaller proportion of smokers were included in the analyses compared to nonsmokers (38% vs 48%, $p<.05$).

Table 3-4 shows that most risk factors were univariately negatively associated with distensibility. The blood pressure and body composition variables showed the strongest correlations with distensibility. CRP was negatively correlated with distensibility ($r= -0.17$, $p <.0001$).

Linear regression models showed that log-transformed CRP was associated with distensibility in both unadjusted and adjusted models (Table 3-5). Before adjustment for confounders, a one unit increase in log CRP was associated with 0.18 lower levels of distensibility ($p<.0001$). After adjustment for age, ethnicity, study site, smoking status, menopausal status, medication use, glucose, triglycerides, and hormone use, a one unit increase in log CRP was associated with 0.13 lower levels of distensibility ($P=.0011$). In addition to CRP, increasing age ($\beta=-0.08$, $p < .0001$), log transformed fasting glucose ($\beta=-0.72$, $p = .03$), and log transformed triglycerides ($\beta=-0.22$, $p = .01$) were all associated with lower distensibility after

adjustment for confounders. Before adjustment for confounders, African American women had a significantly lower mean distensibility than Chinese women (5.95 vs. 6.39, p=.02). After adjustment for confounders, there was no difference in mean distensibility between any of the ethnic groups. There was no difference in mean distensibility between women in different stages of the menopausal transition before or after adjustment for confounders.

Figure 3-1 shows that increasing CRP tertiles were associated with a decrease in adjusted mean distensibility (p for trend = .0031). After adjustment for confounders, the mean distensibility for the bottom tertile was 5.90±0.37, the middle tertile was 5.85±0.37, and the top tertile was 5.56±0.37. When looking at pairwise differences in mean distensibility between the CRP tertile groups, women in the bottom (p=.004) and middle tertiles (p=.006) of CRP had a significantly lower mean distensibility than women in the top tertile. However, women in the bottom and middle tertiles did not significantly differ in distensibility.

When stratifying by ethnicity, the association between log-transformed CRP and distensibility was similar in all ethnic groups (p for interaction = 0.92). After excluding Chinese and Japanese women, there was still no interaction effect between log-transformed CRP and ethnicity (p for interaction = 0.80). Additionally, the increasing trend in mean distensibility across CRP tertiles was similar across all ethnic groups (p for interaction = 0.92). The association between log-transformed CRP and distensibility was similar in women who were later in their transition and women who were earlier in their transition (p for interaction = 0.73).

When excluding women who had only 1 useable distensibility measurement (n=36) or greater than 3 useable distensibility measurements (n=39), continuous (p=.0006) and categorical CRP (p for trend=.003) remained strongly associated with decreasing distensibility. When excluding women with the wrong cuff size used in the distensibility measurement (n=186),

continuous (p=.0003) and categorical (p for trend=.001) CRP remained strongly associated with decreasing distensibility.

Table 3-1. Continuous Descriptive Statistics For Dynapulse Analysis

	N	Mean	Std Dev	Median	Interquartile Range
Age (years)	1069	53.6	2.6	53.4	4.1
Body Composition:					
Height (mm)	1066	161.7	6.7	161.7	9.4
BMI kg/m^2 (kg/m^2)	1064	26.3	5.2	25.4	7.0
Waist Circumference (cm)	1067	83.3	12.8	81.9	18.4
Weight (kg)	1065	69.0	15.2	66.8	20.6
Blood Pressure:					
Systolic Blood Pressure (mmHg)	1064	114.7	14.7	113.0	19.0
Diastolic Blood Pressure (mmHg)	1064	73.3	9.1	73.0	12.0
Pulse Pressure (mmHg)	1064	41.4	10.9	40.0	13.0
Mean Arterial Pressure (mmHg)	1064	87.1	10.0	86.5	13.0
Lipids:					
Total Cholesterol (mg/dL)	1002	209.7	35.6	206.0	47.0
LDL Cholesterol (mg/dL)	978	124.0	32.5	120.0	41.0
HDL Cholesterol (mg/dL)	1001	61.9	15.4	60.0	22.0
Triglycerides (mg/dL)	988	119.7	69.4	99.0	69.0
Glucose Metabolism:					
Fasting glucose (mg/dL)	998	89.5	15.5	87.0	12.0
Fasting insulin (uIU/mL)	918	10.6	6.7	9.0	5.4
Brachial Artery Distensibility (%/mmHg)	1069	6.2	1.2	6.1	1.6
C-Reactive Protein (mg/L)	947	1.8	2.0	1.0	2.2

Table 3-2. Categorical Descriptive Statistics For Dynapulse Analysis

	N	%
Ethnicity		
Blacks	156	14.6
Caucasians	577	54.0
Chinese	124	11.6
Japanese	212	19.8
Menopausal Status		
Pre	29	2.7
E.Peri	280	26.2
L.Peri	111	10.4
Post	586	54.8
Hormone Therapy Use		
Current	171	16.0
Former	217	20.3
Never	681	63.7
Current Smoker	111	10.4
Heart Medication	24	2.3
Blood Pressure Medication	175	16.4
Insulin Medication	29	2.7
Cholesterol Medication	101	9.5
Told to have Diabetes by doctor	37	3.5

Table 3-3. Comparison of Dynapulse Excluded and Included Samples

	Included Sample			Excluded Sample			
	N	**Mean**	**Median**	**N**	**Mean**	**Median**	**P-Value**
Age (years)	1069	53.6	53.4	1251	53.5	53.3	0.29
Body Composition:							
Height (mm)	1066	161.7	161.7	994	162.8	162.8	**0.0003**
BMI kg/m^2 (kg/m^2)	1064	26.3	25.4	990	31.5	30.4	**<.0001**
Waist Circumference (cm)	1067	83.3	81.9	997	94.9	92.6	**<.0001**
Weight (kg)	1065	69.0	66.8	995	83.6	79.9	**<.0001**
Blood Pressure:							
Systolic Blood Pressure (mmHg)	1064	114.7	113.0	986	120.0	118.0	**<.0001**
Diastolic Blood Pressure (mmHg)	1064	73.3	73.0	984	73.5	73.0	0.52
Pulse Pressure (mmHg)	1064	41.4	40.0	984	46.4	45.0	**<.0001**
Mean Arterial Pressure (mmHg)	1064	87.1	86.5	984	89.0	88.0	**<.0001**
Lipids:							
Total Cholesterol (mg/dL)	1002	209.7	206.0	988	202.9	201.0	**<.0001**
LDL Cholesterol (mg/dL)	978	124.0	120.0	933	120.0	117.0	**0.009**
HDL Cholesterol (mg/dL)	1001	61.9	60.0	988	58.0	56.0	**<.0001**
Triglycerides* (mg/dL)	988	119.7	99.0	943	127.7	108.0	**0.03**
Glucose Metabolism:							
Fasting glucose* (mg/dL)	998	89.5	87.0	954	98.1	89.0	**<.0001**
Fasting insulin* (uIU/mL)	918	10.6	9.0	933	16.4	12.6	**<.0001**
C-Reactive Protein* (mg/L)	947	1.8	1.0	847	2.7	1.8	**<.0001**

*Comparisons are for median values

Table 3-4. Relation of Cardiovascular Risk Factors and Brachial Artery Distensibility (Unadjusted Spearman Correlation Coefficients)

Risk Factors	Correlation Coefficient	P-value
Age	-0.15	**<.0001**
Body Composition:		
Weight	-0.17	**<.0001**
Height	0.006	.84
BMI	-0.19	**<.0001**
Waist Circumference	-0.20	**<.0001**
Blood Pressure:		
Systolic Blood Pressure	-0.50	**<.0001**
Diastolic Blood Pressure	-0.21	**<.0001**
Pulse Pressure	-0.54	**<.0001**
Mean Arterial Pressure	-0.36	**<.0001**
Lipids:		
Cholesterol	-0.07	**.03**
LDL	-0.07	**.03**
HDL	0.11	**.0006**
Triglycerides	-0.15	**<.0001**
Glucose Metabolism:		
Fasting Glucose	-0.14	**<.0001**
Fasting Insulin	-0.17	**<.0001**
Inflammatory/ Hemostatic Markers:		
C-Reactive Protein	-0.17	**<.0001**

Table 3-5. Linear Regression Models Testing the Association Between Log-Transformed CRP and Brachial Artery Distensibility

	Regression Estimate (β)	P-value
Unadjusted	-0.18	<.0001
Adjusted*	-0.14	.0004

*Adjusted for age, ethnicity, site, smoking status, menopausal status, hormone therapy use, log transformed triglycerides, log tranformed fasting blood glucose, medications, and diabetes

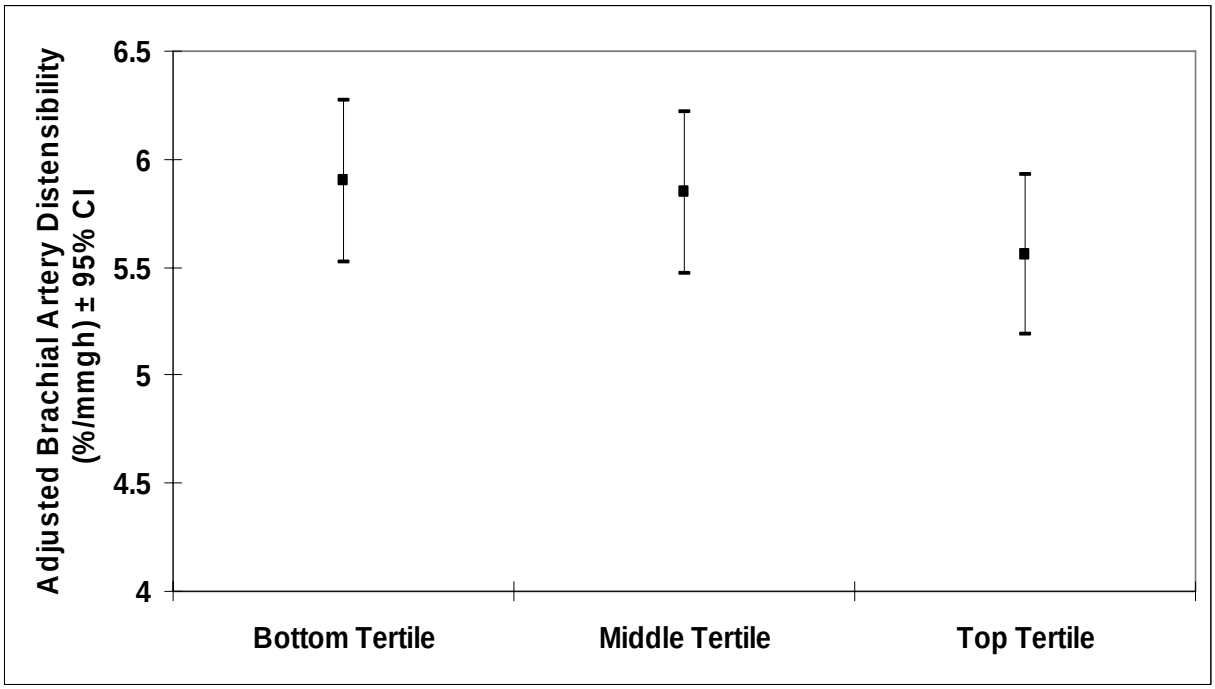

Figure 3-1. Adjusted* Mean Brachial Artery Distensibility by CRP Tertiles†

* Adjusted for age, ethnicity, site, smoking status, menopausal status, hormone therapy use, triglycerides, fasting blood glucose, medications, and diabetes
† Bottom Tertile=0 to 0.5 mg/L; Middle Tertile=0.6 to 1.7 mg/L; Top Tertile > 1.7 mg/L

3.5 DISCUSSION

The primary finding of this study is that CRP was associated with distensibility in a cohort of healthy middle aged women. This relationship persisted whether analyzing CRP as tertiles or as a log transformed continuous variable.

Several studies have found a relationship between CRP and arterial stiffness in a variety of populations. Two recent studies have shown CRP to be related to arterial stiffness in a cohort of untreated hypertensive patients[19,28]. Another study found a number of acute phase proteins (including CRP) to be associated with arterial stiffness in patients with type 2 diabetes mellitus[29]. Several studies have shown a relationship between CRP and arterial stiffness in individuals with an elevated level of systemic inflammation[25,27]. One study found CRP to be a significant positive predictor of increased large artery stiffness in subjects with the metabolic syndrome[21]. The Rotterdam Study found CRP to be associated with increased aortic stiffness in older adults[18]. Finally, two studies examining healthy individuals found a positive relationship between CRP and increased arterial stiffness[22,32]. The current study is the first to examine the relationship between CRP and distensibility.

The Dynapulse parameters have been validated in normotensive[35], hypertensive[36], and hemodialysis patients[8]. Although the research examining the association between brachial artery distensibility and cardiovascular risk factors is limited, a few studies have found an association in healthy populations. Researchers from the Bogulasa Heart Study found a strong negative association between blood pressure and distensibility and weaker associations between age and distensibility among healthy young adults[37]. Additionally, they found that a clustering of cardiovascular risk factors was more strongly associated with a reduction in distensibility than a single risk factor alone[38]. Budoff et al. found an association between decreased brachial

distensibility and higher levels of coronary calcification—indicating the role of distensibility in identifying patients with atherosclerotic burden[39].

There are several potential biological mechanisms that could link increased inflammation to increased arterial stiffness. First, inflammation is known to impair endothelial functioning, which would result in a decreased bioavailability of nitric oxide[40]. Nitric oxide is a vasodilator that controls smooth muscle relaxation, a functional property of elastic arteries. Second, interleukin-6 and tumor necrosis factor α, two cytokines known to stimulate the synthesis of CRP by hepatocytes, may directly cause glomerular damage and loss of kidney function[41,42]. Several studies have shown increased arterial stiffness in individuals with kidney failure as a result of smooth muscle cell calcification[43]. Finally, inflammation could cause structural damage to the vessels by promoting defragmentation of the elastin fibers and increased deposition of the collagen fibers[28].

Because of known ethnic differences in CRP[44], the present study examined whether or not the effect of CRP on distensibility differed by ethnicity. The association between CRP and distensibility was similar across all ethnic groups within this population. However, it must be noted that many of the heavier African American women in this study were excluded because of technical problems in obtaining an appropriately sized cuff.

There are several limitations in the current study. First, the cross-sectional design of the study prevents the determination of the direction of the association between CRP and distensibility. Second, there is a definite bias effect such that the sample used in this study has a better cardiovascular profile than the excluded sample. The main reason for this is the inability to obtain the distensibility measurement on very heavy women due to the arm size exceeding the largest available cuff. Thus, conclusions based on the results of the current study are limited to

women with a generally healthier cardiovascular profile. However, the associations between CRP and distensibility found in this study are probably an underestimate of the true association that would be found with a more representative sample. Third, the SWAN protocol asked for exactly three measurements per woman. Due to the fact that some oscillometric tracings were rejected by the Dynapulse software, some women had less than or greater than three measurements. However, when excluding women with less than two or greater than three measurements, there was still a strong association between CRP and distensibility. Fourth, due to technical issues, an incorrect cuff size was used on several women. However, results were similar after excluding these women from the analyses. Finally, the current study did not adjust for body size or blood pressure variables because they directly incorporated in the formula used to calculate distensibility. Thus, adjustment for these parameters would likely result in an over adjustment.

This study is unique because it explores the relationship between an established marker of inflammation and a new marker of systemic arterial stiffness. Previous studies have found an association between inflammation and arterial stiffness but these studies were not able to establish an effect independent of artery size. In fact, the majority of these studies only focused on the aorta. Because of structural and functional differences between different arteries, this study can provide an early step in identifying CRP as a risk factor for atherosclerosis in different arterial beds. Future prospective studies are needed to explore the nature and direction of this relationship in more detail.

3.6 REFERENCES FOR CHAPTER 3

1. Arnett DK, Evans GW, Riley WA. Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol* 1994;140:669-682.

2. O'Rourke M. Mechanical principles in arterial disease. *Hypertension* 1995;26:2-9.

3. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int* 2003;63:1852-1860.

4. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension* 1998;32:570-574.

5. Shoji T, Emoto M, Shinohara K, Kakiya R, Tsujimoto Y, Kishimoto H, Ishimura E, Tabata T, Nishizawa Y. Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol* 2001;12:2117-2124.

6. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001;37:1236-1241.

7. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 2005;111:3384-3390.

8. Motiwala S, Brewster UC, Perazella MA, Peixoto AJ. Reliability of a noninvasive device to measure systemic hemodynamics in hemodialysis patients. *Blood Press Monit* 2006;11:33-36.

9. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999;138:S419-S420.

10. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-874.

11. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499-511.

12. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973-979.

13. Ridker PM. C-reactive protein and risks of future myocardial infarction and thrombotic stroke. *Eur Heart J* 1998;19:1-3.

14. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-428.

15. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-843.

16. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557-1565.

17. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiol Clin* 2003;21:315-325.

18. Mattace-Raso FU, van der Cammen TJ, van dM, I, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis* 2004;176:111-116.

19. Kampus P, Muda P, Kals J, Ristimae T, Fischer K, Teesalu R, Zilmer M. The relationship between inflammation and arterial stiffness in patients with essential hypertension. *Int J Cardiol* 2005.

20. Duprez DA, Somasundaram PE, Sigurdsson G, Hoke L, Florea N, Cohn JN. Relationship between C-reactive protein and arterial stiffness in an asymptomatic population. *J Hum Hypertens* 2005;19:515-519.

21. Tomiyama H, Koji Y, Yambe M, Motobe K, Shiina K, Gulnisa Z, Yamamoto Y, Yamashina A. Elevated C-reactive protein augments increased arterial stiffness in subjects with the metabolic syndrome. *Hypertension* 2005;45:997-1003.

22. Tomiyama H, Arai T, Koji Y, Yambe M, Hirayama Y, Yamamoto Y, Yamashina A. The relationship between high-sensitive C-reactive protein and pulse wave velocity in healthy Japanese men. *Atherosclerosis* 2004;174:373-377.

23. Pirro M, Schillaci G, Savarese G, Gemelli F, Vaudo G, Siepi D, Bagaglia F, Mannarino E. Low-grade systemic inflammation impairs arterial stiffness in newly diagnosed hypercholesterolaemia. *Eur J Clin Invest* 2004;34:335-341.

24. Arroyo-Espliguero R, Mollichelli N, Avanzas P, Zouridakis E, Newey VR, Nassiri DK, Kaski JC. Chronic inflammation and increased arterial stiffness in patients with cardiac syndrome X. *Eur Heart J* 2003;24:2006-2011.

25. Wong M, Toh L, Wilson A, Rowley K, Karschimkus C, Prior D, Romas E, Clemens L, Dragicevic G, Harianto H, Wicks I, McColl G, Best J, Jenkins A. Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease risk factors and inflammation. *Arthritis Rheum* 2003;48:81-89.

26. Selzer F, Sutton-Tyrrell K, Fitzgerald S, Tracy R, Kuller L, Manzi S. Vascular stiffness in women with systemic lupus erythematosus. *Hypertension* 2001;37:1075-1082.

27. Booth AD, Wallace S, McEniery CM, Yasmin, Brown J, Jayne DR, Wilkinson IB. Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. *Arthritis Rheum* 2004;50:581-588.

28. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 2005;46:1118-1122.

29. Wakabayashi I, Masuda H. Association of acute-phase reactants with arterial stiffness in patients with type 2 diabetes mellitus. *Clin Chim Acta* 2006;365:230-235.

30. Kullo IJ, Seward JB, Bailey KR, Bielak LF, Grossardt BR, Sheedy PF, Peyser PA, Turner ST. C-reactive protein is related to arterial wave reflection and stiffness in asymptomatic subjects from the community. *Am J Hypertens* 2005;18:1123-1129.

31. Nagano M, Nakamura M, Sato K, Tanaka F, Segawa T, Hiramori K. Association between serum C-reactive protein levels and pulse wave velocity: a population-based cross-sectional study in a general population. *Atherosclerosis* 2005;180:189-195.

32. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004;24:969-974.

33. van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension* 2000;35:637-642.

34. Izzo JL, Jr., Shykoff BE. Arterial stiffness: clinical relevance, measurement, and treatment. *Rev Cardiovasc Med* 2001;2:29-40.

35. Brinton TJ, Cotter B, Kailasam MT, Brown DL, Chio SS, O'Connor DT, DeMaria AN. Development and validation of a noninvasive method to determine arterial pressure and vascular compliance. *Am J Cardiol* 1997;80:323-330.

36. Brinton TJ, Kailasam MT, Wu RA, Cervenka JH, Chio SS, Parmer RJ, DeMaria AN, O'Connor DT. Arterial compliance by cuff sphygmomanometer. Application to hypertension and early changes in subjects at genetic risk. *Hypertension* 1996;28:599-603.

37. Urbina EM, Brinton TJ, Elkasabany A, Berenson GS. Brachial artery distensibility and relation to cardiovascular risk factors in healthy young adults (The Bogalusa Heart Study). *Am J Cardiol* 2002;89:946-951.

38. Urbina EM, Kieltkya L, Tsai J, Srinivasan SR, Berenson GS. Impact of multiple cardiovascular risk factors on brachial artery distensibility in young adults: the Bogalusa Heart Study. *Am J Hypertens* 2005;18:767-771.

39. Budoff MJ, Flores F, Tsai J, Frandsen T, Yamamoto H, Takasu J. Measures of brachial artery distensibility in relation to coronary calcification. *Am J Hypertens* 2003;16:350-355.

40. Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, Donald AE, Palacios M, Griffin GE, Deanfield JE, MacAllister RJ, Vallance P. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 2000;102:994-999.

41. Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G. Tumor necrosis factor induces glomerular damage in the rabbit. *Am J Pathol* 1989;134:419-430.

42. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998;339:1448-1456.

43. Safar ME, London GM, Plante GE. Arterial stiffness and kidney function. *Hypertension* 2004;43:163-168.

44. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr., Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol* 2005;46:464-469.

4.0 C-REACTIVE PROTEIN IS ASSOCIATED WITH INCIDENT PERIPHERAL ARTERIAL DISEASE IN AN ELDERLY COHORT

To Be Submitted to Atherosclerosis

4.1 ABSTRACT

Peripheral arterial disease, an indicator of early atherosclerosis, is a highly prevalent disease among older adults. Inflammation appears to play an important role in all stages of atherosclerosis. Few studies have prospectively examined the association between inflammation and PAD and these studies have not looked at racial differences in the association.

The association between three inflammatory markers (C-Reactive Protein, Interleukin-6 and Tumor Necrosis Factor-alpha) and 3 year incidence of PAD was evaluated in 1918 older adults enrolled in the Health Aging, Body, and Composition study (HABC). The mean age of the population was 73.4 (s.d = 2.8). Approximately half of the population was male (48.1%, n=922) and a third was black (35.2%, n=675). C-Reactive Protein was significantly associated with three year higher incidence of PAD after adjustment for confounders (Top Tertile vs Bottom Tertile Odds Ratio = 2.00; Middle Tertile vs Bottom Tertile Odds Ratio = 1.90). Furthermore, there was a significant interaction effect between C-Reactive Protein and ethnicity (p for interaction = .0047) such that the association between C-Reactive Protein and PAD was stronger in whites than in blacks. Tumor Necrosis Factor-alpha and Interleukin-6 were not associated with incident PAD.

High levels of C-Reactive protein were associated with a higher 3 year incidence of PAD in a population based elderly cohort. Measures to reduce inflammation should be tested to determine if this can alleviate the burden of subclinical atherosclerosis within this population.

4.2 INTRODUCTION

Peripheral arterial disease (PAD) is common in older adults and is a strong indicator of generalized atherosclerosis. Because most individuals with PAD are asymptomatic[1,2], the ankle-brachial index (ABI) is a useful non-invasive measure of PAD and shown to predict cardiovascular disease and mortality[3-7].

Inflammation is a feature in the onset, development, and evolution of atherosclerotic lesions[8,9]. Inflammatory markers, including C-reactive protein (CRP), Interleukin-6 (IL-6), and Tumor Necrosis Factor Alpha (TNF-α), have been found to be prospectively associated with cardiovascular outcomes in both clinical and healthy populations[10-18].

Few studies have examined the association between inflammatory markers and PAD and the results from these studies have been equivocal[19-29]. Only one prospective study has examined the association between inflammation and ABI[21] but this study focused on progression of PAD rather than incidence of PAD. Because of the high prevalence in older adults and the long-term consequences associated with the disease, it is essential to discover modifiable risk factors strongly associated with PAD. Given the treatment and prevention methods available to alleviate the burden of inflammation, it may provide a target for reducing the burden of PAD and subsequently cardiovascular disease.

Prevalence rates of PAD have been shown to vary by ethnicity[2,30] with blacks having a higher prevalence than Caucasians. CRP has also been shown to vary by ethnicity with blacks having higher levels than whites[31]. No study has looked at whether or not the association between CRP and incident PAD differs according to ethnicity.

The purpose of this study is to prospectively examine the association between three inflammatory markers (CRP, IL-6, and TNF-α) and 3 year incidence of PAD in a population

based elderly cohort. Furthermore, racial differences in the association between inflammation and incident PAD within this population can be evaluated. The prospective nature of the study allows the evaluation of a temporal relationship between inflammation and PAD.

4.3 METHODS

4.3.1 Study Population

Health ABC is a prospective investigation of interrelationships among health conditions, body composition, social and behavioral factors, and functional change in a population of well-functioning black and white men and women between the age of 70 and 79. Each of the two study sites, Pittsburgh, PA, and Memphis, TN, recruited participants from a list of Medicare beneficiaries between April 1997 and June 1998. The goal of recruitment was to have a cohort of highly functional older people at baseline that was nearly balanced among men and women and white and black individuals. Race status was obtained from the Health Care Financing Administration (now the Centers for Medicare and Medicaid Services) database; recruitment was at random among all age-eligible individuals within each stratum of race (black and white). Inclusion criteria were (1) ability to walk one quarter mile, climb 10 steps, and perform basic activities of daily living without difficulty; (2) absence of life-threatening illness; and (3) plans to remain in the geographic area for at least 3 years. The cohort enrolled 3075 participants who completed baseline evaluations, 42% of whom were black and 48% of whom were men.

The Health ABC study measured ABI at baseline and at a 3 year follow-up visit. Because this study was examining incident PAD, participants with an ABI < 0.9 (indicative of PAD) at baseline were excluded. Participants with an ABI > 1.5, a possible indicator of poorly

compressible arteries[2], were also excluded. Finally, participants who had peripheral revascularization were excluded. Among the 3075 participants at baseline, 2873 had a measured ABI. Among the 202 participants with a missing ABI, 1 was due to an amputated leg, 58 were due to an inability to locate the tibial artery, 10 were due to an ulceration, 67 due to an unoccludable artery, and 66 for other reasons. An additional 86 participants were excluded because they had an ABI > 1.5 (n=35) or peripheral revascularization (n=51). Thus, a total of 2787 participants were included in the baseline analyses. Among the participants with a measured ABI, 2438 had an ABI greater than 0.9 in both legs. At the 3 year follow-up visit, 495 participants who had an ABI measured at baseline did not have it measured. Among these participants, 118 had died, 2 had an amputated leg, 8 were due to an inability to locate the tibial artery, 1 had an ulceration, 12 had unoccludable arteries, and 354 missed the clinic visit for other reasons. Finally, an additional 25 participants were excluded because they had an ABI > 1.5 at the follow-up (n=4) visit or peripheral revascularization at baseline (n=21). Thus, 1918 participants were included in the longitudinal analyses (see Figure 4-1).

4.3.2 Inflammatory Markers

Measures of IL-6, TNF-α, and CRP were performed using ELISA kits from R&D Systems (Minneapolis, MN). Detectable limits were 0.10 pg/ml for IL-6, 0.18 pg/ml for TNF-α, and 0.007 mg/L for CRP. Interassay coefficient of variation were determined by duplicate analyses of 150 specimens; 10.3, 8.0, and 15.8% for IL-6, CRP, and TNF-α, respectively. Participants with CRP values greater than 10.0 mg/L (n=146) could represent cases of acute inflammation and were thus excluded from all analyses involving CRP.

4.3.3 Ankle-Brachial Index

The ABI is a noninvasive procedure used to detect peripheral arterial disease, a condition in which atherosclerosis obstructs blood flow to the legs. It was measured by a hand-held, 8-MHz Doppler probe placed directly over the artery and a conventional mercury sphygmomanometer. Two measurements were taken on the right brachial artery, the right posterior tibial artery, and the left posterior tibial artery. First, the average brachial systolic blood pressure was determined. Next, the average right posterior tibial systolic blood pressure was determined. Then, the average left posterior tibial systolic blood pressure was determined. Finally, the ABI for each leg was calculated as the ratio of the average posterior tibial systolic blood (for the respective leg) to the average brachial systolic blood pressure. Participants with a ratio of less than or equal to 0.9, in either leg, were considered to have a low ABI[32].

4.3.4 Outcome Definition

Since ABI was measured at baseline and 3 year follow-up, we were able to examine the effect of inflammatory markers at baseline on incident PAD. Participants who developed incident PAD were defined as having normal ABI (ABI between 0.9 and 1.5 in at least one leg) at baseline and low ABI (ABI less than 0.9 in the same leg) at the follow up visit with a minimum decline in ABI of 0.15. A minimum value of 0.15 was determined by studies in clinical populations[33-35]. Participants who did not develop incident PAD were defined as either having normal ABI at baseline and normal ABI at the follow up visit or normal ABI at baseline and low ABI at the follow up visit with a less than 0.15 decline in ABI.

4.3.5 Covariates

Information was collected at baseline on sociodemographic fators, lifestyle factors (smoking and drinking status), self-reported comorbid conditions (diabetes defined by use of hypoglycemic agents, self-report, fasting plasma glucose ≥ 126 mg/dl or an oral glucose tolerance test ≥ 200 mg/dl; hypertension defined by either self-report plus use of antihypertensive medications, or measured SBP ≥ 140 or DBP ≥ 90; congestive heart failure, coronary heart disease, cerebrovascular disease, or myocardial infarction by self report). Glucose, insulin, triglycerides total cholesterol, and HDL cholesterol were all measured by a colorimetric technique on a Johnson & Johnson Vitros 950 analyzer. LDL cholesterol was calculated using the Fridewald equation. Baseline blood draws were taken after an 8-h fast. Samples were then aliquotted and stored at -80^0 C until analysis; all transportation was conducted on dry ice. Medications were brought in by the participant and recorded. Carotid-Femoral pulse wave velocity was assessed by Doppler probes placed at the carotid and femoral arteries.

4.3.6 Statistical Methods

All analyses were performed using SAS ver 8.2. Low ABI, an indicator of PAD, was defined as an ABI < 0.9 for at least one leg. Normal ABI was defined as an ABI between 0.9 and 1.5 for both legs. Participants with an ABI > 1.5 in either leg were excluded from all analyses. Chi-squared/Fisher's exact and t-test/Wilcoxon Rank Sum test were used to compare categorical and continuous baseline risk factors between those with and without baseline PAD. Fisher's exact test was used to compare the percent of participants who developed incident PAD between the top and bottom tertile of risk factors. Additionally, this test was used to compare the percent of

participants who developed incident PAD between categories of dichotomous variables. Because of their skewed distribution, inflammatory markers were categorized into tertiles. The tertile categories were as follows: CRP (Bottom Tertile = 0.15 mg/L to 1.08 mg/L, Middle Tertile = 1.09 mg/L to 2.18 mg/L, Top Tertile = greater than 2.18 mg/L), IL-6 (Bottom Tertile = 0.25 pg/ml to 1.333 pg/ml, Middle Tertile = 1.334 pg/ml to 2.190 pg/ml, Top Tertile= greater than 2.190 pg/ml), TNF-α (Bottom Tertile= 0.57 pg/ml to 2.65 pg/ml, Middle Tertile = 2.66 pg/ml to 3.63 pg/ml, Top Tertile = greater than 3.63 pg/ml). Multivariate logistic regression was used to analyze the independent association between tertiles of inflammatory markers and incident PAD while adjusting for confounders. Odds ratios (with 95 % confidence intervals) were calculated to compare the odds of developing PAD for the highest two inflammatory marker tertiles relative to the lowest tertile. An interaction between CRP and race was added to the model to test whether the association between CRP and incident PAD differed according to race. The Hosmer-Lemeshow test was run to assess model fit.

4.4 RESULTS

Characteristics of the population stratified by baseline ABI status are presented in Tables 4-1 and 4-2. Among the participants at baseline, 13.6% (n=380) had low ABI. The low ABI group was significantly older, had higher SBP, heart rate, hemoglobin a1c, fating glucose, pulse wave velocity, CRP, and IL-6 than the normal ABI group. In comparison to the normal ABI group, the low ABI group had a higher percentage of blacks, smokers, participants with a history of stroke, coronary heart disease, congestive heart failure, and participants taking medication. In addition, the low ABI group was less educated and had lower family income than the normal

ABI group. After adjustment for race, gender, age, BMI, SBP, hemoglobin a1c, fasting glucose, income, pulse wave velocity, smoking status, and comorbidities, none of the inflammatory markers were associated with baseline PAD.

The mean duration of study time was 3.03 years. Among the participants with a measured baseline ABI between 0.9 and 1.5 in both legs (n=2407), 79.7% (n=1918) had a calculated follow up ABI that was less than 1.5 in both legs. Within this group, 8.4% (n=162) developed incident PAD at the follow up visit. Table 4-3 compares incidence of PAD between participants in the top and bottom tertiles of a given risk factor. Participants in the top tertile of age, SBP, fasting glucose, hemoglobin, pulse wave velocity, and CRP had significantly higher frequency of incident PAD than participants in the bottom tertile of the respective risk factor. Furthermore, blacks, non-drinkers, participants with lower income, participants with comorbidities, and participants taking insulin had significantly higher frequency of incident PAD than their respective counterparts (Table 4-4).

The baseline participants who were not included in the follow-up analyses (n=869) were significantly older, had higher SBP, hemoglobin a1c, fasting glucose, pulse wave velocity, CRP, IL-6, and TNF-α than those who were included in follow-up analyses (n=1918). Participants who were highly educated, had higher family income levels, and did not smoke were more likely to be included in the follow-up analyses. Additionally, a higher percentage of whites than blacks were included in the follow-up analyses.

Table 4-5 shows results from the multivariate logistic regression models characterizing the association between baseline inflammatory marker tertiles and incident PAD. CRP was univariately associated with incident PAD (Odds Ratio for Top Tertile vs Bottom Tertile = 1.87; Middle Tertile vs Top Tertile = 1.67). However, IL-6 and TNF-α were not significantly

associated with incident PAD. CRP remained significantly associated with incident PAD after adjustment for confounders (Odds Ratio for Top Tertile vs Bottom Tertile = 2.00; Middle Tertile vs Bottom Tertile = 1.90). In addition to CRP, male gender (p=.05), older baseline age (p=.009), higher baseline BMI (p=.04), higher baseline SBP (p=.001), and higher baseline hemoglobin a1c (p=.05) were all multivariately associated with incident PAD. When restricting the sample to individuals without any history of cardiovascular disease, results did not change. There was a significant interaction between race and CRP (p-value for interaction = .0047) such that CRP was associated with incident PAD in whites but not in blacks. In whites, CRP was strongly associated with incident PAD (Odds Ratio for Middle Tertile vs Bottom Tertile = 3.94, Top Tertile vs Bottom Tertile = 5.41). In blacks, CRP was not associated with incident PAD (Odds Ratio for Middle Tertile vs Bottom Tertile = 1.05, Top Tertile vs Bottom Tertile = 0.74). Figure 4-2 shows incidence of PAD across CRP tertiles stratified by ethnicity. Among blacks, incidence of PAD did not differ by CRP (test of trend p-value = 0.68). Among whites, incidence of PAD increased with increasing CRP tertiles (test of trend p-value = 0.0002). When stratifying by gender, a similar trend was found among men and women. However, due to small sample size, the interaction between CRP and ethnicity stratified by gender was not statistically tested.

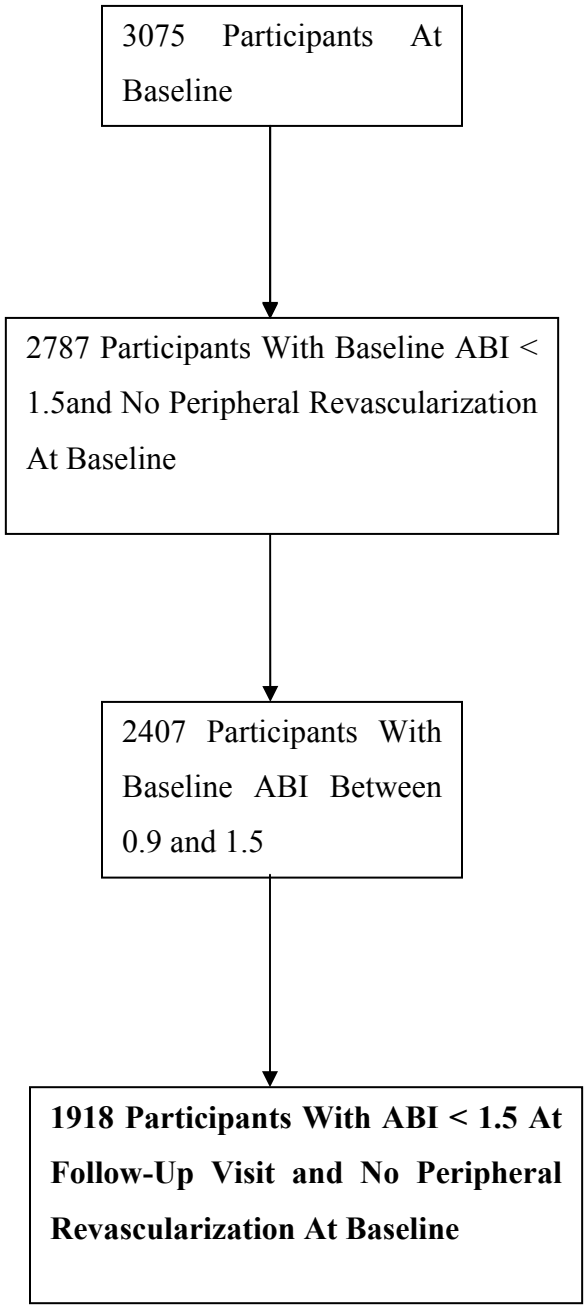

Figure 4-1. Diagram of Participants In the Study

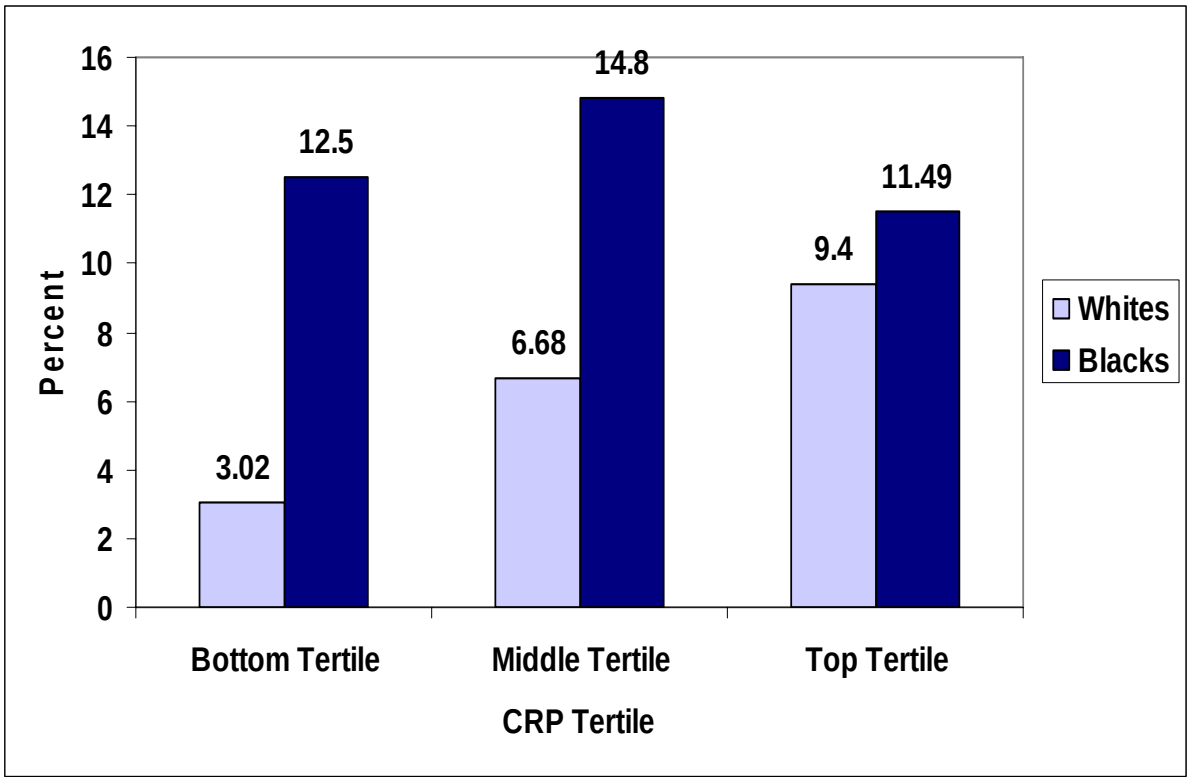

Figure 4-2. Percent of Incident PAD by CRP Tertile and Race

Table 4-1. Continuous Baseline Characteristics of the Health ABC Study Sample

	Full Group (N=2787)	Normal† ABI (N=2407)	Low‡ ABI (N=380)	P-value for Difference
Age (years)	73.6±2.9	73.5±2.9	74.2±2.8	**<.0001**
Body Composition:				
Height (mm)	1661.9±93.5	1662.8±93.5	1656.2±93.0	0.20
BMI (kg/m^2)	27.4±4.8	27.4±4.7	27.3±5.0	0.63
Waist Circumference (cm)	99.5±13.2	99.5±13.3	99.1±13.0	0.59
Blood Pressure:				
Systolic Blood Pressure (mmHg)	135.4±20.6	134.3±19.8	142.9±23.4	**<.0001**
Diastolic Blood Pressure (mmHg)	71.4±11.7	71.4±11.6	71.8±12.0	0.50
Lipids:				
Total Cholesterol (mg/dL)	203.2±38.6	202.8±38.4	205.7±39.6	0.18
LDL Cholesterol (mg/dL)	121.8±34.8	121.2±34.7	125.8±35.0	**0.02**
HDL Cholesterol* (mg/dL)	51.0 (21.0)	51.0 (21.0)	50.0 (19.0)	0.32
Triglycerides* (mg/dL)	119.0 (76.0)	119.0 (76.0)	116.0 (76.0)	0.91
Glucose Metabolism:				
Fasting glucose* (mg/dL)	94.0 (18.0)	94.0 (17.0)	97.0 (28.0)	**0.0008**
Fasting insulin* (uIU/mL)	6.90 (5.40)	6.90 (5.40)	7.30 (5.6)	0.28
Hemoglobin a1c	6.33±1.1	6.26±1.0	6.73±1.3	**<.0001**
PWV* (cm/sec)	804.0 (407.0)	789.6 (398.2)	890.9 (444.0)	**<.0001**
Inflammatory Markers:				
C-Reactive Protein* (mg/L)	1.58 (1.8)	1.52 (1.8)	1.82 (1.9)	**0.005**
Interleukin-6 (pg/mL)*	1.80 (1.5)	1.76 (1.5)	2.19 (2.1)	**0.0006**
Tumor Necrosis Factor Alpha (pg/mL)*	3.16 (1.7)	3.14 (1.6)	3.27 (1.9)	0.12

* Values are median (Interquartile Range)
† ABI between 0.9 and 1.5 for both begs
‡ ABI less than 0.9 in at least one leg

Table 4-2. Categorical Baseline Characteristics of the Health ABC Study Sample

	Full Group (N=2787)		Normal† ABI (N=2407)		Low‡ ABI (N=380)		P-value for Difference
	N	%	N	%	N	%	
Males	1334	47.9	1153	47.9	181	47.6	0.96
Blacks	1123	40.3	895	37.2	228	60.0	**<.0001**
Education:							**<.0001**
< High School	666	24.0	542	22.6	124	32.8	
HS Grad	911	32.8	783	32.6	128	33.9	
Postsecondary	1202	43.3	1076	44.8	126	33.3	
Family Income:							**<.0001**
< 10K	311	12.7	249	11.7	62	18.7	
10K-25K	947	38.6	800	37.7	147	44.3	
25K-50K	793	32.3	699	33.0	94	28.3	
>=50K	402	16.4	373	17.6	29	8.7	
Current Smoking Status	278	10.0	201	8.4	77	20.3	**<.0001**
Current Drinking Status	1395	50.3	1235	51.5	160	42.6	**.002**
Comorbidities:							
History of Stroke	197	7.2	152	6.4	45	12.2	**<.0001**
History of CHD	423	16.0	329	14.4	94	26.3	**<.0001**
History of Congestive Heart Failure	34	1.3	24	1.0	10	2.8	**0.009**
Diabetes	401	14.4	301	12.6	100	26.4	**<.0001**
Hypertension	1202	48.3	985	45.9	217	63.3	**<.0001**
Medication Use:							
ACE Inhibitors	411	14.8	327	13.6	84	22.2	**<.0001**
Insulin Meds	94	3.4	68	2.8	26	6.9	**<.0001**
Statins	362	13.0	296	12.3	66	17.4	**0.008**

† ABI between 0.9 and 1.5 for both begs
‡ ABI less than 0.9 in at least one leg

Table 4-3. Comparison of Incidence of PAD Between the Top and Bottom Tertiles of Risk Factors

	Number (Percent) of Participants In Bottom Tertile Who Developed Incident PAD	Number (Percent) of Participants In Top Tertile Who Developed Incident PAD	P-Value For Difference
Age	40 (6.5)	71 (10.8)	**0.007**
Body Composition:			
Height	52 (8.2)	45 (7.1)	0.46
BMI	62 (9.7)	58 (9.1)	0.78
Waist Circumference	62 (9.7)	51 (8.0)	0.28
Blood Pressure:			
Systolic Blood Pressure	36 (5.7)	84 (12.8)	**<.0001**
Diastolic Blood Pressure	47 (7.4)	64 (10.0)	0.11
Lipids:			
Total Cholesterol	55 (8.8)	60 (9.5)	0.70
LDL Cholesterol	51 (8.2)	60 (9.7)	0.43
HDL Cholesterol	57 (8.9)	56 (8.7)	0.91
Triglycerides	63 (9.8)	50 (7.8)	0.24
Glucose Metabolism:			
Fasting glucose	46 (7.2)	70 (10.8)	**0.03**
Fasting insulin	55 (9.2)	47 (8.0)	0.47
Hemoglobin a1c	35 (5.3)	72 (12.3)	**<.0001**
PWV	20 (3.8)	61 (11.5)	**<.0001**
Inflammatory Markers:			
C-Reactive Protein	35 (5.8)	63 (10.3)	**.0043**
Interleukin-6	49 (8.1)	62 (10.2)	0.20
Tumor Necrosis Factor Alpha	46 (7.7)	51 (8.6)	0.67

Table 4-4. Comparison of Incidence of PAD Between Categorical Risk Factors

	Number Who Developed Incident PAD	% Who Developed Incident PAD	P-Value
Gender			0.74
Males	80	8.7	
Females	82	8.2	
Race			**<.0001**
Blacks	85	12.6	
Whites	77	6.2	
Education			**0.05**
< High School	43	10.7	
Postsecondary	66	7.4	
Income			**<.0001**
<10 K	26	14.4	
> 50 K	11	3.4	
History of Stroke			**0.03**
Yes	22	13.0	
No	140	8.0	
History of Diabetes			**0.03**
Yes	28	12.6	
No	134	7.9	
History of Hypertension			**0.007**
Yes	75	9.9	
No	60	6.2	
Smoker at Baseline			0.20
Yes	16	11.5	
No	146	8.2	
Drinker at Baseline			**0.001**
Yes	66	6.5	
No	96	10.7	

Table 4-5. Odds Ratios (95% C.I.) Describing the Associations Between Inflammatory Markers and Incident PAD

	Unadjusted	Adjusted*
CRP Tertile		
Bottom (reference)	1.00	1.00
Middle	1.67 (1.02,2.58)	1.90 (1.07,3.38)
Top	1.87 (1.22,2.89)	2.00 (1.10,3.62)
IL-6 Tertile		
Bottom (reference)	1.00	1.00
Middle	0.91 (0.60,1.38)	0.78 (0.44,1.38)
Top	1.30 (0.87,1.92)	1.00 (0.58,1.71)
TNF-α Tertile		
Bottom (reference)	1.00	1.00
Middle	1.27 (0.84,1.90)	0.93 (0.55,1.59)
Top	1.12 (0.74,1.70)	0.76 (0.43,1.32)

*Adjusted for Race, Gender, Age, SBP, Baseline ABI, Time Since Baseline, Hemoglobin a1c, Glucose, BMI, Pulse Wave Velocity, Baseline Smoking History, Baseline Family Income Status, and Comorbidities (History of Diabetes, History of Stroke, History of Myocardial Infarction, History of Congestive Heart Failure, History of Coronary Heart Disease)

4.5 DISCUSSION

This study examined the association between three baseline inflammatory markers (CRP, IL-6, and TNF-α) and development of PAD after 3 years in a healthy elderly population. The primary finding of this study is that CRP was associated with the development of incident PAD. Furthermore, the association was stronger in whites than blacks. On the other hand, IL-6 and TNF-α were not associated with the development of PAD.

Several studies have looked at the cross-sectional association between IL-6, CRP, and TNF-α with PAD and findings have been equivocal[19,20,22-24,27-29,36,37]. McDermott et al showed that participants with PAD had higher levels of IL-6 (1.65 vs 1.37 pg/mL, p=.026) and CRP (3.18 vs 2.56 mg/dl, p=.043) compared to those without PAD[20]. In another study, Mcdermott et al. found CRP to be inversely associated with ABI in patients without cardiac or cerebrovascular disease but not in patients with clinically evident cardiac or cerebrovascular disease[19]. Yu et al.[22] found CRP to be associated with PAD in diabetic patients. However, this group did not find a significant relationship between IL-6 and PAD. A few studies have confirmed no relationship between TNF-α and PAD[28,37]. Although there is extensive literature on the cross-sectional association between inflammation and PAD, there have only been a few prospective investigations examining this association[21,25,26]. Furthermore, only one of these studies[21] used ABI as an indicator of PAD, which would capture the asymptomatic cases. However, this study measured ABI continuously and thus focused more on progression than incidence of PAD. Another limitation of this study was its inability to examine ethnic differences in the association.

The incidence of PAD after 3 years of follow-up in this population was 8.4% which is consistent with another study looking at older adults[38]. ABI decline was defined as the difference between ABI at baseline and 3 year follow-up in the same leg. Incident PAD was

defined as a decline in ABI (from baseline to follow-up) to 0.9 of at least 0.15, a value derived from clinical populations[33-35].

In the present study, neither IL-6 nor TNF-α were associated with incident PAD. One prospective study found IL-6 to be a stronger predictor than CRP of atherosclerotic progression[21]. However, another study found CRP and not IL-6 to be associated with PAD in diabetic patients[22]. Other studies have not found IL-6 and TNF-α to be increased in patients affected by uncomplicated PAD[28,37]. It could be theorized that IL-6 and TNF-α act at a later stage in the atherosclerotic process and more involved in progression and rupture of the lesion. Given the age range within this study, any relationship between IL-6 and PAD could be confounded by diabetes.

Contrary to one study[23], this study found that the association between CRP and incident PAD was stronger in whites than in blacks. This is not surprising for an elderly population since many other risk factors could contribute to the development of PAD among blacks. Thus, any effect of CRP would be washed out by confounders.

There are a number of potential biological mechanisms whereby CRP could lead to the development of PAD. There is evidence of a direct role for CRP at all stages of the atherosclerotic process. CRP has been shown to influence vascular vulnerability directly by a variety of mechanisms, including enhanced expression of local endothelial cell surface adhesion molecules[39], monocyte chemoattractant protein-1[39], endothelin-1[40], and endothelial plasminogen activator inhibitor-1[41]. CRP has also been shown to be associated with reduced endothelial nitric oxide bioavailability[40], increased tissue factor induction[42], and increased LDL uptake by macrophages[43].

The present study has several limitations. First, the inflammatory markers were only measured at one time point. This precludes examining how change in inflammatory markers influence incidence of PAD. Second, the follow-up visit was three years after the baseline visit. This may prevent us from picking up a substantial number of cases who would eventually develop PAD. Third, the leg used to define incident PAD was not consistent between individuals. At least one study showed differences in ABI between legs but this study not ascertain whether or not decline in ABI differed by leg[44]. Fourth, this study included some participants with symptoms of intermittent claudication and thus did not completely separate asymptomatic from symptomatic PAD. Fifth, the observational nature of this study limits some of the conclusions that could be made concerning the direction of the relationship between inflammation and PAD. A randomized clinical trial may be necessary to prove that treatment methods aimed at reducing inflammation will also reduce the incidence of PAD. Finally, conclusions based on ethnic differences must be made with caution because of the small sample size. However, the differences in odds ratios between ethnic groups were so overwhelming that we believe the estimates are valid.

In conclusion, CRP was associated with 3 year incident PAD in a population based elderly cohort. Furthermore, the association was much stronger in whites than in blacks. This study did not find any relationship between IL-6 or TNF-α on 3 year incidence of PAD. The primary significance of this study is that it provides the first prospective analysis of inflammation on incidence of PAD, defined by clinically derived cutpoints of the ABI. Given the high prevalence of PAD in the elderly population and its strong association with cardiovascular outcomes, reducing inflammation may be a useful method for reducing the burden of cardiovascular disease in the elderly.

4.6 ACKNOWLEDGMENT

This study was funded by the National Institute on Aging (NIA) contract numbers N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106, and 5-T32-AG00181. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

4.7 REFERENCES FOR CHAPTER 4

1. Criqui MH, Denenberg JO, Bird CE, Fronek A, Klauber MR, Langer RD. The correlation between symptoms and non-invasive test results in patients referred for peripheral arterial disease testing. *Vasc Med* 1996;1:65-71.

2. Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, Wolfson SK. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. *Circulation* 1993;88:837-845.

3. Criqui MH, Coughlin SS, Fronek A. Noninvasively diagnosed peripheral arterial disease as a predictor of mortality: results from a prospective study. *Circulation* 1985;72:768-773.

4. Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. *N Engl J Med* 1992;326:381-386.

5. Vogt MT, McKenna M, Anderson SJ, Wolfson SK, Kuller LH. The relationship between ankle-arm index and mortality in older men and women. *J Am Geriatr Soc* 1993;41:523-530.

6. Zheng ZJ, Sharrett AR, Chambless LE, Rosamond WD, Nieto FJ, Sheps DS, Dobs A, Evans GW, Heiss G. Associations of ankle-brachial index with clinical coronary heart disease, stroke and preclinical carotid and popliteal atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Study. *Atherosclerosis* 1997;131:115-125.

7. Newman AB, Shemanski L, Manolio TA, Cushman M, Mittelmark M, Polak JF, Powe NR, Siscovick D. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group. *Arterioscler Thromb Vasc Biol* 1999;19:538-545.

8. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J* 1999;138:S419-S420.

9. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-874.

10. Ridker PM. C-reactive protein and risks of future myocardial infarction and thrombotic stroke. *Eur Heart J* 1998;19:1-3.

11. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 1998;97:2007-2011.

12. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836-843.

13. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557-1565.

14. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. *Cardiol Clin* 2003;21:315-325.

15. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ* 2000;321:199-204.

16. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation* 2003;108:2317-2322.

17. Volpato S, Guralnik JM, Ferrucci L, Balfour J, Chaves P, Fried LP, Harris TB. Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study. *Circulation* 2001;103:947-953.

18. Rao M, Guo D, Perianayagam MC, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS. Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis* 2005;45:324-333.

19. McDermott MM, Green D, Greenland P, Liu K, Criqui MH, Chan C, Guralnik JM, Pearce WH, Ridker PM, Taylor L, Rifai N, Schneider JR. Relation of levels of hemostatic factors and inflammatory markers to the ankle brachial index. *Am J Cardiol* 2003;92:194-199.

20. McDermott MM, Guralnik JM, Corsi A, Albay M, Macchi C, Bandinelli S, Ferrucci L. Patterns of inflammation associated with peripheral arterial disease: the InCHIANTI study. *Am Heart J* 2005;150:276-281.

21. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. *Circulation* 2005;112:976-983.

22. Yu HI, Sheu WH, Song YM, Liu HC, Lee WJ, Chen YT. C-reactive protein and risk factors for peripheral vascular disease in subjects with Type 2 diabetes mellitus. *Diabet Med* 2004;21:336-341.

23. Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005;96:1579-1583.

24. Unlu Y, Karapolat S, Karaca Y, Kiziltunc A. Comparison of levels of inflammatory markers and hemostatic factors in the patients with and without peripheral arterial disease. *Thromb Res* 2005.

25. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-428.

26. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard

cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001;285:2481-2485.

27. Nasir K, Guallar E, Navas-Acien A, Criqui MH, Lima JA. Relationship of monocyte count and peripheral arterial disease: results from the National Health and Nutrition Examination Survey 1999-2002. *Arterioscler Thromb Vasc Biol* 2005;25:1966-1971.

28. Fiotti N, Giansante C, Ponte E, Delbello C, Calabrese S, Zacchi T, Dobrina A, Guarnieri G. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. *Atherosclerosis* 1999;145:51-60.

29. Cassar K, Bachoo P, Ford I, Greaves M, Brittenden J. Markers of coagulation activation, endothelial stimulation and inflammation in patients with peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2005;29:171-176.

30. Criqui MH, Vargas V, Denenberg JO, Ho E, Allison M, Langer RD, Gamst A, Bundens WP, Fronek A. Ethnicity and peripheral arterial disease: the San Diego Population Study. *Circulation* 2005;112:2703-2707.

31. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr., Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol* 2005;46:464-469.

32. Newman AB, Sutton-Tyrrell K, Kuller LH. Lower-extremity arterial disease in older hypertensive adults. *Arterioscler Thromb* 1993;13:555-562.

33. McLafferty RB, Moneta GL, Taylor LM, Jr., Porter JM. Ability of ankle-brachial index to detect lower-extremity atherosclerotic disease progression. *Arch Surg* 1997;132:836-840.

34. Baker JD, Dix DE. Variability of Doppler ankle pressures with arterial occlusive disease: an evaluation of ankle index and brachial-ankle pressure gradient. *Surgery* 1981;89:134-137.

35. Bird CE, Criqui MH, Fronek A, Denenberg JO, Klauber MR, Langer RD. Quantitative and qualitative progression of peripheral arterial disease by non-invasive testing. *Vasc Med* 1999;4:15-21.

36. Folsom AR, Pankow JS, Tracy RP, Arnett DK, Peacock JM, Hong Y, Djousse L, Eckfeldt JH. Association of C-reactive protein with markers of prevalent atherosclerotic disease. *Am J Cardiol* 2001;88:112-117.

37. Cimminiello C, Arpaia G, Toschi V, Rossi F, Aloisio M, Motta A, Bonfardeci G. Plasma levels of tumor necrosis factor and endothelial response in patients with chronic arterial obstructive disease or Raynaud's phenomenon. *Angiology* 1994;45:1015-1022.

38. Kennedy M, Solomon C, Manolio TA, Criqui MH, Newman AB, Polak JF, Burke GL, Enright P, Cushman M. Risk factors for declining ankle-brachial index in men and women 65 years or older: the Cardiovascular Health Study. *Arch Intern Med* 2005;165:1896-1902.

39. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000;102:2165-2168.

40. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Dhillon B, Mickle DA. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 2002;105:1890-1896.

41. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 2003;107:398-404.

42. Nakagomi A, Freedman SB, Geczy CL. Interferon-gamma and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein: relationship with age, sex, and hormone replacement treatment. *Circulation* 2000;101:1785-1791.

43. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation* 2001;103:1194-1197.

44. Smith FB, Lee AJ, Price JF, van Wijk MC, Fowkes FG. Changes in ankle brachial index in symptomatic and asymptomatic subjects in the general population. *J Vasc Surg* 2003;38:1323-1330.

5.0 OVERVIEW OF DISCUSSION

The following chapter will summarize the results from chapters 2 through 4, identify the strengths and limitations of this research, suggest future avenues of research, and describe the public health significance of the study.

Cardiovascular disease is the leading cause of death in the United States. Older adults and postmenopausal women have an increased risk of developing cardiovascular disease compared to the general population. In 2002, approximately 70% of deaths attributed to some form of cardiovascular disease occurred in individuals over the age of 75[1]. Statistics show that women on average develop cardiovascular disease ten to fifteen years later in life than men—a pattern thought to be attributed to menopause[2].

Most forms of cardiovascular disease are caused by atherosclerosis[3]. Subclinical markers of atherosclerosis are important in detecting early vascular changes that may predispose individuals to clinical cardiovascular disease. Thus, it is important to identify and understand risk factors that are associated with subclinical atherosclerosis in subgroups at an increased risk of developing cardiovascular disease.

In recent years, CRP has been firmly established as a cardiovascular risk factor and has been linked to a myriad of outcomes including clinical cardiovascular disease, type 2 diabetes, hypertension, and renal disease[4]. Although a plethora of studies have examined the association between CRP and clinical cardiovascular events, research looking at the association between

CRP and subclinical atherosclerosis has been scarce (see Appendices for the full literature review).

The purpose of this dissertation was to examine the association between CRP and subclinical markers of atherosclerosis in two distinct population based cohorts. The first part of the dissertation (Chapters 2 and 3) focused on delineating the cross-sectional relationship between CRP and arterial stiffness, an indicator of the sclerotic component of atherosclerosis, in a cohort of women transitioning through menopause. The second part of the dissertation (Chapter 4) characterized the prospective association between CRP and subclinical PAD, an indicator of the atheroma component of atherosclerosis, in an elderly cohort.

5.1 INFLAMMATION AND ARTERIAL STIFFNESS

Several studies have found an association between CRP and central arterial stiffness but none have thoroughly examined this association in women transitioning through menopause. In the first analysis (Chapter 2), it was hypothesized that higher levels of CRP would be associated with higher central arterial stiffness, assessed by carotid-femoral pulse wave velocity. This analysis also examined whether or not the aforementioned association differed according to the woman's stage of menopausal transition. The primary finding from this analysis was that higher levels of CRP (examined both continuously and categorically) were significantly associated with higher levels of pulse wave velocity and this association was stronger in women later in their menopausal transition than women earlier in their menopausal transition. Women classified as "late in their menopausal transition" were defined as not having a menstrual bleed in the three month prior to the start of the study whereas women classified as "early in their transition" were

defined as having a bleed in the three months prior to the start of the study. Although CRP levels were greater in women who were later in their transition compared to women earlier in their transition, the difference was not statistically significant. A previous study in this cohort showed an increase in CRP with hormone therapy use[5]. However, they did not find an association between CRP and endogenous levels of estradiol. Another study found evidence of an increase in expression and secretion of proinflammatory cytokines (IL-1, IL-6, and TNF- α), which are thought to promote the synthesis of CRP by the liver[6], with a reduction in endogenous estradiol[7]. More research is needed to explain why CRP may be differentially associated with exogenous and endogenous estrogens. Because of the skewed distribution of the variable, CRP was examined as tertiles and as a logarithmically transformed variable. The results were consistent regardless of how CRP was examined—further strengthening the findings. Previous studies looking at the association between CRP and arterial stiffness have used clinically defined cutpoints for CRP (low risk=0 to1 mg/L, moderate risk=1 to 3 mg/L and high risk > 3.0 mg/L)[8]. However, more recent studies have promoted categorization into tertiles because of the lack of generalizability associated with the clinically defined cutpoints[9].

Several biological mechanisms could support the findings from this study. First, increased inflammation due to estrogen deficiency could inhibit the synthesis of the smooth muscle relaxant nitric oxide. Estradiol has cardioprotective effects which may be mediated by activation of the estrogen receptor-α and estrogen receptor-β, both of which are thought to have vasodilatory effects[10,11]. Studies have shown that enzymes responsible for the synthesis of nitric oxide are associated with reduced arterial stiffness[12]. These studies have also found an association between nitric oxide inhibitors and increased arterial stiffness[12]. A second potential mechanism is that an increase in vessel diameter due to menopausal-induced stiffening could

make the vessel more susceptible to risk factors associated with increased inflammation. Third, because diabetes is more prevalent among postmenopausal women than premenopausal women[13], it could act as an indirect mechanism explaining the strong association CRP and pulse wave velocity in women who were later in their transition. Our results seem to support this theory because the addition of interaction terms between glucose and insulin with menopausal status diminished the interaction effect between CRP and menopausal status. Furthermore, exclusion of women with metabolic syndrome, which is known to be associated with diabetes, attenuated the interaction effect between CRP and menopausal status. It isn't clear whether diabetes would act as a confounder or component of the mechanism linking CRP with increased central arterial stiffness. However, a number of studies seem to indicate that CRP may be a risk factor as opposed to a risk marker for diabetes[14-17]—a fact that would implicate diabetes as part of the mechanism.

In the second analysis (Chapter 3), it was hypothesized that higher levels of CRP would be associated with higher systemic arterial stiffness, defined by a lower distensibility. Research looking at distensibility and cardiovascular risk are limited and no previous study has explored the association between CRP and distensibility. Distensiblity, defined as the percent change in the cross-sectional area of the artery per change in pressure, was measured by the Dynapulse 5000A monitoring instrument (Pulse Metric, Inc., San Diego, CA). The Dynapulse instrument used a pulse dynamic pattern recognition methodology to determine distensibility from the oscillometric signal of a standard blood pressure cuff. The pressure waveform that was generated from the cuff was calibrated and incorporated into a physical model of the cardiovascular system, assuming a straight tube brachial artery and a T-tube aortic system. The formula for calculating distensibility incorporated the participant's effective cuff width and

brachial artery diameter, which was estimated using an empirically derived model based on gender, height, weight, and mean arterial blood pressure. Consequently, distensibility provided a measure of systemic arterial stiffness that inherently controlled for body size, arterial size, and arterial pressure.

The primary finding from the second analysis was that higher levels of CRP were associated with lower levels of distensibility in a cohort of women transitioning through menopause. This finding persisted whether CRP was analyzed continuously or categorically. The association between CRP and distensibility did not significantly differ according to the stage of the menopausal transition. In light of the findings from the first study, this result is surprising. The measurement of distensibility in SWAN was conducted at a later stage in the study than carotid-femoral pulse wave velocity. Therefore, there was a relative increase in postmenopausal and decrease in premenopausal women in the second analysis compared to the first. Among the women who were considered late in their transition, a greater proportion were postmenopausal in the second analysis compared to the first (n=586, 86% vs n=68, 79%). There is at least some evidence that cardiovascular risk in women is greater during perimenopause than postmenopause[18]. Thus, women who were defined as late in their transition in the second analysis may have in fact been healthier than the same group of women in the first analysis.

Because of the cross-sectional nature of this study, we can only speculate on the potential biological mechanisms linking CRP to systemic arterial stiffness. Interleukin-6 and tumor necrosis factor α, two cytokines known to stimulate the synthesis of CRP by hepatocytes, may directly cause glomerular damage and loss of kidney function[19-21]. Several studies have shown increased arterial stiffness in individuals with kidney failure as a result of smooth muscle cell calcification[22]. Inflammation could also cause structural damage to the vessels by promoting

defragmentation of the elastin fibers and increased deposition of the collagen fibers. We are not able to speculate whether or not CRP may have a differential affect on different sites of the arterial wall. Central and peripheral arteries are known to have different structural and functional properties and established risk factors are known to act differentially on their vessel walls[23,24]. Further research is needed to explore the mechanisms linking CRP to arterial stiffness in different vascular beds.

5.2 INFLAMMATION AND PERIPHERAL ARTERIAL DISEASE

In the third analysis it was hypothesized that increased levels of baseline CRP would be associated with increased three year incidence of PAD in a cohort of older adults. This study also examined the relationship between two pro-inflammatory cytokines (IL-6 and TNF-α) and incident PAD. The ABI, a noninvasive measure of PAD, was calculated at baseline and a three year follow-up visit and incidence of PAD was based on a clinically derived formula confirmed from previous research[25]. The prevalence of PAD at baseline was 14% and the three year incidence of PAD was approximately 8%. Similar percentages have been found in other elderly cohorts[25]. Participants in the top tertile of CRP had approximately two times the odds of developing PAD compared to participants in the bottom tertile. IL-6 and TNF-α, cytokines responsible for the synthesis of CRP by hepatocytes, were not significantly associated with incident PAD. Because of known ethnic differences in CRP[26], we tested whether or not the association between CRP and PAD differed by ethnicity. We found the association to be much stronger in whites than blacks and this pattern persisted after additional stratification by gender. A previous study discovered the cross-sectional association between CRP and PAD to be

stronger in blacks than whites—a finding that contradicted our results[27]. However, their population was younger and the prevalence of PAD (4.9%) was consequently much lower. In our population, it could be hypothesized that other risk factors contributed more to the development of PAD among blacks than whites. Thus, any effect of CRP may have been washed out by the other risk factors among the blacks but not whites. Although the analyses adjusted for these risk factors, there may have been an additive effect that we were unable to statistically control for. Surprisingly, IL-6 and TNF-α were not associated with increased incidence of PAD. Previous studies looking at these associations have produced conflicting results[28-31]. It could be hypothesized that CRP was the only inflammatory marker sensitive enough to predict PAD in an older population with a high burden of disease.

There are a number of potential mechanisms that could link CRP to the development of PAD. PAD has been found to be associated with reduced levels of physical activity[32,33] and lower levels of physical activity are associated with increased inflammation[34]. It is unclear whether physical activity acts as a confounder or is directly involved in the mechanism linking CRP with PAD. We would speculate that CRP is associated with increased PAD resulting in a reduction of physical activity and further increase in inflammation. CRP has also been shown to act directly at all stages of the atherosclerotic process. Expression of adhesion molecules by endothelial cells, the first major step in the atherosclerotic process, is induced by CRP. Studies have implicated CRP as an activator of complement[35-37] and stimulator of tissue factor production[38,39], both of which are associated with PAD[40,41]. CRP has also been found to be associated with an increased uptake of oxidized LDL by macrophages[42]. A previous study found the macrophage uptake of oxidized LDL to be higher in patients with PAD compared to

controls[43]. CRP appears to be associated with reduced endothelial nitric oxide bioavailability[44], which is linked to increased PAD[45].

In summary, the three analyses conducted as part of this dissertation all showed a strong association between higher levels of systemic inflammation and increased subclinical atherosclerosis in two distinct populations at increased risk of developing cardiovascular disease. The following section aims to identify limitations in the current study and possible avenues of future research.

5.3 LIMITATIONS/FUTURE RESEARCH--ANALYSES 1 AND 2

There are a number of existing limitations in the first two analyses that support the need for future research. The analyses both utilized a cross-sectional study design. Thus, the direction of the association between CRP and arterial stiffness could not be tested. To our knowledge, no study has prospectively examined the association between CRP and arterial stiffness and future research should focus on generating more longitudinal studies in this area. Furthermore, studies that examine within woman change in the menopausal transition could more accurately distinguish the effects of age and menopause on the association between CRP and arterial stiffness.

Although we selected a high risk subgroup for cardiovascular disease, the results may not have been representative of the general population of women transitioning through menopause because women with clinical cardiovascular disease were excluded. However, both analyses found that levels of fasting glucose and insulin, total cholesterol, and LDL cholesterol were higher in the women who were later in their transition compared to women who were earlier in

their transition. In the second analysis, systolic blood pressure and mean arterial pressure were also higher in women who were later in their transition than women who were earlier in their transition. This provided evidence that the women who were later in their transition had an inferior cardiovascular risk profile than women who were earlier in their transition.

The method used for classifying menopausal status presented another limitation. The variables for menopausal status were all derived from an annual interviewer administered questionnaire and based on the participants most recent menstrual bleed date. Although this may have increased the likelihood of recall bias, it is the most feasible way of deriving menopausal status in large population based cohort studies. Another limitation with the classification of menopausal status was that duration of time spent within a status category was not considered. Future studies need to examine whether or not the cumulative time spent in a given menopausal status category influences the association between CRP and arterial stiffness.

Both analyses either controlled for or excluded hormone therapy users in the multivariate models. There was insufficient power to detect whether or not hormone therapy use impacted the association between CRP and arterial stiffness. There has been a great deal of controversy on the effect of hormone therapy use on cardiovascular risk. Observational studies in the past have suggested that postmenopausal hormone therapy was associated with a reduction in risk of coronary heart disease by 40 to 50 percent[46,47]. However, recent evidence from clinical trials have provided no evidence of cardiac protection and even some deleterious effects of hormone therapy use[48-50]. Studies looking at the association between hormone therapy use and either arterial stiffness or inflammation have also been inconsistent[51-54]. These inconsistencies could be attributed to differences in clinical characteristics of the study populations, including age, years since menopause, and risk of coronary heart disease. Future studies are needed to ascertain

whether or not certain regimens of hormone therapy use may be more beneficial than others in reducing inflammation and arterial stiffness and how this translates into reduction of cardiovascular risk.

There is evidence that the perimenopausal stage may confer a greater cardiovascular risk than the postmenopausal stage[18]. Due to the small sample size of perimenopausal women, our study was not able to test differences in risk factors between perimenopausal and postmenopausal women. In the first analysis, we showed that the association between inflammation and central arterial stiffness was stronger in women who were later in their transition than women who were earlier in their transition. However, we were forced to collapse the late perimenopausal with the postmenopausal women and the early perimenopausal with the premenopausal women. Future studies should examine tripartite differences between premenopausal, perimenopausal, and postmenopausal women. This would help to identify the exact stage of the menopausal transition that begins to modify the effect of CRP on arterial stiffness.

A limitation with the first analysis relates to the methodology used to measure carotid-femoral pulse wave velocity. The distance portion of the equation for pulse wave velocity was calculated as the distance between the carotid and femoral recordings measured over the surface of the body. Because the proximal and distal pulse waves travel in opposite directions, the distance measured over the surface of the body may have increased error in the measurement. In order to circumvent this problem, some authors have suggested subtracting the distance between the sternal notch to the carotid location from the total carotid-femoral distance. However, this method would require two measurements of distance which could conceivably increase the error[55].

A limitation with the second analysis was the composition of the population. As a result of not having a large enough cuff size to cover the upper arm of a number of women, we were forced to exclude a disproportionate number of black and overweight women from the analysis. Thus, women from this study represented a healthy cohort and the results were not generalizable to all middle-aged women transitioning through menopause. Because the heavier women constitute a high risk group, the association between CRP and distensibility would likely have been even stronger if these women had been included. We are currently attempting to obtain measurements of distensibility on these women by either creating a larger cuff size or conducting the measurement on the forearm rather than the upper arm. We conducted a reproducibility study to compare the distensibility measured in the upper arm with the distensibility measured in the forearm. A total of twelve women between the ages of 24 and 63 had distensibility measured on the upper arm and forearm. The mean upper arm value for the twelve volunteers was significantly greater than the mean forearm value. A plot of the data showed that the distensibility measured in the upper arm was greater than the distensibility measured in the forearm in ten of the twelve women. This would suggest the possibility of applying a correction formula to equate measurements from the upper arm with the forearm. However, larger reproducibility studies are needed on a more homogeneous sample of women to justify the use of a correction formula.

5.4 LIMITATIONS/FUTURE RESEARCH—ANALYSIS 3

The third analysis prospectively examined the association between CRP and PAD in a cohort of older adults who were considered well functioning at the baseline visit of the study. In order to

be eligible for the study, participants were required to walk one quarter mile, climb 10 steps, and perform basic activities of daily living without difficulty and not have any life-threatening illness. Therefore, this cohort may not have been representative of the general population of older adults. The population in this study may reflect a survival bias such that many of the unhealthy participants had died prior to the follow-up visit. In this analysis, the participants who were excluded (many of who had died at the follow-up visit) had a worse baseline cardiovascular profile than participants who were included. Future studies are needed to examine the association between CRP and PAD in unselected higher risk older individuals.

The inflammatory markers and most covariates tested in this analysis were measured at a single baseline visit and the outcome variable (ABI) was measured at the baseline visit and a three year follow-up visit. Thus, we were not able to test how the change in certain inflammatory markers affected the incidence of PAD. Because the inflammatory markers and majority of covariates were only measured at baseline, we only adjusted for baseline covariates in the analysis. Thus, we could not accurately control for the change in cardiovascular profile between the two visits (approximately 3 years). However, when excluding individuals who had developed a clinical cardiovascular event between the two visits, we found similar results. Future studies are needed to test whether or not the change in CRP is associated with incidence of PAD.

Because of the already limited number of incident PAD cases, we chose not to exclude participants who presented symptoms of intermittent claudication. There is at least some evidence that individuals with intermittent claudication experience a greater decline in ABI over time than individuals without symptoms[56]. However, our study did not have a large enough number of individuals with intermittent claudication to separate asymptomatic from symptomatic

PAD. Therefore, future studies are needed to test whether or not the association between CRP and incident PAD is different among asymptomatic and symptomatic individuals.

This study examined the association between baseline CRP markers and three year incidence of PAD. Among the participants who had a high level of CRP but no incidence of PAD, it would be important to identify how many developed PAD in the future. Three years may not have provided long enough time for many of these participants to develop PAD. Future studies should be aimed at examining the association between CRP and incidence of PAD at multiple time points.

Previous studies testing the association between proinflammatory cytokines and PAD have been inconsistent. Our study did not find an association between IL-6 and TNF-α with incidence of PAD. One study found IL-6 to be a stronger predictor than CRP of decrease in ABI over time. However, a large percentage (24%) of their population already had PAD at the baseline visit and these participants were not excluded from the follow-up analysis. Thus, it could be hypothesized that IL-6 acts at a later stage of the atherosclerotic process and CRP is a more sensitive marker of early atherosclerotic events. Future prospective studies are needed to further distinguish the effects of different inflammatory markers on different stages of the atherosclerotic process.

5.5 SUMMARY

In summary, this research found a strong positive association between CRP and subclinical atherosclerosis in two distinct populations at increased risk of cardiovascular disease. CRP was strongly associated with increased central and systemic arterial stiffness in a cohort of women

transitioning through menopause. Additionally, CRP was associated with increased incidence of PAD in a cohort of older adults. The strength of this study was in its ability to test the association between systemic inflammation and subclinical atherosclerosis in large multi-ethnic population based cohorts. This research has provided evidence that CRP may directly promote early vascular damage in various arterial beds.

5.6 PUBLIC HEALTH SIGNIFICANCE

Early vascular damage is thought to precede clinical cardiovascular events in high risk populations. CRP appears to be directly involved in promoting vascular damage. Several behavioral interventions including weight loss[57,58], physical activity[59], diet[4], and smoking cessation[60] have been linked to lower CRP levels. Additionally, pharmacological interventions including statins and angiotensin converting enzyme (ACE) inhibitors have also been recognized as having anti-inflammatory effects[4]. This dissertation has provided strong evidence that CRP may promote early vascular damage in individuals at high risk for developing cardiovascular disease. This is important because a reduction in CRP may reverse or prevent early detrimental vascular changes. Given the overwhelming evidence that levels of CRP can be reduced with behavioral and pharmacological treatment, future randomized clinical trials should determine whether this reduction translates into diminished cardiovascular risk.

5.7 REFERENCES FOR CHAPTER 5

1. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng ZJ, Flegal K, O'Donnell C, Kittner S, Lloyd-Jones D, Goff DC, Jr., Hong Y, Adams R, Friday G, Furie K, Gorelick P, Kissela B, Marler J, Meigs J, Roger V, Sidney S, Sorlie P, Steinberger J, Wasserthiel-Smoller S, Wilson M, Wolf P. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006;113:e85-151.

2. Rossouw JE. Hormones, genetic factors, and gender differences in cardiovascular disease. *Cardiovasc Res* 2002;53:550-557.

3. Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868-874.

4. Bassuk SS, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 2004;29:439-493.

5. Sowers MR, Matthews KA, Jannausch M, Randolph JF, McConnell D, Sutton-Tyrrell K, Little R, Lasley B, Pasternak R. Hemostatic factors and estrogen during the menopausal transition. *J Clin Endocrinol Metab* 2005;90:5942-5948.

6. Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimburger O, Cederholm T, Girndt M. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. *Kidney Int* 2005;67:1216-1233.

7. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 2002;23:90-119.

8. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004;24:969-974.

9. Mattace-Raso FU, van der Cammen TJ, van dM, I, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis* 2004;176:111-116.

10. Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol* 2002;89:12E-17E.

11. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801-1811.

12. Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. *Hypertension* 2004;44:112-116.

13. Curtis J, Wilson C. Preventing type 2 diabetes mellitus. *J Am Board Fam Pract* 2005;18:37-43.

14. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003;52:1799-1805.

15. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 1999;353:1649-1652.

16. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001;286:327-334.

17. Han TS, Sattar N, Williams K, Gonzalez-Villalpando C, Lean ME, Haffner SM. Prospective study of C-reactive protein in relation to the development of diabetes and

metabolic syndrome in the Mexico City Diabetes Study. *Diabetes Care* 2002;25:2016-2021.

18. Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF. Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. *Stroke* 2001;32:1104-1111.

19. Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G. Tumor necrosis factor induces glomerular damage in the rabbit. *Am J Pathol* 1989;134:419-430.

20. Remuzzi G, Bertani T. Pathophysiology of progressive nephropathies. *N Engl J Med* 1998;339:1448-1456.

21. Arici M, Walls J. End-stage renal disease, atherosclerosis, and cardiovascular mortality: is C-reactive protein the missing link? *Kidney Int* 2001;59:407-414.

22. Safar ME, London GM, Plante GE. Arterial stiffness and kidney function. *Hypertension* 2004;43:163-168.

23. Izzo JL, Jr., Shykoff BE. Arterial stiffness: clinical relevance, measurement, and treatment. *Rev Cardiovasc Med* 2001;2:29-40.

24. van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension* 2000;35:637-642.

25. Kennedy M, Solomon C, Manolio TA, Criqui MH, Newman AB, Polak JF, Burke GL, Enright P, Cushman M. Risk factors for declining ankle-brachial index in men and women 65 years or older: the Cardiovascular Health Study. *Arch Intern Med* 2005;165:1896-1902.

26. Criqui MH, Vargas V, Denenberg JO, Ho E, Allison M, Langer RD, Gamst A, Bundens WP, Fronek A. Ethnicity and peripheral arterial disease: the San Diego Population Study. *Circulation* 2005;112:2703-2707.

27. Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005;96:1579-1583.

28. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. *Circulation* 2005;112:976-983.

29. Yu HI, Sheu WH, Song YM, Liu HC, Lee WJ, Chen YT. C-reactive protein and risk factors for peripheral vascular disease in subjects with Type 2 diabetes mellitus. *Diabet Med* 2004;21:336-341.

30. Fiotti N, Giansante C, Ponte E, Delbello C, Calabrese S, Zacchi T, Dobrina A, Guarnieri G. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. *Atherosclerosis* 1999;145:51-60.

31. Cimminiello C, Arpaia G, Toschi V, Rossi F, Aloisio M, Motta A, Bonfardeci G. Plasma levels of tumor necrosis factor and endothelial response in patients with chronic arterial obstructive disease or Raynaud's phenomenon. *Angiology* 1994;45:1015-1022.

32. McDermott MM, Greenland P, Liu K, Guralnik JM, Celic L, Criqui MH, Chan C, Martin GJ, Schneider J, Pearce WH, Taylor LM, Clark E. The ankle brachial index is associated with leg function and physical activity: the Walking and Leg Circulation Study. *Ann Intern Med* 2002;136:873-883.

33. McDermott MM, Ohlmiller SM, Liu K, Guralnik JM, Martin GJ, Pearce WH, Greenland P. Gait alterations associated with walking impairment in people with peripheral arterial disease with and without intermittent claudication. *J Am Geriatr Soc* 2001;49:747-754.

34. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. *Am J Epidemiol* 2001;153:242-250.

35. Volanakis JE, Kaplan MH. Interaction of C-reactive protein complexes with the complement system. II. Consumption of guinea pig complement by CRP complexes: requirement for human C1q. *J Immunol* 1974;113:9-17.

36. Volanakis JE. Complement activation by C-reactive protein complexes. *Ann N Y Acad Sci* 1982;389:235-250.

37. Jiang H, Robey FA, Gewurz H. Localization of sites through which C-reactive protein binds and activates complement to residues 14-26 and 76-92 of the human C1q A chain. *J Exp Med* 1992;175:1373-1379.

38. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood* 1993;82:513-520.

39. Ramani M, Khechai F, Ollivier V, Ternisien C, Bridey F, Hakim J, de Prost D. Interleukin-10 and pentoxifylline inhibit C-reactive protein-induced tissue factor gene expression in peripheral human blood monocytes. *FEBS Lett* 1994;356:86-88.

40. Szeplaki G, Prohaszka Z, Duba J, Rugonfalvi-Kiss S, Karadi I, Kokai M, Kramer J, Fust G, Kleiber M, Romics L, Varga L. Association of high serum concentration of the third component of complement (C3) with pre-existing severe coronary artery disease and new vascular events in women. *Atherosclerosis* 2004;177:383-389.

41. Makin AJ, Chung NA, Silverman SH, Lip GY. Thrombogenesis and endothelial damage/dysfunction in peripheral artery disease. Relationship to ethnicity and disease severity. *Thromb Res* 2003;111:221-226.

42. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation* 2001;103:1194-1197.

43. Ramirez-Tortosa MC, Urbano G, Lopez-Jurado M, Nestares T, Gomez MC, Gonzalez J, Mir A, Ros E, Mataix J, Gil A. Lifestyle changes in free-living patients with peripheral vascular disease (Fontaine stage II) related to plasma and LDL lipid composition: a 15 month follow-up study. *Clin Nutr* 1999;18:281-289.

44. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Dhillon B, Mickle DA. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 2002;105:1890-1896.

45. Jacob T, Ascher E, Vorsanger M, Hingorani A, Kallakuri S, Yorkovich W, Schuzter R. Decreased production of nitric oxide by peripheral blood mononuclear cells of patients with peripheral vascular disease. *Vasc Endovascular Surg* 2005;39:175-181.

46. Grodstein F, Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. *Prog Cardiovasc Dis* 1995;38:199-210.

47. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med* 1992;117:1016-1037.

48. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 1998;280:605-613.

49. Herrington DM, Reboussin DM, Brosnihan KB, Sharp PC, Shumaker SA, Snyder TE, Furberg CD, Kowalchuk GJ, Stuckey TD, Rogers WJ, Givens DH, Waters D. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med* 2000;343:522-529.

50. Clarke SC, Kelleher J, Lloyd-Jones H, Slack M, Schofiel PM. A study of hormone replacement therapy in postmenopausal women with ischaemic heart disease: the Papworth HRT atherosclerosis study. *BJOG* 2002;109:1056-1062.

51. Maas AH, van der GY, van der Schouw YT, Grobbee DE. HRT and heart disease: problems and prospects. *Maturitas* 2004;47:255-258.

52. Stork S, van der Schouw YT, Grobbee DE, Bots ML. Estrogen, inflammation and cardiovascular risk in women: a critical appraisal. *Trends Endocrinol Metab* 2004;15:66-72.

53. Tanaka H, DeSouza CA, Seals DR. Arterial stiffness and hormone replacement use in healthy postmenopausal women. *J Gerontol A Biol Sci Med Sci* 1998;53:M344-M346.

54. Gorgulu S, Eren M, Celik S, Dagdeviren B, Uslu N, Suer N, Tezel T. The effects of hormonal therapy on aortic stiffness and left ventricular diastolic function. *Acta Cardiol* 2003;58:1-8.

55. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26:485-490.

56. Smith FB, Lee AJ, Price JF, van Wijk MC, Fowkes FG. Changes in ankle brachial index in symptomatic and asymptomatic subjects in the general population. *J Vasc Surg* 2003;38:1323-1330.

57. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003;289:1799-1804.

58. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, D'Andrea F, Molinari AM, Giugliano D. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002;105:804-809.

59. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 2000;21:21-24.

60. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol* 2002;89:1117-1119.

APPENDIX A

DETAILED LITERATURE REVIEW OF STUDIES EXAMINING THE ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND ARTERIAL STIFFNESS

A.1 HEALTHY POPULATIONS

Several studies have found an association between CRP and arterial stiffness in healthy populations. One study examined the association between CRP and aortic pulse wave velocity in a cohort of 427 healthy subjects between the ages of 16 and 83 years[1]. The gender distribution in the study was approximately equal (55% male, n=232). This study excluded subjects with diabetes, renal disease, and cardiovascular disease. The authors examined CRP both as a categorical variable (using clinically defined cutpoints) and a log transformed continuous variable. The mean CRP was 4.0 mg/L and 35.3% of the participants had a CRP greater than 3.0 mg/L, a level that is considered high risk for future cardiovascular disease. The primary finding was that mean aortic pulse wave velocity increased with increasing CRP risk groups (7.0 m/s for low risk CRP group, 7.6 m/s for moderate risk CRP group, 8.2 m/s for high risk CRP group, P-value for trend=0.001). After adjustment for age, mean arterial pressure, and gender, log transformed CRP was linearly associated with aortic pulse wave velocity (β=0.127, P=0.004). A major limitation with this study was that the authors did not stratify by age. Given the broad age range, it would be of interesting to know the contribution of age as an effect modifier. Given the

inclusion of healthy subjects, the CRP levels in this study were surprisingly high. This may be due to the fact that authors included participants with acute inflammation (CRP greater than 10.0). Clinically defined cutpoints for CRP are considered to be outdated because they are not representative of most populations. The authors did repeat the analyses using tertiles but did not present the results.

A community based study examined the association between CRP and arterial stiffness in 214 asymptomatic Caucasian men and women[2]. The mean age of the study was 59.6 ± 10.2 years and the median CRP was 2.0. Participants with a history of bypass surgery, angioplasty, coronary surgery, myocardial infarction, or stroke were excluded from the study. After adjustment for age, heart rate, mean arterial pressure, BMI, and total and HDL cholesterol, log transformed CRP was associated with aortic pulse wave velocity (β=0.74, P<0.001). Similar to the previous study, a major limitation is that the current study did not exclude participants with acute inflammation. Although the subjects were considered asymptomatic, a large percentage (44%) was taking antihypertensive medications. However, the authors made no mention of adjusting for medication use in the models.

Two population based studies in healthy Japanese older adults tested the association between CRP and brachial ankle pulse wave velocity[3,4]. The first study recruited 870 males and females between the ages of 40 and 79 years[3]. The authors excluded subjects with heart disease or an ABI less than 0.9, women on hormone therapy, and individuals taking statins. The mean age of the population was 59 years, the mean CRP was 0.82 mg/L, and approximately half of the population was male. After adjustment for confounders, CRP was associated with log transformed pulse wave velocity (β=0.049, P<0.01). The mean levels of CRP increased with increasing quartiles of pulse wave velocity (p for trend < 0.01). A limitation of this study was

that the authors used the brachial ankle pulse wave velocity to represent aortic stiffness. Although it has been found to be correlated with aortic pulse wave velocity, studies are needed to test whether or not it can be used as a surrogate measure. The second study examined the association between CRP and brachial ankle pulse wave velocity in a cohort of 2,678 Japanese men between the ages of 30 and 74 years[4]. The mean age and CRP of the population was 43 years and 1.2 mg/L respectively. After adjustment for age, BMI, pulse pressure, and systolic blood pressure, log transformed CRP was not found to be associated with pulse wave velocity. Participants in the top quartile of pulse wave velocity had a significantly higher unadjusted mean CRP than subjects in the other three quartiles ($p < 0.01$). The lack of association between continuous CRP and pulse wave velocity could have resulted from overadjusment due to inclusion of both pulse pressure and systolic blood pressure in the multivariate models. The authors' recruitment of a "healthy" population was questionable as they made no mention of excluding participants with clinical cardiovascular disease. Additionally, they elicited confusion by mentioning the exclusion of women on hormone therapy.

A.2 OLDER ADULT POPULATION

The Rotterdam Study was the only population based study to examine the association between CRP and arterial stiffness in older adults who were not screened for cardiovascular disease[5]. The population consisted of 866 men and women over the age of 55 years. The mean age was 70.9 ± 5.4 years and approximately half of the population was male. After adjustment for age and gender, CRP was associated with increased carotid-femoral pulse wave velocity (β=0.138, 95% CI = 0.063 to 0.213). After adjustment for additional confounders, the association was

attenuated but still significant. When analyzing CRP as tertiles, the authors found an increase in adjusted mean pulse wave velocity with increasing tertiles of CRP. One limitation of the study was that the authors chose to exclude logarithmic CRP values which were considered outliers. The transformation of data is used to normalize the distribution (and thus reduce outliers) and outliers should have been eliminated from the original untransformed data. Another limitation is that the authors did not actually use the log transformed CRP in the multivariate regression models. This could have resulted in violations of linear regression assumptions including linearity and normality.

A.3 PATIENT STUDIES

Several studies have investigated the association between CRP and arterial stiffness in patients with a systemic inflammatory disease. One study recruited 53 patients over the age of 18 with rheumatoid arthritis (15 of which had coronary artery disease) and 53 age and sex matched controls without rheumatoid arthritis (15 of which had coronary artery disease) from two public and private clinics in Australia[6]. Arterial stiffness was assessed by small and large artery elasticity, both of which are inversely related to pulse wave velocity. The mean age of the patients and controls was 55 and 54 years respectively. CRP was significantly greater in patients than controls (10.0 mg/L vs. 1.6 mg/L, $p<0.001$). In the combined group of patients and controls, CRP was significantly inversely associated with both small and large artery elasticity after adjustment for age and sex ($p<0.001$ and $p=.035$ respectively). However, when the groups were considered separately, no association was found. It could be hypothesized that the study did not have enough power to test the association in patients. However, an increase in sample

size may not have mattered because patients with rheumatoid arthritis in this study already had significant vascular damage. Thus, levels of CRP in this subgroup may not have been associated with further damage to the arteries. The authors in this study did not mention the techniques used to measure small and large artery elasticity. Therefore, comparisons in the association between CRP and arterial stiffness according to artery size were not clear.

Another study looked at the association between CRP and aortic pulse wave velocity in 60 untreated hypercholesterolaemic patients and 25 age and sex matched controls[7]. The investigators excluded participants with clinical cardiovascular disease, hypertension, or evidence of increased inflammation. Additionally, smokers and participants taking anti-inflammatory medications were excluded. The mean age of the population was 57 years in both cases and controls and CRP was significantly higher in cases than controls (1.65 mg/L vs 0.7 mg/L, p=0.03). After adjustment for confounders, log transformed CRP was associated with increased pulse wave velocity in hypercholesterolaemic patients (β=0.30, p=0.03) and the combined sample (β=0.32, p=0.008). Participants with a CRP greater than 3.0 mg/L had a higher age adjusted mean pulse wave velocity than the rest of the population. One major limitation with this study was that the authors did not test whether the association between CRP and arterial stiffness was different in patients and controls. Another limitation was reflected in the strict exclusion criteria. The authors excluded patients with any history of hypertension, cardiovascular disease, or inflammation. Additionally, smokers and participants taking antiplatelet, anti-inflammatory, or hypolipidemic agents were excluded. Therefore, this population may have represented a healthy subgroup of patients with hypercholesterolaemia.

Investigators from a specialized vasculitis clinic studied the association between CRP and aortic pulse wave velocity in a cohort of patients with small vessel vasculitis (n=31) and controls

(n=32) matched on age, sex, height, blood pressure, and cholesterol[8]. The patients were separated into two groups—those with active disease (n=15) and those in remission (n=16). The population ranged in age from 18 to 85 years with a mean of 55 years. The unadjusted mean CRP was significantly higher in the active disease group (16.0 mg/L) compared to the remission (2.9 mg/L) and control groups (1.1 mg/L). There was no significant difference in CRP between the remission and control groups. After adjustment for confounders, log transformed CRP was significantly associated with increased pulse wave velocity (p=0.01). As with the previous patient studies, small sample size was a major limitation. Although the authors recruited cases and controls, they did not have the power to test whether or not the association between CRP and pulse wave velocity was different between the groups. The finding that CRP levels were much lower in the remission group compared to the active disease group provides evidence that inflammation can be reduced with treatment.

One study recruited 42 patients (83% male, n=35) with untreated hypertension and 42 age and sex matched controls[9]. Individuals with diabetes, coronary artery disease, heart failure, hypercholesterolaemia, renal disease, acute inflammation (characterized by a CRP greater than 5.0 mg/L), or anemia were excluded. All participants were nonsmokers and none were taking vasocative or anti-inflammatory medications. The authors used the travel time of the pulse wave to the periphery as a surrogate marker of aortic stiffness that is inversely related to the pulse wave velocity. The mean age of the population was 52 years. CRP was found to be significantly higher in the hypertensive group than the controls (1.27 mg/L vs 0.74 mg/L, p<.001). After adjustment for confounders, the authors did not find a significant association between log transformed CRP and travel time of the pulse wave in either the controls or the hypertensive patients. There were several notable limitations in this study. Because of the stringent exclusion

criteria, the sample size in the study was small and may have affected the results. A second limitation is that the study used an indirect measure of arterial stiffness that has not been well established as a surrogate marker. Finally, the authors did not explain the apparent selection bias characterized by the disproportionate number of males.

In summary, the current literature has provided compelling evidence of a strong relationship between CRP and arterial stiffness. However, the role of ethnicity and the menopausal transition on this relationship has not been investigated. This dissertation extends upon the literature by establishing a strong association between CRP and arterial stiffness in women transitioning through menopause. Furthermore, this dissertation provides preliminary evidence that CRP is associated with systemic arterial stiffness. Future longitudinal studies in ethnic diverse populations are needed to verify our findings.

APPENDIX B

DETAILED LITERATURE REVIEW OF STUDIES EXAMINING THE ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND PERIPHERAL ARTERIAL DISEASE

B.1 CROSS-SECTIONAL STUDIES

Several population based studies have looked at the association between CRP and peripheral arterial disease. The InCHIANTI Study recruited 955 men and women over the age of 60 from two Italian communities[10]. This study used an ABI less than 0.9 as an indicator of PAD. Participants with PAD were older (78.27 vs 73.78 years, $p<0.001$) and had higher levels of CRP (3.60 mg/dL vs 2.52 mg/dL, $p=.001$) than participants without PAD. The association between CRP and PAD was attenuated but remained significant after adjustment for age, sex, BMI, smoking, comorbidities, and cholesterol. Additional adjustment for physical activity further attenuated the association and results were no longer significant. Although physical activity attenuated the association, the magnitude of the effect was extremely small. The lack of significance may have simply been a result of a reduced sample size. It would have been useful to know how many individuals were missing a physical activity score.

Another study tested the association between CRP and PAD in 30 diabetic patients with PAD (defined by an ABI < 0.9) and 60 age and BMI matched controls (defined by an ABI between 1.0 and 1.3)[11]. The authors excluded individuals with acute or chronic infectious

diseases, creatinine > 2.0 mg/dl, or chronic inflammatory diseases. The mean age of the population was 67 years and the male to female ratio was approximately two to one. The unadjusted median CRP was significantly greater in diabetic patients with PAD compared to diabetic patients without PAD (0.282 mg/dl vs 0.102 mg/dl, $p<0.001$). Participants in the top tertile of CRP had a significant greater odds of having PAD than participants in the bottom tertile of CRP (OR=2.93, p=0.028). A major limitation of this study is that the authors did not clearly define how and which confounders were adjusted for. From the multivariate model, it is unclear whether they adjusted for age and smoking status. Another limitation is that the authors made no mention of why individuals with an ABI between 0.9 and 1.0 were excluded from the analyses.

A study of 370 men and women over the age of 55 with PAD (ABI < 0.9) and 321 without PAD (ABI between 0.9 and 1.5) assessed the relationship between CRP and PAD[12]. Among participants without a history of cardiac or cerebrovascular disease, median levels of CRP were higher in PAD patients compared to controls (0.30 mg/dl vs 0.20 mg/dl, $p < 0.01$). However, CRP was not associated with PAD in patients with a history of cardiovascular or cerebrovascular disease. After adjustment for confounders, log transformed CRP was only associated with decreasing ABI in patients without a history of cardiovascular or cerebrovascular disease (β= -0.034, p=0.026). The major limitation of this study was that the authors did not perform the multivariate analysis with ABI as a dichotomous variable. The authors simultaneously adjusted for several hemostatic factors but did not examine an additive effect. Furthermore, the inclusion of several hemostatic markers in a single model may have raised questions of colinearity.

One study investigated the association between CRP and PAD in 89 healthy individuals without cardiovascular disease, diabetes, cancer, or kidney disease[13]. The mean age of this

population was 51 years and approximately 85% was male. The median CRP was higher in the patients with PAD compared to controls (4.95 vs 0.9, $p < 0.001$). Among patients with PAD, the mean CRP increased with increasing severity of PAD. One of the major limitations in this study is an obvious selection bias. The authors gave no explanation for the disproportionate number of men in the study. Another limitation is that the authors only presented the unadjusted results. Finally, the authors classified the study as prospective cohort but provided no information on the duration of the study.

Another study examined the cross-sectional association between CRP and PAD along with the additive effect of CRP and PAD on clinical cardiovascular outcomes[14]. This study recruited 110 men and women over the age of 18 years and followed them up for myocardial infarction, stroke, revascularization, or death. At baseline, median levels of CRP were greater in patients with PAD compared to those without PAD (3.83 vs 2.11, $p=0.019$). There appeared to be an additive effect of CRP and PAD on risk of outcome such that participants with high CRP and PAD had the highest risk of incurring an event. One limitation of this study was that the authors did not give the follow-up time for the participants. They provided the mean follow-up time but it would have been relevant to also provide the maximum follow-up time. Additionally, it may have been more suitable to show a survival curve rather than an odds ratio. Another limitation is that the authors only presented the unadjusted results for the association between CRP and PAD.

The NHANES Study assessed the relationship between CRP and PAD among 4,787 men and women over the age of 40 years[15]. To explore associations across range of ABI levels, the authors categorized ABI into 4 levels: less than 0.9 (patients with PAD) and tertiles of ABI among participants without PAD (ABI greater than 0.9). The mean CRP was found to decrease

across increasing categories of ABI (p for trend <0.001). The age standardized prevalence of PAD increased with increasing quartiles of CRP ($p < 0.01$). After adjustment for confounders, participants in the top quartile had a higher odds of prevalent PAD than those in the bottom quartile (OR=2.14, $p<0.001$). The association between CRP and PAD was much stronger in Non-Hispanic blacks compared to Non-Hispanic whites (OR=3.10 vs 1.50, p for interaction = 0.049) and younger adults compared to older adults (OR=5.59 vs 0.98, p for interaction = 0.018). The strongest finding of this study was the notable subgroup differences in the association between CRP and PAD. Contrary to our study, this study showed a stronger association among blacks compared to whites. This could be related to the younger and healthier population that these authors studied. In addition to the authors' categorization of ABI, it may have been interesting to categorize ABI according to severity of PAD. However, sample size restrictions may have limited their ability to do this.

B.2 PROSPECTIVE STUDIES

The Physicians' Health Study examined the association between CRP and future risk of developing PAD among 288 healthy men[16]. Cases of PAD were defined as individuals who self-reported symptoms of intermittent claudication or had revascularization procedures. Controls were randomly selected participants matched on age, smoking habit, and length of follow-up. The mean age of the population was 63 years. Participants in the highest quartile of CRP had twice the risk of developing PAD compared to those in the lowest quartile. One limitation is that the authors used prospective data but selected cases and controls a priori. Another limitation is

that they only looked at symptomatic cases of PAD, which would severely underestimate the true incidence of PAD.

The Edinburgh Artery Study is the only other study that has looked at the prospective association between CRP and subclinical PAD, assessed by the ABI[17]. This study examined CRP at baseline in 1,582 men and women between the ages of 55 and 74 years. Additionally, they measured ABI at baseline, a 5 year, and 12 year follow-up visit. The authors used progression of ABI, defined as change in ABI from baseline to each follow-up visit, as their primary outcome variable. The mean age of the population at baseline was 64.8 years and approximately half (50.9%) of the population was male. At baseline, mean CRP was higher in individuals with symptomatic PAD (intermittent claudication as assessed by WHO) compared to individuals with asymptomatic PAD (ABI < 0.9 but no symptoms of intermittent claudication) or controls. After adjustment for risk factors, baseline levels of CRP were associated with decreasing 12 year decline in ABI (β= -0.018, $p < 0.01$) but not 5 year decline in ABI. The primary limitation of this study is that the authors used ABI as a continuous measure but did not exclude individuals with PAD at baseline. Thus, their conclusions related to progression rather than incidence of PAD. It might have been interesting for the authors to stratify the analysis according to baseline levels of ABI and compare the differential association between CRP and PAD.

In summary, several studies have examined the association between CRP and PAD both cross-sectionally and prospectively. The prospective studies were either restricted to symptomatic cases of PAD or focused on progression of PAD. Our study adds to the literature

by focusing on the relationship between CRP and incidence of PAD in both asymptomatic and symptomatic individuals. Furthermore, our study was able to examine ethnic differences in this association.

BIBLIOGRAPHY

1. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004;24:969-974.

2. Kullo IJ, Seward JB, Bailey KR, Bielak LF, Grossardt BR, Sheedy PF, Peyser PA, Turner ST. C-reactive protein is related to arterial wave reflection and stiffness in asymptomatic subjects from the community. *Am J Hypertens* 2005;18:1123-1129.

3. Nagano M, Nakamura M, Sato K, Tanaka F, Segawa T, Hiramori K. Association between serum C-reactive protein levels and pulse wave velocity: a population-based cross-sectional study in a general population. *Atherosclerosis* 2005;180:189-195.

4. Tomiyama H, Arai T, Koji Y, Yambe M, Hirayama Y, Yamamoto Y, Yamashina A. The relationship between high-sensitive C-reactive protein and pulse wave velocity in healthy Japanese men. *Atherosclerosis* 2004;174:373-377.

5. Mattace-Raso FU, van der Cammen TJ, van dM, I, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis* 2004;176:111-116.

6. Wong M, Toh L, Wilson A, Rowley K, Karschimkus C, Prior D, Romas E, Clemens L, Dragicevic G, Harianto H, Wicks I, McColl G, Best J, Jenkins A. Reduced arterial elasticity in rheumatoid arthritis and the relationship to vascular disease risk factors and inflammation. *Arthritis Rheum* 2003;48:81-89.

7. Pirro M, Schillaci G, Savarese G, Gemelli F, Vaudo G, Siepi D, Bagaglia F, Mannarino E. Low-grade systemic inflammation impairs arterial stiffness in newly diagnosed hypercholesterolaemia. *Eur J Clin Invest* 2004;34:335-341.

8. Booth AD, Wallace S, McEniery CM, Yasmin, Brown J, Jayne DR, Wilkinson IB. Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. *Arthritis Rheum* 2004;50:581-588.

9. Kampus P, Muda P, Kals J, Ristimae T, Fischer K, Teesalu R, Zilmer M. The relationship between inflammation and arterial stiffness in patients with essential hypertension. *Int J Cardiol* 2005.

10. McDermott MM, Guralnik JM, Corsi A, Albay M, Macchi C, Bandinelli S, Ferrucci L. Patterns of inflammation associated with peripheral arterial disease: the InCHIANTI study. *Am Heart J* 2005;150:276-281.

11. Yu HI, Sheu WH, Song YM, Liu HC, Lee WJ, Chen YT. C-reactive protein and risk factors for peripheral vascular disease in subjects with Type 2 diabetes mellitus. *Diabet Med* 2004;21:336-341.

12. McDermott MM, Green D, Greenland P, Liu K, Criqui MH, Chan C, Guralnik JM, Pearce WH, Ridker PM, Taylor L, Rifai N, Schneider JR. Relation of levels of hemostatic factors and inflammatory markers to the ankle brachial index. *Am J Cardiol* 2003;92:194-199.

13. Unlu Y, Karapolat S, Karaca Y, Kiziltunc A. Comparison of levels of inflammatory markers and hemostatic factors in the patients with and without peripheral arterial disease. *Thromb Res* 2005.

14. Beckman JA, Preis O, Ridker PM, Gerhard-Herman M. Comparison of usefulness of inflammatory markers in patients with versus without peripheral arterial disease in predicting adverse cardiovascular outcomes (myocardial infarction, stroke, and death). *Am J Cardiol* 2005;96:1374-1378.

15. Wildman RP, Muntner P, Chen J, Sutton-Tyrrell K, He J. Relation of inflammation to peripheral arterial disease in the national health and nutrition examination survey, 1999-2002. *Am J Cardiol* 2005;96:1579-1583.

16. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998;97:425-428.

17. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. *Circulation* 2005;112:976-983.