EVALUATING NOVEL RISK FACTOR ASSOCIATIONS FOR SUBCLINICAL CARDIOVASCULAR DISEASE

by

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Globally, cardiovascular disease (CVD) is the leading cause of death. Increased risk for CVD can be attributed to smoking, high blood pressure, poor lipid profiles, obesity and psychosocial factors. Markers of subclinical CVD are non-invasive measures that detect early atherosclerotic changes. The purpose of this dissertation was to evaluate novel risk factor associations for subclinical CVD in three distinct populations.

The protective effect of HDL-c for subclinical CVD was diminished in a population of postmenopausal women compared to premenopausal women. Furthermore, the concentration of small HDL particles was higher among postmenopausal women. Lipid profile changes with the menopausal transition may in part explain the increased risk of CVD seen after menopause.

The protective effect of education for subclinical CVD was evident only among females from an Afro-Caribbean population. Educational differences in SBP and lipids varied for males and females providing insight into potential mechanisms for the education-subclinical CVD relationship observed on the island of Tobago.

Tonic cardiac sympathetic activity and parasympathetic reactivity were independent predictors of subclinical CVD in a population of overweight and obese young adults. The effect of C-reactive protein (CRP) on subclinical CVD is potentially explained by the autonomic anti-inflammatory mechanisms linking heart rate variability and CRP.

Identifying novel risk factor associations for subclinical CVD in various populations supports the important public health objective of reducing the global burden of CVD morbidity and mortality through early detection of atherosclerosis.

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1.0 DISSERTATION OVERVIEW AND OBJECTIVES

Globally, cardiovascular disease (CVD) is the leading cause of death. Increased risk for CVD can be attributed to smoking, high blood pressure, poor lipid profiles, obesity and psychosocial factors. Markers of subclinical CVD are non-invasive measures that detect early atherosclerotic changes. These measures are associated with traditional CVD risk factors and have shown to predict future cardiovascular events.

The intention of this dissertation is to evaluate novel risk factor associations for subclinical CVD in three distinct populations. The three aims and study populations are described below:

1. As women transition through menopause their risk for CVD increases. It is plausible that adverse lipid profile changes during the transition may explain a portion of this increased risk. SWAN Heart, an ancillary study to SWAN (Study of Women's Health Across of the Nation), assessed subclinical CVD during the menopausal transition. The aim of this manuscript was to determine if the relationship between lipids and subclinical CVD varies by menopausal status. Understanding the mechanisms that promote early CVD in women may lead to new preventative efforts or delay disease onset in the postmenopausal years.

2. Education is protective against adverse health outcomes; however the effect of education on CVD has shown to be steeper among females. This manuscript aimed to determine the association between education and subclinical CVD in a

community-based sample of Afro-Caribbean males and females from the Tobago Family Health Study. Differences in CVD risk factors by education may offer potential mechanism for gender differences in the education-subclinical CVD relationship.

3. Heart rate variability (HRV) and C-reactive protein (CRP) are known markers of CVD. Autonomic nervous system activity, as measured by HRV, modulates inflammation. The primary aim of this manuscript was to determine which CVD risk factors predicted HRV, CRP and subclinical CVD and how HRV and CRP together predicted subclinical CVD in a population of moderately overweight and obese young adult men and women from the study to Slow Adverse Vascular Effects (SAVE). Using measures of tonic sympathetic and parasympathetic outflow simultaneously and including HRV reactivity to standard autonomic challenges may provide further evidence into how the autonomic nervous system relates to early atherosclerotic changes.

2.0 GENERAL INTRODUCTION

2.1 CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the leading cause of death in the world [1]. In 2004, 32% of mortality among females and 27% among males were due to myocardial infarction and stroke. A dramatic increase from 17.5 million CVD deaths in 2005 to 24.2 million in 2030 is projected [2]. The estimated cost of CVD in the United States is $475.3 billion for 2009 [3]. Worldwide, over 80% of CVD mortality occurs in low to middle income developing countries [4]. There is tremendous economic growth potential lost in these countries because CVD death occurs among the working aged [5]. Given the large economic impact and premature death, prevention is imperative in reducing the global burden of CVD. Identifying novel risk factor associations with subclinical CVD can facilitate the stratification of individuals at increased risk for early CVD.

2.2 ATHEROSCLEROSIS

Atherosclerosis is the initial process that ultimately leads to myocardial infarction and stroke [6, 7]. Luminal occlusion begins with repeated damage to the endothelium. Inflammation, toxins and oxidized LDL-c are hypothesized mechanisms of vascular injury. Following injury, monocytes attach to the endothelium due to increased adhesion molecule expression and migrate into the intimal layer where they develop into macrophages. These

macrophages accumulate lipids, referred to as foam cells, and together with lymphocytes, become fatty streaks. If these streaks are allowed to progress, as the result of continued CVD risk factor exposure, they will eventually become plaques. Fibrous plaques are composed of foam cells and layers of smooth muscle cells. Atherosclerotic changes progress to incident CVD as a result of cumulative plaque burden, arterial lumen narrowing and vulnerability of the lesions [8]. Large lesions with thin fibrous caps and vascular remodeling are factors that promote plaque rupture and subsequent hemorrhage and thrombosis, resulting in vessel occlusion [9].

2.3 SUBCLINICAL CARDIOVASCULAR DISEASE

Subclinical CVD measures are non-invasive techniques that allow for the early detection of vascular changes due to aging and other risk factors. The following measures are used in this dissertation and appear in chapters 3, 4 and 5.

2.3.1 Carotid Intima Media Thickness

Carotid intima media thickness (IMT) is a measure of the inner two layers of the arterial wall. B-mode ultrasound is a non-invasive technique that is used to capture reliable measures of IMT [10]. Arterial wall hypertrophy and dilation occur with vascular adaptation to increased blood pressure and atherosclerosis [11, 12]. These changes maintain shear and tensile stresses. IMT is primarily a result of intimal hypertrophy [13]. However, ultrasound techniques are not sensitive enough to capture only the intimal layer and therefore the intima and media are measured collectively [10].

IMT has shown to be an independent predictor of incident CVD: myocardial infraction, stroke and mortality [14-20]. In addition, IMT is associated with traditional CVD risk factors such as age, male gender, lipids, smoking, glucose and blood pressure [14, 17, 21-26]. A linear increase in IMT has been observed with increasing age among healthy adults [24]. The increased risk of incident CVD seen with increasing IMT is similar to the observed increase with age, suggesting IMT is good measure of vascular aging [27]. IMT changes have been noted with SBP above 120 mmHg and these changes were independent of other CVD risk factors [25]. A 40 mg/dL increase in LDL-c was associated with a 0.02 to 0.05 mm increase in IMT [26]. Small changes in IMT indicate large changes in risk. For example, the odds of myocardial infarction and stroke increased 25% and 34%, respectively, over a 3 year period for a 1 SD increase in IMT (0.163mm) [28]. Although IMT is not necessarily synonymous with atherosclerosis, it is likely the result of similar factors as plaque formation given the strong associations with traditional risk factors [10]. This in conjunction with its ability to predict incident CVD makes IMT a valuable subclinical measure of CVD.

2.3.2 Arterial and Lumen Diameter

As with intima media thickness, adventitial and lumen diameter (AD and LD, respectively) are obtained from B-mode ultrasound imaging. The AD is the distance between the adventitial and medial interfaces on the near and far wall, while the LD is the distance between the intima and lumen interfaces. An intraclass correlation coefficient of 0.99 has been previously reported for AD [29]. Traditional CVD risk factors, such as age, weight, SBP, glucose and HDL-c have been shown to be independently associated with AD in a population of older adults from the EVA study [30].

Larger diameters result from outward remodeling due to age related decreases in arterial wall elastin and mechanical stress [31, 32]. In a subset of non-smoking ARIC participants free of known CVD, diabetes, hypertension, obesity or hyperlipidemia, AD was positively associated with age, suggesting outward remodeling is independent of known CVD risk factors. Besides age associated remodeling, AD increases with hemodynamic changes seen with plaque formation. AD was shown to be higher among postmenopausal women with carotid plaque compared to women without plaque in a case-control study matched on age and adjusted for HDL-c and SBP [33].

In addition to outward remodeling, hypertrophy occurs as a result of risk factor exposure. Consequently, increases in AD may mask IMT changes. Therefore, AD and IMT should be evaluated simultaneously as predictors of incident CVD. In a subset of ARIC participants, AD was an independent predictor of incident CVD after adjusting for traditional risk factors and carotid IMT [34].

2.3.3 Carotid Plaque

Eccentric carotid plaque develops as a result of atherosclerotic progression within the vessel wall. Plaque is often distinguished from IMT on ultrasound imaging as focal protrusion greater than 50% of the surrounding arterial wall. IMT is a potential precursor for carotid plaque given its positive correlation independent of traditional risk factors and its modest predictability of carotid plaque ($R^2 = 0.23$) [35, 36]. Traditional CVD risk factors, such as age, SBP, smoking, BMI and glucose have shown to predict carotid plaque [37]. In addition, among several large longitudinal studies, carotid plaque has predicted myocardial infarction, stroke and death [19, 38, 39].

2.3.4 Aortic Calcification

Electron beam computed tomography (EBCT) is a non-invasive method that requires minimal radiation exposure to detect calcified aortic plaques. Age, gender, diabetes, lipids, SBP and smoking have been shown to predict aortic calcification (AC) [40]. The Framingham Heart study observed a 6 to 8 fold increase in AC over a 25 year follow up [41]. Unlike coronary calcification, where males tend to have much higher rates compared to females, AC differences by gender were less striking in the Rotterdam Study [22]. A 20mg/dL increase in LDL-c was associated with an OR for AC of 1.33 (95% CI: 1.0, 1.8) whereas a 10mg/dL increase in HDL-c was associated with an OR of 0.70 (95% CI: 0.47, 1.0) [42].

2.3.5 Pulse Wave Velocity

Pulsatile blood flow is converted to continuous flow by stored energy in compliant vessel walls and pressure wave reflection [43]. Arterial stiffening due to vascular aging and hypertrophy of the vessel wall leads to noncompliant vessels and subsequently increased pulse pressure and decreased coronary blood supply during diastole [44, 45]. Pulse wave velocity (PWV) is a highly reproducible method of choice for assessing arterial stiffening [46, 47]. The Rotterdam study observed a significant association between PWV and IMT, carotid plaque, aortic calcification and peripheral artery disease [48]. In addition, to predicting other measures of subclinical atherosclerosis, increased PWV is related to CVD events and mortality [49-51]. The Health ABC study observed a > 50% increase in incident CVD among those in the top 25th percentile of PWV.

2.4 CARDIOVASCULAR RISK FACTORS

A vast number of risk factors have been established as independent predictors for subclinical and clinical CVD [52]. These risk factors include, but are not limited to: age, lipids, weight, gender, socioeconomic status and heart rate variability. Assessment of these factors among various populations can help with risk stratification and determine mechanism for disease development. The following risk factors are evaluated in this dissertation and appear in chapters 3, 4 and 5.

2.4.1 Menopause

Menopause is the cessation of menses due to ovarian follicular inactivity [53]. The menopausal transition begins with changes to the regular menstrual cycle and ends when menstruation has stopped for one year. Natural menopause occurs between 45 to 55 years of age and lasts 4 to 5 years on average [53, 54]. Hormones are known to fluctuate on a day-to-day basis as women transition through menopause, with a gradual decrease in estrogen and an increase in follicular stimulating hormone (FSH) over time. Estrogen is known to increase vasodilation, reduce vascular injury and reduce smooth muscle cell proliferation. In addition, a decrease in LDL-c, an increase in HDL-c and a reduction of LDL-c oxidation have been documented. Therefore, menopause is associated with a decrease in a hormone that has cardioprotective effects.

The decline in estrogen that accompanies menopause is thought to contribute to the increased risk for CVD that is observed among postmenopausal women [55, 56]. However, it is also possible that the increased risk that accompanies the 4-5 year menopausal transition is a function of older age. Thus, the role of age should be considered in examining whether

menopausal changes in hormones contribute to CVD risk. Simple adjustment may not account for the entire effect of age if the range is too wide and there is little overlap between the pre- and postmenopausal groups. Therefore, careful consideration in recruitment needs to be addressed in order to reduce bias and differentiate risk associated with hormonal changes from age-related increases in risk [57].

Growing evidence shows that a number of factors contribute to menopause-related increases in CVD risk that are independent of age [58]. Both total cholesterol and LDL-c have been shown to be higher among the post- than pre-menopausal group [59]. The Study of Women's Health Across the Nation showed a significant difference in LDL-c from pre- to post-menopause (116.3 vs. 123.4 mg/dL, $p < 0.0001$). Weight gain over the menopausal transition is most likely due to chronological aging; however, increases in visceral adiposity and decreases in lean mass have been attributed to menopause [60, 61]. In the Vermont Longitudinal Study of the Menopause, postmenopausal women had higher fat mass and higher intra-abdominal adiposity compared to premenopausal women (18 vs. 23 kg, $p<0.01$ and 65 vs. 77 cm^2, $p<0.05$).

2.4.2 Lipoproteins

Low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) are often referred to as the *'bad'* and *'good'* cholesterol; however, they are not types of cholesterol, but rather different transporters of lipids and proteins [62]. LDL-c has shown to be directly related to the risk of CHD in a large population of Japanese men and women [63]. However, LDL-c levels alone may not accurately characterize lipid profiles [64, 65]. Two individuals may have similar LDL-c levels; however, the number of particles carrying that cholesterol may vary greatly. NMR spectroscopy measures the total number of terminal

methyl groups in each lipoprotein particle, thereby approximating the diameter of the lipoprotein [64]. This method provides an estimate of the particle concentration rather than the amount of cholesterol being carried by the particles.

Several studies have concluded small LDL particles are more atherogenic compared to large particles [65]. However, the MESA study, a large population of relatively healthy men and women, ages 45 to 84 years, demonstrated that both small and large LDL particles were predictors of subclinical CVD, and only when the inverse correlation between small and large particles was accounted for was this association apparent [66]. It has been suggested that LDL particle number is a more informative risk factor compared to LDL-c or LDL particle size [65].

HDL-c is inversely related to CVD. It exerts its protective effects through reverse cholesterol transport from the periphery to the liver for secretion with bile [67]. In addition, HDL-c is known for its anti-atherogenic properties, such as inhibition of adhesion molecules and LDL-c oxidation [68]. The protective properties of HDL-c may not be equally shared between large and small particles [69]. In a population of adult men with a known history of myocardial infarction, small HDL particles were directly related to the progression of coronary atherosclerosis, while large particles were inversely related [70]. Therefore, HDL particle size, as a CVD risk factor, may provide additional information beyond HDL-c.

2.4.3 Education

Socioeconomic status (SES) is a complex combination of education, income and occupation [71, 72]. Using more than one measure of SES is ideal because the three measures contribute different information, however, education is the variable most often used in epidemiology research [73]. It has shown to be reliable and valid [74]. Education is a

measure of social, psychological and economic resources. It influences behavior, problem solving and values. Since education is attained early in life, it does not reflect changes in SES or accumulation of SES in adulthood [75]. Income and occupation reflect a more current status of adulthood SES. However, income and occupation are difficult to analyze among the unemployed, homemakers or retired. In addition, it is possible that health status could influence occupation and subsequently income. Education may improve health related knowledge and allow for favorable occupation and income in adulthood.

Education has been shown to be a strong predictor of traditional CVD risk factors and disease. In a study of Scottish males, occupation was a stronger predictor of all cause mortality, but when individual causes of mortality were explored, education was strongly associated with cardiovascular mortality [73]. Using US census data, education was inversely associated with mortality [76]. Income did not account for additional variation in mortality. When income was substituted for education, traditional CVD risk factors explained the majority of the association between income and CVD events [77].

2.4.4 Autonomic Nervous System

The autonomic nervous system (ANS) is a component of the peripheral nervous system consisting of two main branches, the sympathetic and the parasympathetic. The sympathetic and parasympathetic branches innervate the sinoatrial node of the heart and regulate heart rate variability [78]. Vasculature tone is achieved via sympathetic vasoconstriction and parasympathetic vasodilation [79]. The ANS is responsible for maintaining vascular homeostasis when the system is disturbed. For example, during a rapid standing position, blood will pool in the lower extremities. As a result, there is decreased cardiac return and subsequently decreased cardiac output. This drop in blood

pressure triggers the sympathetic branch to increase heart rate and peripheral resistance in order to restore the arterial pressure [80].

Heart rate variability (HRV) is a well known and validated method to measure the ANS. This method has produced reproducible results in reliability studies [81]. Heart rate and respiratory rate variability, from the electrocardiogram and bioimpedance plethysmography, respectively, is converted to quantitative estimates of ANS function via spectral analysis [82-84]. From the classic frequency domain analysis, the high frequency peak (HF) has been shown to correlate with parasympathetic (vagal) activity; however, the low frequency peak (LF) is believed to be a combination of sympathetic and parasympathetic activity. These interpretations are based on pharmacologic studies observing a reduction in the LF peak with sympathetic blockade, but a reduction in both LF and HF peaks with parasympathetic antagonist [78]. Another concern is slower respiratory rates can cause false elevations in LF [85, 86]. To address these problems, a new method was created to take into account respiratory rate. By isolating the respiratory peak on the respiratory rate spectral analysis, and centering the HF component at this frequency, parasympathetic and sympathetic activity estimates are improved [86].

Measures of HRV have been associated with increased risk of CHD and mortality. The ARIC study, a population based cohort of adult men and women, ages 45 to 64 years, found a significant inverse association (RR = 1.72, 95% CI: 1.17-2.51) for CHD in the lower quartile of HF compared to the upper three quartiles [87]. No significant association was observed for LF or LF/HF ratio, a possible measure of sympathovagal balance. A decrease in all HRV measures, except LF/HF ratio, was associated with a significant increase in the risk of incident CVD among the Framingham Heart Study cohort [88]. The lack of association of CHD and LF in the ARIC study and the inverse association in the Framingham study may reflect the mixture of sympathetic and parasympathetic activity within the LF component.

2.4.5 C-Reactive Protein

C-Reactive Protein (CRP) is a marker for low-grade systemic inflammation that can be measured in peripheral circulation [89]. Inflammation is involved in the atherosclerotic process from vascular injury and endothelial dysfunction to foam cell development and plaque rupture. Cytokines produced during atherosclerotic progression, specifically, IL-1, IL-6, and TNF-α, are known to trigger the acute phase response, which includes the production of CRP by hepatocytes [90]. In addition, IL-6 and CRP are produced by adipocytes, which may account for the positive associations of weight with circulating levels of CRP [91].

CRP is an inexpensive and widely available marker of general inflammation [92]. The mechanism by which CRP is related to the progression of atherosclerosis is complex. A significant association has been observed with age, smoking, BMI, lipids, clotting factors and glucose [93]. CRP has proven to be a sensitive marker of incident CVD in a general population of British men [94]. The adjusted odds ratio for CHD was 2.13 (95% CI: 1.38, 3.28) for the top tertile of CRP compared to the bottom. The Women's Health Study showed an adjusted relative risk of 1.5 (95% CI: 1.1, 2.1) for CVD events (myocardial infarct, stroke, revascularization and death) in a population of postmenopausal women [95]. Despite its predictability, the benefit of therapeutic reduction in CRP is debatable given the lack of a casual association observed in a genetics study [83]. In addition, CRP was not found to improve risk stratiifcation over convential factors in a large population of Swedish men and women [96]. Nevertheless, CRP can assist in the explanation of atherosclerotic disease mechanims.

2.5 STATISTICS

2.5.1 Multinomial Logistic Regression

Multinomial logistic regression (MLR) and ordinal logistic regression (OLR) are used when the outcome measure is a categorical variable with more than two levels [97]. Nominal outcomes are analyzed with MLR and ordinal outcomes by OLR. OLR takes into account the rank order of the outcome when calculating the odds ratio, but requires that the predictor variables have the same effect on each ordered outcome level. When the logistic regression model does not meet this assumption of proportional odds, results can be misleading and MLR should be used [98]. MLR generates an odds ratio for each pair of outcome measures; however, it does not consider the order of the outcome measure.

In chapter 3, aortic calcification, a three level categorical measure (none, moderate and high), is one of the key outcome variables assessed. The three levels are ordinal in nature; however, the assumption of proportional odds was violated. Therefore, MLR was the method of choice when analyzing aortic calcification.

2.5.2 Collider Bias

Collider bias can occur when exposure and disease influence a third variable (*C*) and the association between exposure and disease is conditioned upon *C* [99, 100]. If sample selection or stratification is based on *C* this can create a collider bias; the results from these data analyses may misrepresent the true relationships between the variables and false conclusions may be drawn regarding causality. The direction of the associations between exposure, disease and *C* is critical. If *C* causes disease and *C* is related to exposure then

stratifying on *C* would remove bias (confounding). However, if exposure and disease affect *C* then the observed change in the association estimate is biased. The effect of exposure on *C* or disease on *C* does not have to be direct, it can occur through other measured or unmeasured variables.

The manuscripts in chapters 3 and 4 both include interactions in the model; therefore, it was necessary to rule out the possibility for collider bias. Chapter 3 assessed the association between HDL-c and subclinical CVD by menopausal status. It is unlikely that HDL-c (exposure) or subclinical CVD (disease) influences menopausal status (*C*), therefore, the risk for collider bias is small. However, it is plausible that other measured or unmeasured factors may affect menopausal status; thus the potential for collider bias is still possible. In addition, chapter 4 evaluated the effect of education on subclinical CVD among a population of Tobagonian males and females. Since education (exposure) and subclinical CVD (disease) do not determine ones gender (*C*), conditioning on gender is acceptable.

3.0 THE MENOPAUSAL TRANSITION MODIFIES THE CONTRIBUTION OF LIPIDS TO EARLY ATHEROSCLEROSIS IN SWAN HEART WOMEN

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3.1 ABSTRACT

Background: The risk of cardiovascular disease increases after menopause. Changes in the lipoprotein profile may in part be responsible for this trend. Evaluating the contribution of HDL cholesterol (HDL-c) to subclinical cardiovascular disease across the menopausal transition would provide insight for this increased risk. Methods: Aortic calcification (AC), carotid plaque and intima media thickness (IMT) were measured in Pittsburgh and Chicago women enrolled in an ancillary study to the Study of Women's Health Across the Nation (SWAN). Women were stratified into two menopause groups; premenopausal or early perimenopausal (Pre/EP) and late perimenopausal or postmenopausal (LP/Post). Results: On average Pre/EP women were 49 years of age and LP/Post women were 52 years of age. LP/Post women had slightly higher HDL-c levels compared to Pre/EP women (59 vs. 56 mg/dL, p = 0.04). Across all women, HDL-c was negatively associated with high and moderate AC, any carotid plaque and IMT in univariate analysis. However, interactions between menopause status and HDL-c were statistically significant for AC and IMT in fully adjusted models (p = 0.01, 0.03), but borderline for any carotid plaque (p = 0.08). In multivariable models, Pre/EP women demonstrated a negative association between HDL-c levels and moderate AC (OR = 0.97, 95%CI 0.94, 1.00), high AC (OR = 0.98, 95%CI 0.94, 1.02), any carotid plaque (OR = 0.97, 95%CI 0.94, 1.00) and IMT (β = -0.001, 95%CI -0.002, -0.0002), however only IMT showed a significant association. In LP/Post women, a positive association between HDL-c levels and moderate AC (OR = 1.03, 95%CI 1.00, 1.06), high AC (OR = 1.05, 95%CI 1.01, 1.09), any carotid plaque (OR = 1.01, 95%CI 0.98, 1.03) and IMT (β = 0.0002, 95%CI -0.0006, 0.0009) was observed, but was only significant for high AC. In the small sub sample of Pittsburgh women, LP/Post women had more small

HDL particles (HDL-p) compared to Pre/EP women. Conclusion: In conclusion, the protective effect of HDL-c appears to diminish in LP/Post women compared to Pre/EP women. This loss of protection may be due to increased concentrations of small HDL-p.

3.2 INTRODUCTION

Cardiovascular disease (CVD) contributes to 39 percent of mortality in American women [1]. As women age they are increasingly exposed to major CVD risk factors, including a poor lipid profile and weight gain. In addition to chronological aging, women experience the biological changes of the menopausal transition. The largest increase in CVD rates around the menopausal transition adds additional risk above and beyond chronologic aging [2]. Decreasing estrogen levels have been linked to endothelial dysfunction and poor vascular tone [3-5]. However, there may be additional mechanisms through which the menopausal transition confers CVD risk.

Lipids are known to change in association with both age and the menopausal transition [6, 7]. Understanding these changes, including how they contribute to early vascular disease, may provide evidence to the underlying mechanism(s) that increases CVD risk following the transition. During the transition, low density lipoprotein cholesterol (LDL-c) levels increase, while high density lipoprotein cholesterol (HDL-c) levels tend to remain stable or increase slightly [8-12]. It is widely accepted that increasing levels of LDL-c promote CVD while increasing levels of HDL-c are protective. However, no studies have yet evaluated whether or not these associations are consistent across the stages of menopause. One study has suggested that the protective effects of HDL-c on IMT may diminish over time in middle aged-women [13].

The Study of Women's Health Across the Nation (SWAN) provides a unique opportunity to determine if the relationship between lipids and CVD varies by menopausal status. SWAN has explored biological changes as women have transitioned through menopause, including subclinical CVD measures in two of the seven sites. Subclinical CVD measures are reliable measures of the atherosclerotic process [14]. Accordingly, the purpose of this study was to evaluate whether the menopausal transition modifies the contribution of lipids to subclinical CVD, specifically, aortic calcification (AC), carotid plaque and intima media thickness (IMT). These non-invasive subclinical measures have been shown to predict clinical events including stroke, myocardial infarction and cardiovascular death in large population studies [15-19]. These studies have shown independent positive associations between subclinical measures and the following traditional CVD risk factors: increasing age, African American race, hypertension, obesity, hyperlipidemia, diabetes and smoking [20]. Therefore, subclinical measures can be used to evaluate risk factor associations and possible differences in these associations by menopausal status. Understanding mechanisms that promote early CVD in women may lead to new preventative efforts or delay CVD in the postmenopausal years.

3.3 METHODS

3.3.1 Study Population

The study population for this analysis included 540 women who participated in SWAN Heart, an ancillary study to the Study of Women's Health Across the Nation. Detailed study design and recruitment descriptions for SWAN have previously been published [21]. In brief, this multi-center, community-based, longitudinal study recruited women from 7 clinical sites

(Chicago, IL; Pittsburgh, PA; Boston, MA; Detroit, MI; Newark, NJ; Oakland, CA; and Los Angeles, CA) to assess the biological and psychological changes associated with the menopause transition. Baseline enrollment of 3302 women between the ages of 42 and 52 years took place during 1996 to 1997. Only women who had an intact uterus and at least one ovary, menstruated in the prior 3 months and not taken hormone therapy or oral contraceptives in the prior 3 months were eligible for the parent project. Women who were pregnant or breast feeding were excluded.

The ancillary study, SWAN Heart, evaluated 608 women for subclinical CVD. Baseline enrollment at the Pittsburgh and Chicago sites occurred between 2001 and 2003 (corresponding to the 4^{th} - 7^{th} annual SWAN visits). Women were ineligible for SWAN Heart if they had a history of CVD (angina, myocardial infarction, congestive heart failure, stroke, transient ischemia attack or vascular surgery), had a hysterectomy or bilateral oophorectomy since the start of SWAN, were pregnant or were taking diabetic or hormone therapy. Women were excluded from this analysis for CVD history (n=2), hysterectomy/oophorectomy (n=16), diabetes (n=1) and hormone therapy (n=32). An additional 17 women were excluded due to missing menopausal status or no subclinical vascular measures collected, resulting in a final sample size of 540 (89%) for this analysis.

Research protocols for SWAN and SWAN Heart were approved by the site specific institutional review boards and all women provided informed consent prior to enrollment.

3.3.2 Subclinical Measures

Aortic calcification (AC) was quantified using electron beam computed tomography (EBCT; Imatron C-150 Ultrafast CT Scanner, GE Imatron, San Francisco, CA). An initial scout scan was done to identify anatomical landmarks. Next, the scanner obtained cross-sectional 6

mm images with a 300 ms exposure time from the aortic arch to the iliac bifurcation. Participants were exposed to a total of 2.45 rads during the aortic scan. All scans were saved to optical disc and read centrally at the University of Pittsburgh using a DICOM workstation and Acuimage software (South San Francisco, CA). Using the Agatston scoring method, 3 contiguous pixels > 130 Hounsfield units was used to determine the presence of lesions within the aorta. A total calcium score was calculated for the entire vessel by one blinded physician trained in EBCT.

Carotid intima media thickness (IMT) and carotid plaque assessment were made using B-mode ultrasound (Pittsburgh site: Toshiba American Medical Systems, Tustin, CA and Chicago site: Hewlett Packard, Andover, MA). Right and left carotid arteries were scanned to obtain a total of 8 images: near and far wall of common carotid (1 cm proximal to bulb), far wall of carotid bulb (starting from the point where the common carotid walls are no longer parallel and ending at the flow divider) and far wall of internal carotid artery (distal 1 cm from flow divider). Semi-automated reading software (AMS system developed in Sweden by Dr. Thomas Gustavsson) took 140 measurements of IMT from the leading edge of the intima to the trailing edge of the media over the 1 cm segment. The mean IMT of each of the 8 IMT segments was determined, and the average of these 8 mean measures was computed for the outcome variable in this analysis. Carotid plaques were identified as discrete focal protrusions > 50% of the surrounding wall thickness. These plaques were graded from 1 to 3 based on the size and number. The cumulative plaque grades for the left and right carotid arteries were summed to generate a plaque index. Image readings were performed centrally at the University of Pittsburgh Ultrasound Research Laboratory (URL; Pittsburgh, PA). Reproducibility of IMT during annual recertifications and plaque readings from previous work in the URL are as follows: intraclass correlation coefficients for IMT 0.98 and plaque 0.86 to 0.93 [22, 23].

3.3.3 Covariates

SWAN collected annual self- and interviewer-administered questionnaires, fasting blood glucose and lipid laboratory measures, anthropometric measures and blood pressure readings. Data from these annual SWAN clinic visits (4^{th} visit: n=272, 5^{th} visit: n=231, 6^{th} visit: n=20 and 7^{th} visit: n=17) were matched to participant's baseline SWAN heart subclinical measures in this cross-sectional analysis. Demographic factors such as age, race (only Caucasian and African American race for the Pittsburgh and Chicago sites), alcohol and smoking status were obtained through questionnaires. Participants were asked if they had ever smoked on a regular basis defined as at least 20 packs in a lifetime or 1 cigarette per day for at least one year. They were also asked the number of servings of beer, wine or liquor they consumed on an average day, week or month. Based on self-reported bleeding history, menopausal status was classified as premenopausal (Pre; menses in the last 3 months with no irregularity), early perimenopausal (EP; menses in the last 3 months with irregularity), late peri-menopausal (LP; no menses for at least 3 months, but less than 12 months) and postmenopausal (Post; no menses for at least 12 months). Blood pressure was collected by a standard sphygmomanometer in the right arm of participants after a 5 minute rest period. A total of three pressures were collected and the last two were averaged. Waist circumference was collected to the nearest 0.1 cm at the narrowest portion of the torso in each participant. Fasting blood samples were collected and analyzed at the Medical Research Laboratories (Lexington, KY). A hexokinase-coupled reaction was used to measure glucose levels (Boehringer Mannheim Diagnostics, Indianapolis, IN). EDTA treated plasma was used to analyze lipids; isolation of HDL-c

(mg/dL) was done with heparin-2M manganese chloride and LDL-c (mg/dL) was estimated with the Friedewald equation [24].

Lipoprotein assays were conducted on blood samples from women at the Pittsburgh site (sample collection dates from 1997 to 2001). Lipoprotein subclasses were determined on stored EDTA plasma using an automated nuclear magnetic resonance (NMR) spectroscopic assay (LipoScience Inc., NC), with a modification of the method previously reported [25, 26]. Briefly, the particle concentrations of the lipoprotein subclasses were derived from the measured amplitudes of the characteristic lipid methyl group NMR signals they emit. The following subclasses were analyzed for this study: large LDL particles (LDL-p) (21.2-23 nm), small LDL-p (18-21.2 nm), large HDL particles (HDL-p) (8.8-13 nm) and small HDL-p (7.3-8.8 nm). Subclasses were summed to provide total LDL-p and total HDL-p, and mean LDL and HDL particle sizes are weighted-averages (i.e. the diameter of each subclass multiplied by its relative concentration). NMR-determined LDL and HDL subclass distributions are correlated with those determined by gradient gel electrophoresis [27, 28]. A total of 71 SWAN Heart women had lipoprotein data and subclinical CVD measures available. Participants had up to three lipoprotein assays conducted; however, the latest date was chosen to match to SWAN Heart subclinical measures. Those with lipoprotein data collected more than 16 months apart from their subclinical measures were excluded from this analysis. Of the remaining 53 women, average collection time from subclinical measures was 184.5 days for Pre/EP and 236.3 days for LP/Post women.

3.3.4 Statistical Analysis

The distribution of each continuous variable was examined and variables were log transformed or categorized if the distribution was not approximately normal. Glucose,

alcohol consumption, AC and carotid plaque were identified as having skewed distributions and large proportions of zero values. Glucose was log transformed; however, medians (IQR) are presented in Table 1 for descriptive purposes. Alcohol and carotid plaque were dichotomized into any or none, and AC was classified as none, moderate and high calcification (0, 1-74 and 75+, respectively). High AC was set at the 75th percentile [29]. Menopausal status was dichotomized into Pre plus EP menopause (Pre/EP) and LP plus Postmenopausal (LP/Post) to maintain a moderate sample size [30]. All remaining variables met the guidelines for normality and were treated as continuous. Two sample t-test and chi-square test were utilized to compare participant characteristics between Pre/EP and LP/Post.

Univariate and multivariable logistic and linear regression were used to assess the association between traditional CVD risk factors and AC, carotid plaque and IMT. For AC, multinomial logistic regression rather than ordinal logistic regression was preferred due to unmet assumptions of proportional odds [31]. No AC and no carotid plaque were the reference groups for the multinomial and binary logistic regression, respectively. Stepwise regression selection was used to evaluate the inclusion of traditional CVD risk factors in the multivariable linear and logistic regression models. Default p-value entry and exit (p-value = 0.15) were used, however, final models were chosen to be parsimonious. Age, site and race were variables of interest and therefore kept in the final models. Waist circumference was included in multivariable models since it explained more of the variation compared to BMI. Interactions between HDL-c and menopausal status and LDL-c and menopausal status were tested in multivariable models with and without adjustments for other covariates. Further exploration of the HDL-c interactions was done using a reparameterized model to include the effects of HDL-c among Pre/EP and LP/Post women in a single multivariable model. The sub sample of Pittsburgh women (n=53) with available lipoprotein data was

compared across menopausal status using a two sample t-test. These data were not used in the regression analysis due to small sample size. All statistical analyses were completed with SAS 9.1. Two-sided p-values ≤ 0.05 were considered statistically significant.

3.4 RESULTS

Of the 540 SWAN Heart participants included in this analysis, 316 (59%) were Pre/EP and 224 (41%) were LP/Post women (Table 3.1). Average age was 50 years, with Pre/EP women being 3 years younger than LP/Post women. Approximately 39% were African American, 62% drank alcohol and 16% had ever smoked regularly. These characteristics did not differ by menopausal status. LP/Post women had higher mean HDL-c, LDL-c, SBP and glucose compared to Pre/EP. Following age-adjustment, mean HDL-c and SBP no longer differed significantly. Average BMI for the total group was 29.4 kg/m^2. There was a trend towards a higher mean waist circumference in LP/Post women. In addition, LP/Post women had more AC, more carotid plaque and higher IMT on average, however, once adjusted for age, only carotid plaque differed between the two groups.

In univariate analysis (Table 3.2) the odds of high AC increased with age, LDL-c, SBP, glucose, waist circumference, smoking and postmenopausal status. Higher HDL-c and alcohol use were significantly associated with lower odds of high AC. African American race was significantly associated with higher odds of moderate AC, but was not statistically significant associated with high AC. For carotid plaque, only age, LDL-c and postmenopausal status significantly increased the odds of any carotid plaque, while alcohol use lowered the odds of any carotid plaque. Thicker IMT was seen with increasing age, LDL-c, SBP, glucose, waist circumference, African American race and postmenopausal status, while IMT decreased with increasing HDL-c and alcohol use.

In the multivariable models adjusted for age, site, race, HDL-c, LDL-c, SBP, glucose, waist circumference and smoking, the effect of HDL-c significantly varied by menopausal status (interaction p-value = 0.01 for high and moderate AC). To better visualize the interactions, the models were reparameterized to the following: subclinical measure = Menopausal Status + Effect for HDL-c for Pre/EP women + Effect for HDL-c for LP/Post women + Covariates (Figure 3.1). For every 1 mg/dL increase in HDL-c the odds of high AC among Pre/EP women was decreased by 2% (OR = 0.98, 95% CI = 0.94, 1.02) while the odds for LP/Post women was increased by 5% (OR = 1.05, 95% CI = 1.01, 1.09). For the carotid plaque model, the interaction p-value was 0.08 after adjustment for age, site, race, HDL-c and LDL-c. Although not significant, a similar pattern of HDL-c being protective for Pre/EP women, but not for LP/Post women was seen in the reparameterized model (OR = 0.97, 95% CI = 0.94, >1.00 vs. OR = 1.01 95% CI = 0.98, 1.03). After adjusting for age, site, race, HDL-c, LDL-c, SBP and waist circumference, the interaction in the IMT model reached statistical significance (p-value = 0.03). Reparameterization of the adjusted model showed that HDL-c was negatively associated with IMT in Pre/EP women, but positively associated in LP/Post women (not significant). For every 1 mg/dL increase in HDL-c there was an associated 0.001 mm decrease in IMT for Pre/EP women (-0.002, -0.0002) and a 0.0002 mm increase for LP/Post women (-0.0006, 0.0009).

The interaction between LDL-c and menopausal status was tested, but did not reach statistical significance for any of the three subclinical measures (data not shown). However, in multivariable analyses stratified by menopausal status, the odds ratio of high AC associated with a 1 mg/dL increase in LDL-c was 1.02 (<1.00, 1.04) for Pre/EP women and 1.03 (1.01, 1.05) for LP/Post women. An odds ratio associated with LDL-c of 1.00 (0.99, 1.01) and 1.02 (>1.00, 1.03) for Pre/EP and LP/Post women respectively was observed for

any carotid plaque. The β coefficient estimate for IMT was -.00005 (-.0004, .0003) among Pre/EP women and .0003 (-.0001, .0007) among LP/Post women for LDL-c.

Similar analyses were conducted with coronary artery calcification as an outcome. However, HDL-c was protective for Pre/EP and LP/Post women alike (results not shown).

To explore lipoprotein profile difference by menopausal status, the lipoprotein NMR spectroscopy data for a small sub sample of Pittsburgh women (n = 19 for Pre/EP and n = 34 for LP/Post) was used. LP/Post women had more small HDL-p, more total HDL-p and a smaller HDL particle size on average (Table 3.3). Large HDL-p concentrations were lower in LP/Post women, however this was not significant. Finally, LP/Post women had significantly more total LDL-p than Pre/EP women.

3.5 DISCUSSION

This study demonstrates that the potency of lipids as CVD risk factors changes from premenopause to postmenopause. Statistically significant interactions between menopausal status and HDL-c were seen for AC and IMT, while the interaction for carotid plaque was borderline. Among LP/Post women, HDL-c appeared to lose its protective effect against subclinical CVD measures. These results are in accord with Fan *et al*. who report that the protective effects of HDL-c on IMT may diminish around the age of menopause [13]. This analysis demonstrated positive associations between HDL-c and AC, any carotid plaque and IMT in LP/Post women after adjustments for traditional risk factors; where as HDL-c had a traditionally protective effect in Pre/EP women. For LDL-c, there was a trend for a stronger association with subclinical measurements among LP/Post women; however this interaction did not reach statistical significance.

The traditional protective effect of HDL-c is exerted through reverse cholesterol transport, inhibition of LDL-c oxidation, decreased adhesion molecule expression and increased endothelial function [32-35]. In men, this protective effect is maintained longitudinally, suggesting the phenomenon observed in this analysis among LP/Post women is most likely not due to age and corresponds to changes due to the menopausal transition [13]. Additional evidence of a detrimental effect of HDL-c in women comes from the EUROSTROKE study, a collaboration between several large European cohort studies. This study reported that among women pooled from the Novosibirsk and Rotterdam cohorts, higher HDL-c was associated with increased odds of stroke [36]. The average age of the cohorts were 51.6 and 72.8 years respectively; therefore these effects were seen among women who have recently transitioned through menopause and those who have been postmenopausal for years.

Early studies report substantial rises in HDL-c with the menopausal transition. Kim *et al.* and Do *et al.* explained that these studies, showing increases in HDL-c with the transition, are not accounting for the rise due to age. The majority of researchers agree there is little net change in HDL-c when age is accounted for appropriately [8, 10, 12, 37]. A moderately higher HDL-c was observed in LP/Post compared to Pre/EP women in this analysis; however, this difference disappears once adjusted for age.

The change in risk association between HDL-c and the menopausal transition may be explained by compositional changes in HDL-c lipoproteins. Conventional methods of measuring HDL-c only reflect the concentration of cholesterol and not the HDL concentration [38]. The lipoprotein particles are believed to provide more information in predicting CHD [39]. Using NMR spectroscopy to capture lipoprotein particle concentrations, it has been shown that small HDL-p are less protective than the large HDL-p. The traditional protective effect of HDL-c on CVD is mostly due to the large HDL-p [34, 40]. In the

Framingham Offspring Study and Milwaukee Cardiovascular Data Registry, having more small HDL-p were associated with higher odds of having coronary heart disease [28,34, 35, 41]. However, these observations between HDL-c lipoproteins and CVD focused primarily on men, diabetics or drug therapies.

A recent study exploring the relationship between HDL-c lipoproteins and the risk of CHD in postmenopausal women found no association with HDL-c, however, when the lipoprotein sub fractions were examined a negative association with large HDL-p and a positive association with small HDL-p was determined [39]. Similar results were obtained in Women's Health Study for large HDL-p, but small HDL-p was not significant, however, this sample was a mixture of pre and postmenopausal women [42]. The small sub sample of NMR spectroscopy data available for Pittsburgh women in SWAN Heart showed that LP/Post women had more small HDL-p and smaller HDL particle size on average compared to Pre/EP women. This lipoprotein subclass profile could potentially explain the loss of HDL-c protection observed in this analysis. Another study found similar results where postmenopausal women had significantly more small HDL-p and fewer large HDL-p compared to premenopausal women [43]. The Healthy Women Study saw a significant protective association between coronary calcification and large HDL-p and HDL particle size in postmenopausal women, whereas small HDL-p were not correlated with coronary calcium [44]. In addition, postmenopausal women with aortic or coronary calcification had lower large HDL-p and higher small HDL-p [45]. It has been reported that increased levels of small HDL-p and smaller HDL particle size were correlated with thicker IMT measures of Finnish men and women [46].

One potential mechanism responsible for these lipoprotein profile changes is enzymatic activity. Large HDL-p are converted to small HDL-p by increased hepatic lipase [47]. It is shown that estrogen inhibits this enzyme; therefore decreases in estrogen with

menopause would lead to a higher ratio of small HDL-p to large HDL-p. In support of this theory, postmenopausal women have been found to have higher hepatic lipase activity that covaried inversely with large HDL-p. Although not statistically significant, LP/Post women in this SWAN Heart analysis had larger waist circumference compared to Pre/EP women. This rise in central adiposity, most likely due to chronological aging, could account for additional increases in hepatic lipase activity leading to more small HDL-p. One study found intra-abdominal fat to be positively associated with hepatic lipase activity in obese women [48].

In addition to increasing hepatic lipase, decreased estrogen hinders several protective vascular mechanisms. Estrogen is believed to accelerate reendothelialization in the face of vascular injury thereby inhibiting smooth muscle proliferation and subsequently medial thickening [49]. Vascular tone is maintained by estrogen through several pathways, including nitric oxide and prostacyclin [4]. Therefore, decreased estrogen following the menopausal transition leaves the vasculature vulnerable to CVD risk factors. SWAN Heart, has previously shown that lower estrogens are directly related to larger carotid arterial diameter [30]. Larger vessel diameters lead to less ability to compensate for hemodynamic changes due to blood pressure or arterial wall thickening and thus leave the vessel more vulnerable [30, 50]. Indirect effects of estrogen on the vasculature include maintenance of cardioprotective lipid profiles, decreased coagulation and reduced LDL-c oxidation [3].

Other lipoprotein profile changes include a significant increase in LDL-c as women transition through menopause [8-11]. The difference in LDL-c was statistically significant in the LP/Post compared to Pre/EP women. Menopausal status did not significantly modify the contribution of LDL-c on subclinical measures, but a similar pattern of LDL-c as a stronger risk factor among postmenopausal women was suggested for all three subclinical measures. Given the moderate sample size in this analysis, further studies examining this

possibility are indicated. The NMR data in this analysis showed that LP/Post women have higher total LDL-p. This coincides with the literature that suggests the quantity of LDL-p, rather than the particle size is a stronger predictor of CHD risk [42]. Thus it is possible that the potency of LDL-c as a risk factor does increase after the transition.

This analysis is comprehensive because we were able to analyze the contribution of menopausal status, HDL-c and LDL-c and lipoprotein subclasses in the same study. A positive association with HDL-c among LP/Post women was consistently observed across 3 subclinical measures. The NMR data provides initial evidence that supports the potential mechanism of lipoprotein compositional changes being responsible for this association. This analysis is cross sectional, and thus a causal relationship between menopause status and HDL changes cannot be proven. However, acute changes in calcification, carotid plaque and IMT have been reported previously, supporting the potential for subclinical disease development over the menopausal transition [36, 51-53]. Premature and early menopause ranged from 1 - 1.4% and 2.9 - 3.7% respectively, in SWAN Caucasian and African American women [54]. It is plausible that the lipid changes attributed to the menopausal transition may instead be explained by altered lipid patterns due to premature and early menopause [55]. Despite the modest sample size for detecting interactions, the HDL-c by menopausal status interaction was significant for AC and IMT, suggesting differences effect by menopausal status. The borderline and non-significance observed for carotid plaque and LDL-c require larger sample sizes for confirmation. The protective effect of HDL-c to coronary artery calcification among LP/Post women could be due to the low amount of plaque detected in the SWAN Heart population (2.4%) [29]. A loss of HDL-c protection for coronary artery calcification might be observed in a population with more extensive disease and therefore further studies need to be conducted in this area. Lastly,

since all participants did not have NMR data, lipoproteins could not be use in the regression models.

In conclusion**,** the protective effect of HDL-c is reduced among postmenopausal women most likely related to changes in the lipid profile seen with the menopausal transition. This, in addition to a more vulnerable vessel caused by age, decreased estrogen and adipose tissue redistribution, may partially explain the increased CVD risk seen after menopause. Future studies evaluating lipid profiles longitudinally through the menopause transition are needed.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES AND FIGURES

Table 3.1 Participant characteristics by menopausal status

Characteristic	n	Total	Pre/EP *n=316*	LP/Post *n=224*	p-value	Age Adjusted p-value
Age (years)	540	50.2 (2.9)	48.9 (2.2)	52.1 (2.7)	**< .0001**	—
African American (%) †	540	38.7 (209)	36.1 (114)	42.4 (95)	0.14	0.31
Alcohol (yes/no, %) †	537	61.6 (331)	61.5 (193)	61.9 (138)	0.92	0.50
Smoker (ever regular, %) †	488	16.0 (78)	16.6 (47)	15.2 (31)	0.69	0.32
HDL-c (mg/dL)	472	57.5 (15.0)	56.2 (13.8)	59.3 (16.4)	**0.04**	0.23
LDL-c (mg/dL)	474	119.0 (32.8)	113.5 (28.7)	126.6 (36.5)	**< .0001**	**< .0001**
SBP (mmHg)	519	119.7 (16.7)	118.2 (16.3)	121.7 (17.0)	**0.017**	0.94
Glucose (mg/dL) *	480	88 (83, 96)	87 (81, 95)	91 (84, 98)	**0.0003**	**0.01**
Waist Circumference (cm)	518	89.2 (14.4)	88.3 (14.7)	90.5 (14.0)	0.089	0.69
BMI (kg/m^2)	518	29.4 (6.4)	29.1 (6.5)	29.7 (6.3)	0.35	0.85
Aortic Calcification (%) † None (0) Moderate (1-74) High (75 +)	515	 30.3 (156) 44.7 (230) 25.0 (129)	 34.3 (104) 44.9 (136) 20.8 (63)	 24.5 (52) 44.4 (94) 31.1 (66)	**0.0096**	0.28
Any Carotid Plaque (%) †	535	15.1 (81)	11.5 (36)	20.4 (45)	**0.0047**	**0.04**
Intima Media Thickness (mm)	529	0.67 (0.1)	0.66 (0.1)	0.68 (0.1)	**0.040**	0.77

Values are presented as mean (SD); p-values generated with t-test
† Values are presented as % (n); p-values generated with chi-square test
* Non-normally distributed; values presented as median (IQR); p-values generated with t-test with log of glucose

Table 3.2 Univariate associations between traditional CVD risk factors and subclinical measures

Risk Factor	Subclinical measure			
	Aortic Calcification n = 515		**Any Carotid Plaque** n = 535 *OR (95% CI)*	**Carotid IMT** (mm) n = 529 *β Estimates (95% CI)*
	Moderate AC *OR (95% CI)*	High AC *OR (95% CI)*		
Age (years)	1.09 (1.01, 1.17) †	1.18 (1.09, 1.28) *	1.09 (>1.00, 1.18) †	0.006 (0.003, 0.009) *
African American (%)	1.90 (1.23, 2.93) *	1.61 (0.98, 2.64)	0.94 (0.58, 1.53)	0.033 (0.016, 0.050) *
Alcohol (yes/no, %)	0.69 (0.44, 1.06)	0.53 (0.32, 0.86) *	0.62 (0.38, 0.99) †	-0.033 (-0.051, -0.017) *
Smoker (ever regular, %)	1.00 (0.51, 1.96)	3.05 (1.58, 5.88) *	1.03 (0.52, 2.01)	-0.021 (-0.044, 0.002)
HDL-c (mg/dL)	0.97 (0.95, 0.98) *	0.97 (0.95, 0.98) *	0.99 (0.97, 1.01)	-0.001 (-0.002, -0.0005) *
LDL-c (mg/dL)	1.02 (1.01, 1.03) *	1.02 (1.01, 1.03) *	1.01 (>1.00, 1.02) *	0.0004 (0.0002, 0.001) *
SBP (mmHg)	1.03 (1.01, 1.04) *	1.04 (1.03, 1.06) *	1.00 (0.99, 1.02)	0.001 (0.001, 0.002) *
Glucose (mg/dL) #	1.06 (1.04, 1.08) *	1.06 (1.04, 1.09) *	1.00 (0.99, 1.01)	0.115 (0.062, 0.168) *
Waist Circumference (cm)	1.14 (1.11, 1.17) *	1.18 (1.14, 1.22) *	1.01 (0.99, 1.02)	0.002 (0.002, 0.003) *
Late Peri/Postmenopausal	1.38 (0.91, 2.11)	2.10 (1.30, 3.39) *	1.97 (1.23, 3.18) *	0.018 (0.001, 0.035) †

Values are odds ratios (95% CI) for AC and carotid plaque and β Estimates (95% CI) for IMT.
Reference value for both moderate and high AC was no calcification.
Glucose was log transformed in linear regression model for carotid IMT
* < 0.01, † <0.05

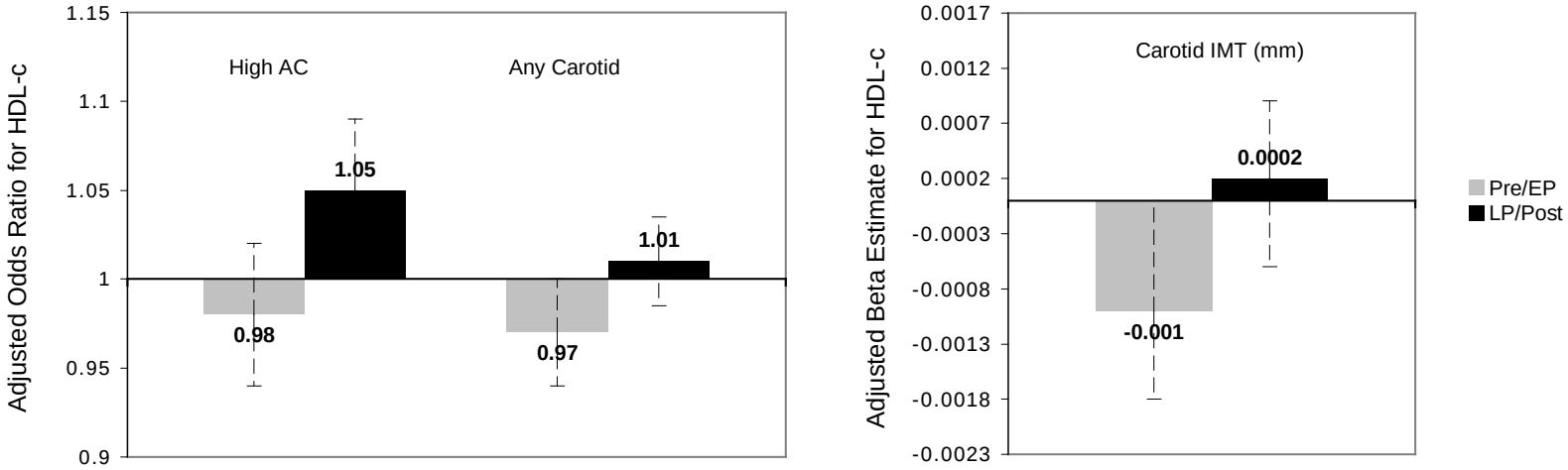

Figure 3.1 Multivariable association between HDL-c and subclinical measures

High AC - values are OR (95% CI) of high AC for HDL-c; adjusted for age, site, race, LDL-c, SBP, glucose, waist circumference and smoking.
Any Carotid Plaque - values are odds ratios (95% CI) of carotid plaque for HDL-c; adjusted for age, site, race and LDL-c.
Carotid IMT - values are β coefficient estimates (95% CI) of IMT for HDL-c; adjusted for age, site, race, LDL-c, SBP and waist circumference.
* < 0.01, † <0.05 for Pre/EP versus LP/Post

Table 3.3 Lipoprotein levels by menopausal status for Pittsburgh SWAN Heart sub sample

Lipid Variable	**Pre/EP** (n=19)	**LP/Post** (n=34)	**p-value**
HDL-c (mg/dL)	49.1 (15.0)	49.3 (14.0)	0.97
Small HDL Particles (µmol/L)	21.6 (5.7)	26.6 (5.7)	**0.0035**
Large HDL Particles (µmol/L)	6.7 (2.9)	6.1 (3.1)	0.49
Total HDL Particles (µmol/L)	28.3 (6.4)	32.7 (7.6)	**0.037**
HDL Particle Size (nm)	9.4 (0.5)	9.1 (0.4)	**0.0078**
IDL-p (nmol/L)	41.9 (35.5)	70.9 (52.7)	**0.037**
Small LDL Particles (nmol/L)	478.2 (247.1)	620.5 (351.7)	0.13
Large LDL Particles (nmol/L)	575.4 (193.7)	620.3 (227.1)	0.47
Total LDL Particles (nmol/L)	1095.6 (189.3)	1311.6 (325)	**0.0036**
LDL Particle Size (nm)	21.6 (0.5)	21.5 (0.7)	0.50

Values are presented as mean (SD); p-values generated with t-test

3.8 REFERENCES FOR CHAPTER THREE

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4.0 GENDER DIFFERENCES IN RISK FACTORS ASSOCIATED WITH SUBCLINICAL CARDIOVASCULAR DISEASE: THE TOBAGO FAMILY HEALTH STUDY

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4.1 ABSTRACT

Background: Education is protective against risk factors and incident cardiovascular disease (CVD). The protective effect of education for CVD has suggested to be stronger among females. The association between education and subclinical CVD was assessed for males and females from the Tobago Family Health Study. Differences in CVD risk factors by education were evaluated as potential mechanism for gender differences in the education-subclinical CVD relationship. Methods: A community-based sample of Afro-Caribbean males and females were evaluated for intima media thickness (IMT), adventitial diameter (AD) and lumen diameter (LD). Education, age, waist circumference, SBP, HDL-c, LDL-c and other traditional CVD risk factors were collected. Results: The average age was 43.7 years and ranged from 18 to 86 years. More females had ≥ secondary education compared to males (52% versus 34%, p-value = 0.0093). Mean IMT, AD and LD were higher among males. The interaction between education and gender was significant for AD and LD. The adjusted mean AD and LD were lower among females that had ≥ secondary education compared to females with < secondary education (AD: 6.94 vs. 7.29 mm, p-value = 0.0051 and LD: 5.51 vs. 5.90 mm, p-value = 0.0010). There was no significant difference observed for males. Females with ≥ secondary education also had lower age adjusted SBP compared to females < secondary education. Males with ≥ secondary education had lower HDL-c and higher LDL-c compared to males < secondary education. Conclusion: Gender differences in the association between education and subclinical CVD are evident in a Tobago population. Educational differences in SBP for females and lipids for males may potentially explain some of the gender difference in the education-subclinical CVD relationship.

4.2 INTRODUCTION

It is well established that education is inversely related to health in developed countries. Those with lower education have higher rates of CVD risk factors such as obesity, hypertension and atherogenic lipid profiles [1]. In addition, lower education is associated with higher risk of all cause-mortality, cerebrovascular disease and cardiovascular disease (CVD). The inverse association observed between education and health outcomes has been shown to vary by gender. Higher education and other measures of social position are protective for all cause mortality, with several studies showing a steeper gradient among males [2-7]. On the other hand, the protective effect of education and social position for CVD is steeper among females [1-3, 8-12].

The relationship between traditional CVD risk factors and education varies by gender. In developed countries, education tends to be protective against hypertension and obesity among females, whereas a significant association is not always evident for males [13]. The gender difference in the education-SBP relationship is even more striking in developing countries. Males in the Caribbean have been shown to have a direct association between education and SBP [14, 15].

In 2004 approximately 25% of deaths in Trinidad and Tobago were due to CVD, making it the leading cause of death [16]. Intima media thickness (IMT) and adventitial and lumen diameter (AD and LD, respectively) are markers of early atherosclerosis and vascular remodeling [17-20]. Increases in IMT, AD and LD are associated with CVD risk factors and clinical outcomes such as myocardial infarction and stroke, making these measures quality subclinical markers for CVD [17, 21-24].

Few studies have examined the association between education and CVD by gender in developing countries, and no study to date has evaluated this relationship in a population

of African descent. Using a community-based population of Afro-Caribbean males and females from the Tobago Family Health Study, the gender difference in the education-subclinical CVD relationship was assessed. Potential mechanisms explaining this relationship was further evaluated by exploring education differences in traditional CVD risk factors for males and females.

4.3 METHODS

4.3.1 Study Population

The Tobago Family Health Study began recruitment in 2003 on the Caribbean Island of Tobago. Study design and recruitment have been previously described [25]. The purpose of this study was to assess the role of inheritance, lifestyle and body composition in the etiology of chronic disease, including diabetes, obesity and CVD. A total of 471 individual participants were recruited from 8 large multi-generational families of Afro-Caribbean ancestry. To be eligible for the Tobago Family Health Study, a proband had to be Afro-Caribbean (reported that all 4 of grandparents were Afro-Caribbean), have a spouse willing to participate in the study and at least 6 living offspring and/or siblings ($\geq$ 18 years of age) residing in Tobago. In order to obtain a community-based sample, representative of the island of Tobago, no health status eligibility criteria were defined. An ancillary study to the Tobago Family Health Study, invited participants back for an ultrasound scan of their carotid arteries. Of the 471 participants originally recruited for the parent study, 415 returned for an ultrasound scan. To date, 255 of these images have been read and were available for this analysis.

The Tobago Ministry of Health and University of Pittsburgh institutional review boards approved research protocols for the Tobago Family Health study. All participants provided written informed consent prior to enrollment.

4.3.2 Subclinical Measures

All Tobago Family Health Study participants were invited back for a B-mode carotid ultrasound scan conducted between May 2007 and November 2008. The common carotid artery (CCA) was imaged using an Acuson Cypress portable ultrasound machine (Siemens Medical Solutions, Malvern, PA). Images were taken from the near and far walls of the distal CCA (one centimeter proximal to the carotid bulb). Intima media thickness (IMT) measures were obtained electronically using a semi-automated reading software (AMS system developed in Sweden by Dr. Thomas Gustavsson). Lines were traced between the lumen-intima interface and the media-adventitia interface across the 1 cm segment. The computer then generated one measurement for each pixel over this area, for a total of about 140 measures. The mean of the near and far wall IMT measurements from the left and right CCA comprises the average IMT. Diameter measurements were derived from the same 1 cm CCA segment. Specifically, the CCA inter-adventitial diameter was measured as the average distances between the adventitial-medial interface on the near wall and the medial-adventitial interface on the far wall. Similarly, the CCA intra-lumen diameter was measured using the two lumen-intima interfaces. Inter-sonographer reproducibility was conducted on 35 Tobago Family Health Study participants. The intraclass correlation (ICC) was 0.97 for average IMT and 0.95 for average AD. All images were read centrally at the Department of Epidemiology's Ultrasound Research Laboratory (University of Pittsburgh, Pittsburgh, PA). Inter-reader ICC was 0.99 for IMT and AD.

4.3.3 Education Measure

Participants were asked what was their highest grade or level of schooling completed. Categories included: no formal education, primary (age 5 – 11 years), secondary – *O level* (age 11 – 16 years), secondary – *A level* (16 – 18 years), technical vocational training, some university or associate degree, university graduate (4 or 5 year program), master's degree (or other post-graduate training) or doctoral degree (MD, MBBS, PhD, EDD, DVM, DDS, JD).

4.3.4 Covariates

Trained and certified clinical staff conducted standardized interviewer-administered questionnaires. Race was self-defined by the participant in conjunction with a detailed ascertainment of the ethnic origin of their parents and grandparents. According to recent census data, 97% of the island of Tobago is of West African origin, therefore, this analysis was limited to those who reported Afro-Caribbean race (n=232, 91% of those participants with a carotid scan) [26]. Participants were asked how many alcoholic beverages (beer, wine or liquor) they consumed on a weekly basis. Smoking status was defined as ever smoked (> 100 cigarettes in a lifetime) or never smoked. Physical activity was reported as the number of minutes walked per week. Marital status was categorized as 1) married/ or living as married or 2) widowed, divorced, separated or never married.

Blood pressure was measured three times in the right arm following a 5 minute rest period using the Omron HEM-705 automatic monitor. An average of the last two readings was taken. Weight (measured to the nearest 0.1 kg, wearing indoor clothing, but no shoes) was assessed using a balance beam scale and standing height (measured to the nearest

0.1 cm, without shoes) with a wall-mounted stadiometer. An average of two readings was used for final weight and height measurements. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). An inelastic tape measure around the abdomen, at the point of the umbilicus, was used to determine waist circumference (cm).

Venipuncture blood samples were collected on participants who had been fasting for 12 hours. All biochemical assays were performed in the Heinz Nutrition Laboratory at the University of Pittsburgh. Serum glucose, triglycerides, total cholesterol and HDL were quantitatively determined utilizing enzymatic reactions [27-29]. LDL-c was calculated indirectly using the Friedewald equation [30]. CRP was measured by a commercial calorimetric competitive enzyme-linked immunosorbent assay (from Diagnostic Systems Laboratories, Inc).

4.3.5 Statistical Analysis

The distribution of each continuous variable was examined and log transformed if needed to approximate normality. Triglycerides, glucose and CRP were log transformed; however, medians (IQR) are presented in Table 1 for descriptive purposes. All remaining variables met the guidelines for normality and were treated as continuous. Highest level of completed education was dichotomized as < secondary education and ≥ secondary education. Alcohol was categorized into zero drinks or ≥ one drink per week; physical activity into ≤ 30 minutes or > 30 minutes of walking per week; and marital status into married or not married. These variables were collapsed into these cutoffs to maintain adequate sample size in each category. Physical activity was tested in univariate models, but was not retained in multivariable modeling due to large number of missing values (n=66). Two sample t-test

and chi-square test were utilized to compare participant characteristics between males and females.

Univariate and multivariable linear regression were used to assess the association between traditional CVD risk factors and IMT, AD and LD. Stepwise regression selection was used to evaluate the inclusion of traditional CVD risk factors in the multivariable linear regression models. Age, height, gender and education were variables of interest and therefore kept in the final models. Waist circumference was chosen in multivariable models because it explained more of the variation compared to BMI. Models repeated using BMI showed similar results to those including waist circumference. The interaction between education and gender was tested in multivariable models with and without adjustments for other covariates. Further exploration of the education interaction was done using a reparameterized model to include the effects of education among males and females in a single multivariable model. Adjusted least squares means were obtained from generalized linear models. Statistical analyses were completed with SAS 9.1. Two-sided p-values ≤ 0.05 were considered statistically significant.

4.4 RESULTS

Of the 232 Tobago Family Health Study participants used in this analysis, 152 (66%) were female (Table 1). The average age was 43.7 years (range 18 to 86). Forty-six percent of participants had ≥ secondary education, with females having a higher percentage than males (52% versus 34%, p-value = 0.0093). On average, males drank more alcohol, smoked more cigarettes, were more likely to be married and had higher blood pressures then females. Mean LDL-c, CRP and BMI were higher among females. Mean IMT, AD and LD were higher among males.

Univariate analyses were conducted to determine which CVD risk factors were associated with subclinical CVD (Table 2). Having ≥ secondary education was associated with lower mean IMT, AD and LD in univariate analyses, whereas SBP and waist were associated with higher means of all three subclinical measures. Mean IMT and AD were positively associated with age, marital status, DBP, LDL-c and glucose. Female gender was associated with lower mean AD and LD in unadjusted models. Alcohol, physical activity and CRP were not associated with any of the subclinical measures.

Multivariable models for subclinical CVD risk factors included age, height, gender, education and other CVD risk factors that remained significant (Table 3). Having ≥ secondary education continued to be protective only for LD in adjusted models. Waist circumference was the only risk factor that remained significant in multivariable analysis for all three outcomes. Female gender remained independently associated only with mean IMT in multivariable modeling.

Interactions between gender and SBP, waist circumference and education were tested for all three subclinical measures (Table 4). For AD and LD, the gender and education interaction was significant (unadjusted p-value = 0.0066 and 0.0014, respectively). The interaction remained significant for LD and borderline for AD after adjusting for age, SBP, waist circumference and height (adjusted p-value = 0.021 and 0.061, respectively). To demonstrate this interaction further, adjusted mean AD and LD are shown in Figure 1. AD and LD means were significantly lower for females with ≥ secondary education compared to those with < secondary education (AD: 6.94 vs. 7.29 mm, p-value = 0.0051 and LD: 5.51 vs. 5.90 mm, p-value = 0.0010). These means were adjusted for age, SBP, height and waist circumference. There were no significant differences between education levels for AD or LD among males. No significant interactions were found for IMT.

Finally, age adjusted CVD risk factors were examined by education level for males and females (Figure 2). Waist circumference did not vary by education for males or females. Mean SBP was significantly higher among females with < secondary education than ≥ secondary education (126.0 vs. 115.7 mmHg, p-value = 0.0048). Adjusting for waist circumference did not attenuate the difference (data not shown). The lipid profile was worse for males with ≥ secondary education than < secondary education: lower HDL-c and higher LDL-c (34.9 vs. 45.0 mg/dL, p-value = 0.0025 and 136.4 vs. 117.7 mg/dL, p-value = 0.041, respectively). Triglycerides, glucose, CRP, smoking and alcohol did not vary by education level (data not shown).

4.5 DISCUSSION

It was observed that the effect of education on subclinical CVD varied by gender; education was protective for females, but this pattern was not seen for males. This interaction remained significant for LD and borderline for AD after adjustment for CVD risk factors. This was in accord with Rosvall *et al.* who demonstrated a significant inverse association with subclinical CVD for females, but not males after adjustment for CVD risk factors in a general population of adults 46 to 68 years of age from Sweden [9, 10]. When age adjusted CVD risk factors were evaluated by education for males and females from the Tobago population, some interesting trends were seen. The only CVD risk factor that differed by education level for females was SBP, which was lower for those with a higher education. Interestingly, among males with ≥ secondary education, HDL-c was lower and LDL-c was higher compared to males with < secondary education. These patterns in education differences by gender have been documented previously [13, 31-33]. In a population of males and females from Trinidad ages 24 to 89 years, an inverse association for education-SBP

was only observed among females [32]. Higher LDL-c and lower HDL-c have been observed among Chinese, Polish and Russian men with higher education levels [33].

The association between education and incident CVD has been shown to vary by gender, with females having a stronger association than males [1-3, 12, 34]. In a population of adults from Finland, 35 to 64 years of age, females showed the largest education difference for CVD deaths [3]. Mackenbach *et al*. explored this gender difference in seven countries and observed a larger inequality in CVD mortality between low and high education levels for females in five of the countries [2]. Consistent with previous literature, both studies showed the opposite pattern for all-cause mortality, with males having a steep education-mortality gradient compared to females. It has been argued that much of this gender difference in the education-CVD relationship can be explained by BMI [12]. In the Tobago population, the effect of education for AD and LD varied by gender, however, adjustment for waist circumference did not significantly attenuate this finding.

Other markers of social position, neighborhood status and occupation, have shown similar gender differences seen for education-CVD relationship. Females, ages 25 to 64 years, from the Glasgow MONICA coronary event register, who lived in more deprived neighborhoods had higher rates of myocardial infarctions and coronary deaths compared to those in better neighborhoods [11]. This association was not as strong for males. A large sample (n=12,000) representing the general population of the Netherlands showed a marked difference in CVD morbidity (angina, infarction and neurovascular incidence) by occupation for females only [8].

Not all studies have shown a gender difference in the relationship between education and CVD. NHANES I showed that for circulatory deaths, education was protective for males and females equally [35]. However, significantly more males died compared to females during the 20 year follow up. Since females live longer than males, it

is plausible that not enough circulatory events were observed among females to see a gender difference in the association between education and mortality.

Similar to incident CVD, a stronger association between education and subclinical CVD has been observed among females. Thicker IMT and more carotid stenosis were seen among females, from a subcohort of the population-based Malmö Diet and Cancer Study, with lower education or lower occupational status after adjustment for CVD risk factors [9]. This observation was not apparent for males. In the same subcohort, life-course occupation, a combination of parental and adulthood occupation, was associated with carotid stenosis for females only [10]. The association between neighborhood poverty, childhood socioeconomic position or adult socioeconomic positioning (a summary score for income, education and wealth) and carotid IMT also varied by gender [36]. All three socioeconomic positioning measures were inversely associated with IMT among females.

There are several explanations for the greater protective effect of education on subclinical CVD among females compared to males. Females depend on education more for health than males [37]. A higher education results in higher expectations of health and better ability to estimate risk. Results from the NHANES I study showed that education modified the contribution of cardiovascular risk factors to self-rated health for females; highly educated females had higher odds of poor self-reported health among those with high glycosylated hemoglobin or high cholesterol [38]. Education was not related to self-reported health among males. Females with lower education have been shown to have higher rates of psychological disadvantages including: low income, depression, single parenting and unemployment [12]. Therefore, poorer health among low educated females may be due to higher psychological stressors compared to males with low education.

Delayed gratification is another factor that contributes to the protective effect of education for females. The higher rate of college education among females in the US is

partially due to personal and family related returns [39]. These female-favorable trends are in the form of income, marital returns and insurance against poverty. More females in the Tobago population had ≥ secondary education compared to males. Gender disparities in education attainment are reversing in Latin America and the Caribbean, with female education rates now exceeding that of males [40]. In 2005 the ratio of females to males completing primary education was 1.09 on the islands of Trinidad and Tobago. It is plausible that personal and family returns may account for the higher rate of schooling among females, and the larger dependence of education for health among females may contribute to the protective effect of education on subclinical CVD observed in the Tobago population.

Currently, the Republic of Trinidad and Tobago is a high-income developing nation: the wealthiest independent country in the Caribbean [41]. The economic transition experienced by developing countries alters patterns in CVD risk factors. The current transition has led to increased obesity and non-communicable diseases among developing countries [42]. This transition is a result of urbanization and globalization, which increases food production, mass media advertisements, supermarkets, technology and transportation [42, 43]. These changes lead to a rapid shift to diets high in fat, sugar and salt. These types of foods tend to be high energy dense and low cost [44]. Decreased energy expenditure due to less labor intensives occupations and more television watching also aid in increased CVD risk. Initially with economic prosperity, increased CVD risk occurs among high social classes, but with time this reverses and lower social classes share this increased risk [13].

It is possible that the way in which CVD manifests itself varies by gender. Risk factors may play diverse roles and have varying potencies in the pathogenesis of atherosclerotic changes for males and females [4]. Gender differences in CVD risk factors by education level may in part explain the difference in education-CVD relationship observed

for males and females [2]. Evaluation of education differences for Tobago males and females revealed varying effects of education on SBP, HDL-c and LDL-c, but not waist circumference. In addition to the effect of education on risk factors, the economic transition may be responsible for some of the observed differences in risk factors.

The relationship between education and SBP varies by gender and varies by the economic status of the country where the population was sampled. The protective effect of education on SBP for females tends to be independent of age, but largely explained by BMI for both developed and developing countries; however, adjustments for waist circumference in the Tobago population did not explain the difference [13]. The education gradient in SBP was further explained with salt intake and physical activity among a population of Trinidad females [32]. It is plausible that these two behaviors are influenced by education and the effect of education on salt intake and physical activity varies by gender. Colhoun *el at.* reviewed the socioeconomic status-blood pressure relationship in 57 studies from developed and 13 from developing countries [13]. In developed countries, an inverse association was more consistent with females. Similar to developed countries, education was inversely related to SBP for females and not significantly related for males among a population of Trinidadians [32]. The direct association observed for males in developing countries such as Jamaica and St. Vincent, may reflect the low income economic status of the country and likely represents an earlier time point in the economic transition [14, 15]. The 10 mmHg difference seen between education levels in the Tobago females is large compared to the usual difference of 2 to 3 mmHg reported by Colhoun *el at.*, however, the NHANES II study observed a difference of 10 mmHg among females [13 45].

The association between lipids and schooling varies across countries based on their societal economic development more for males. Among US white males there was an inverse association between education and LDL-c and a direct association with HDL-c [33].

When US black males and males from China, Israel, Poland and Russia were pooled together, the opposite was observed: direct relationship with LDL-c and inverse relationship with HDL-c. In Jackson, Mississippi, a poor, southern urban community that may parallel a developing country, males who lived in "poorer" neighborhoods who had higher individual socioeconomic status had higher serum cholesterol, while those who lived in "richer" neighborhoods had lower cholesterol with higher socioeconomic status [31]. It is plausible that males in developing countries, who have more education and or resources, tend to eat diets high in fat, whereas educated females avoid such diets. The effect of physical activity and alcohol consumption on lipid profiles for males and females may vary by education. However, alcohol consumption did not vary by education level for males or females in the Tobago population.

Although a strong predictor of subclinical CVD, waist circumference did not vary by education for the Tobago population. A strong inverse association between education and obesity has been observed among females of developed countries whereas the association among males is nonsignificant [46]. Obesity is positively associated with higher socioeconomic status in low-income developing countries, but as the country becomes economically developed the relationship reversed with higher socioeconomic status being protective against obesity [47]. Those in the transition have a mixed picture. Therefore, the nonsignificant difference for obesity by education level seen in the Tobago population for may be explained by the economic transition. Females weighted significantly more than males in the Tobago population. Therefore, the protective effect of education may been ameliorated among females due to their high rates of obesity [43].

A few aspects of this study need to be considered when interpreting the results. First, the Tobago Family Health Study was cross-sectional, however, education begins in childhood and continues on into early adulthood. It is unlikely that subclinical CVD

influences the level of education attained and therefore reverse causality is unlikely. The association between education and subclinical CVD for males could have occurred due to a type II error. Low educated males may have died of competing risks or were too sick to participant in the study. Therefore the education-subclinical CVD relationship needs to be reevaluated in a larger sample of males and females. The higher subclinical CVD among males compared to females may account in part for the observed gender difference. Specifically, a smaller effect of education on subclinical CVD is plausible with a higher prevalence of disease. Another consideration is during vascular remodeling, AD can mask IMT changes [48]. Given the large age range and young average age of the study population (44 years), it is possible that there simply was not enough IMT change to observed gender differences in the education-IMT relationship. Exploration of IMT may need to be conducted in a sample of older adults. Given the wide age range it is also possible that a cohort effect masked the results. The education older adults received may vary compared to younger adults. Future analyses with a larger sample size should stratify the interaction by age categories [49]. Given the design of the Tobago Family Health study, 8 large multi-generational families, observations are not independent; therefore, results may be biased. Lastly, there were several variables not collected in this study. Previous literature has shown that income and education did explain additional variability between education and health outcomes and education was a stronger predictor compared to income or occupation [50-54]. Dietary and psychosocial health data was not available and a large portion of the physical activity data was missing. These factors could potentially further explain the mechanism linking education and subclinical CVD; therefore, residual confounding could be present.

In conclusion, gender differences in the association between education and subclinical CVD are evident in a Tobago population. Educational differences in SBP were

evident for females, and waist circumference did not account for this pattern. Additional research needs to be conducted to assess differences in dietary salt intake and physical activity. A poorer lipid profile was observed among males with higher education. Fat intake and physical activity could be plausible mechanisms for this pattern. Educational differences in risk factors are further complicated by the economic transition of Tobago and the global impact of obesity. Gender disparities in education are reversing in Trinidad and Tobago. As higher education rates increase with economic development, special interest needs to be taken in order to maintain gender parity in education to help reduce CVD. Early education should include healthy lifestyles messages to encourage such behaviors in childhood.

4.6 TABLES AND FIGURES FOR CHAPTER FOUR

Table 4.1 Participant characteristics by gender.

Characteristic	**Total** (n = 232)	**Female** (n = 152)	**Male** (n = 80)	**p-value**	**Age adjusted p-value**
Age (years)	43.7 (15.9)	43.6 (15.8)	44.0 (16.1)	0.87	—
Education (≥ secondary)	45.5 (105)	51.7 (78)	33.8 (27)	**0.0093**	**0.0037**
Alcohol (> 1 drink/wk)	36.4 (84)	25.2 (38)	57.5 (46)	**<0.0001**	**<0.0001**
Smoke (100+ cigs)	8.2 (19)	2.6 (4)	19.0 (15)	**<0.0001**	**<0.0001**
Walking (> 30 mins/wk)	45.8 (76)	43.8 (46)	49.2 (30)	**0.51**	**0.46**
Married	44.6 (103)	40.1 (61)	53.2 (42)	0.059	**0.045**
SBP (mmHg)	124.2 (24.2)	120.8 (25.2)	130.6 (21.0)	**0.0041**	**0.0007**
DBP (mmHg)	76.5 (12.7)	75.5 (12.8)	78.5 (12.2)	0.093	0.064
HDL (mg/dL)	40.0 (12.9)	39.3 (13.6)	41.6 (11.3)	0.23	0.24
LDL (mg/dL)	133.5 (41.4)	137.8 (43.3)	124.8 (35.7)	**0.034**	**0.028**
Triglycerides (mg/dL) *	76.0 (59, 103)	75.0 (59, 95)	79.0 (59, 108)	0.26	0.21
Glucose (mg/dL) *	83.0 (76, 91)	83.0 (76, 93)	84.0 (76, 89)	0.35	0.33
CRP (mg/dL) *	1.0 (0.4, 2.2)	1.3 (0.5, 3.6)	0.7 (0.3, 1.5)	**<0.0001**	**<0.0001**
Height (cm)	170.3 (8.1)	166.8 (6.4)	176.3 (6.5)	**<0.0001**	**<0.0001**
BMI (kg/m^2)	29.0 (6.7)	30.1 (7.4)	26.9 (4.6)	**<0.0001**	**0.0004**
Waist (cm)	90.7 (15.2)	90.7 (16.7)	90.7 (12.2)	0.98	0.98
Mean IMT (mm)	0.72 (0.16)	0.71 (0.15)	0.75 (0.17)	0.070	**0.030**
Mean AD (mm)	7.2 (0.7)	7.1 (0.7)	7.4 (0.7)	**0.0012**	**0.0009**
Mean LD (mm)	5.8 (0.6)	5.7 (0.6)	6.0 (0.6)	**0.0025**	**0.0026**

Values presented as mean (SD) or percents (n); p-values generated with t-test or chi-square test
* Non-normally distributed; values presented as median (IQR); p-values generated with t-test for log of triglycerides, log of glucose or log of CRP

Table 4.2 Univariate linear regression between participant characteristics and subclinical CVD measures.

Characteristic	**Mean IMT** (n = 229)	**Mean AD** (n = 231)	**Mean LD** (n = 232)
Age (years)	0.007 (0.006, 0.008) ¥	0.015 (0.010, 0.021) ¥	0.001 (-0.04, 0.006)
Female	-0.039 (-0.082, 0.003)	-0.328 (-0.526, -0.130) †	-0.260 (-0.427, -0.092) †
Education (≥ secondary)	-0.107 (-0.146, -0.069) ¥	-0.436 (-0.621, -0.250) ¥	-0.214 (-0.376, -0.053) †
Alcohol (> 1 drink/week)	0.001 (-0.041, 0.043)	-0.005 (-0.206, 0.196)	0.003 (-0.167, 0.172)
Smoke (100+ cigs)	0.056 (-0.016, 0.129)	0.508 (0.168, 0.848) †	0.389 (0.098, 0.680) †
Walking (> 30 mins/wk)	-0.015 (-0.064, 0.033)	-0.015 (-0.247, 0.216)	0.022 (-0.169, 0.214)
Married	0.094 (0.055, 0.133) ¥	0.280 (0.089, 0.470) †	0.077 (-0.087, 0.241)
SBP (mmHg)	0.003 (0.003, 0.004) ¥	0.011 (0.007, 0.015) ¥	0.005 (0.001, 0.008) †
DBP (mmHg)	0.005 (0.004, 0.007) ¥	0.015 (0.008, 0.022) ¥	0.005 (-0.002, 0.011)
HDL (mg/dL)	-0.001 (-0.002, 0.001)	-0.006 (-0.014, 0.001)	-0.005 (-0.011, 0.002)
LDL (mg/dL)	0.001 (0.001, 0.002) ¥	0.003 (0.0003, 0.005) †	0.0003 (-0.002, 0.002)
Log Triglycerides (mg/dL)	0.124 (0.075, 0.174) ¥	0.212 (-0.032, 0.456)	-0.024 (-0.233, 0.186)
Log Glucose (mg/dL)	0.205 (0.122, 0.289) ¥	0.746 (0.344, 1.149) †	0.324 (-0.031, 0.679)
Log CRP (mg/dL)	0.013 (-0.003, 0.029)	0.064 (-0.012, 0.141)	0.037 (-0.029, 0.103)
Height (cm)	-0.002 (-0.005, 0.0003)	0.015 (0.003, 0.027) *	0.019 (0.009, 0.029) †
Waist (cm)	0.003 (0.002, 0.005) ¥	0.017 (0.011, 0.023) ¥	0.011 (0.006, 0.016) ¥

Values presented as β coefficient estimates (95% CI)
¥ p-value < 0.0001, † p-value < 0.01, * p-value <0.05

Table 4.3 Multivariable linear regression between participant characteristics and subclinical CVD measures.

Covariate	**Mean IMT** (n=216)	**Mean AD** (n = 218)	**Mean LD** (n = 219)
Age (years)	0.006 (0.005, 0.007) ¥	0.008 (0.001, 0.016) *	-0.005 (-0.011, 0.002)
Female	-0.040 (-0.078, -0.002) *	-0.091 (-0.326, 0.143)	-0.033 -0.247, 0.181)
SBP (mmHg)	0.001 (0.0002, 0.002) *	0.005 (0.001, 0.010) *	0.004 (-0.0005, 0.008)
Waist (cm)	0.001 (0.0004, 0002) †	0.012 (0.007, 0.018) ¥	0.010 (0.005, 0.015) †
Height (cm)	-0.0003 (-0.003, 0.002)	0.018 (0.004, 0.032) *	0.017 (0.004, 0.029) *
Education (≥ secondary)	0.021 (-0.012, 0.054)	-0.168 (-0.372, 0.036)	-0.200 (-0.387, -0.014) *

Values presented as β coefficient estimates (95% CI)
¥ p-value < 0.0001, † p-value < 0.01, * p-value <0.05

Table 4.4 Single multivariable linear regression models between participant characteristics and subclinical CVD measures illustrating the effect of education for females and males

Covariate	**Mean IMT** (n = 216)	**p -value** (interaction)
Age	0.006 (0.005, 0.007) ¥	
Female	-0.042 (-0.086, 0.001)	
SBP	0.001 (0.0002, 0.0002) †	
Height	-0.0003 (-0.003, 0.002)	
Waist	0.001 (0.0004, 0.002) †	
Education for Females	0.024 (-0.015, 0.062)	0.82
Education for Males	0.016 (-0.037, 0.069)	

Covariate	**Mean AD** (n = 218)	**p -value** (interaction)
Age	0.009 (0.002, 0.017) *	
Female	0.033 (-0.234, 0.299)	
SBP	0.004 (-0.00004, 0.009)	
Height	0.016 (0.002, 0.030) *	
Waist	0.012 (0.006, 0.017) ¥	
Education for Females	-0.283 (-0.519, -0.047) *	0.061
Education for Males	0.074 (-0.251, 0.398)	

Covariate	**Mean LD** (n = 219)	**p -value** (interaction)
Age	-0.004 (-0.011, 0.003)	
Female	0.106 (-0.136, 0.349)	
SBP	0.003 (-0.001, 0.007)	
Height	0.015 (0.003, 0.028) *	
Waist	0.010 (0.004, 0.015) †	
Education for Females	-0.330 (-0.544, -0.115) †	**0.021**
Education for Males	0.072 (-0.223, 0.367)	

Values presented as β coefficient estimates (95% CI)
Unadjusted interaction between gender and education for IMT (p-value = 0.83)
Unadjusted interaction between gender and education for AD (p-value = **0.0066)**
Unadjusted interaction between gender and education for LD (p-value = **0.0014)**
¥ p-value < 0.0001, † p-value < 0.01, * p-value <0.05

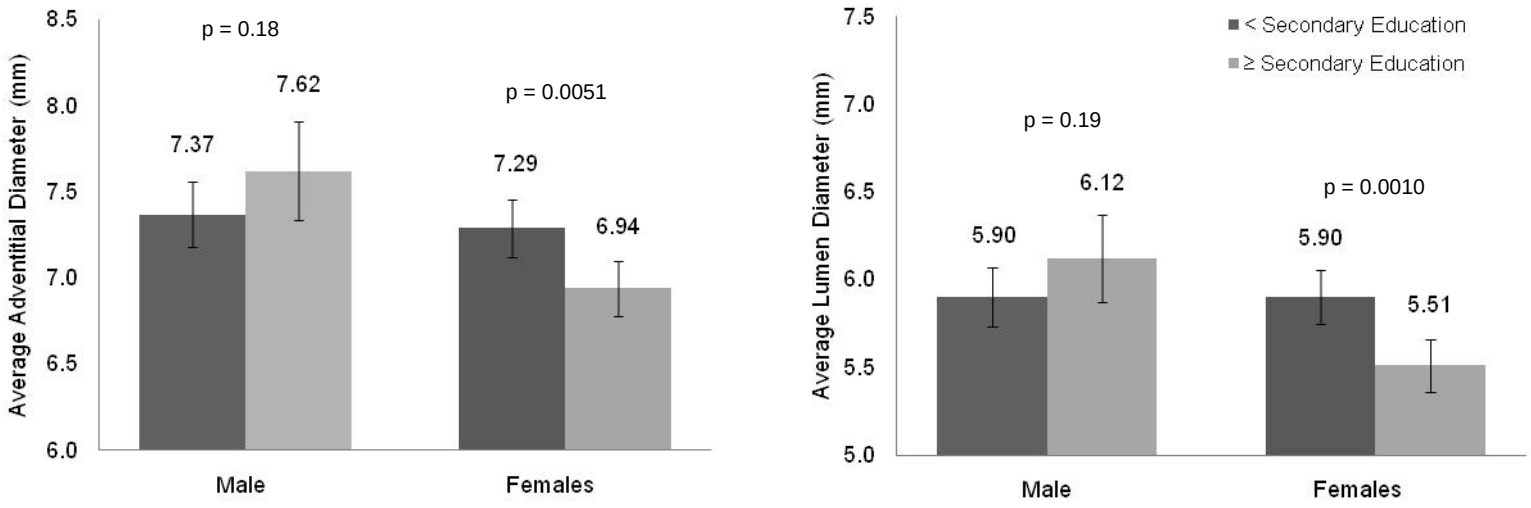

Figure 4.1 Adjusted means for average adventitial and lumen diameters by education level for males and females

Mean diameters adjusted for age, SBP, height and waist circumference.

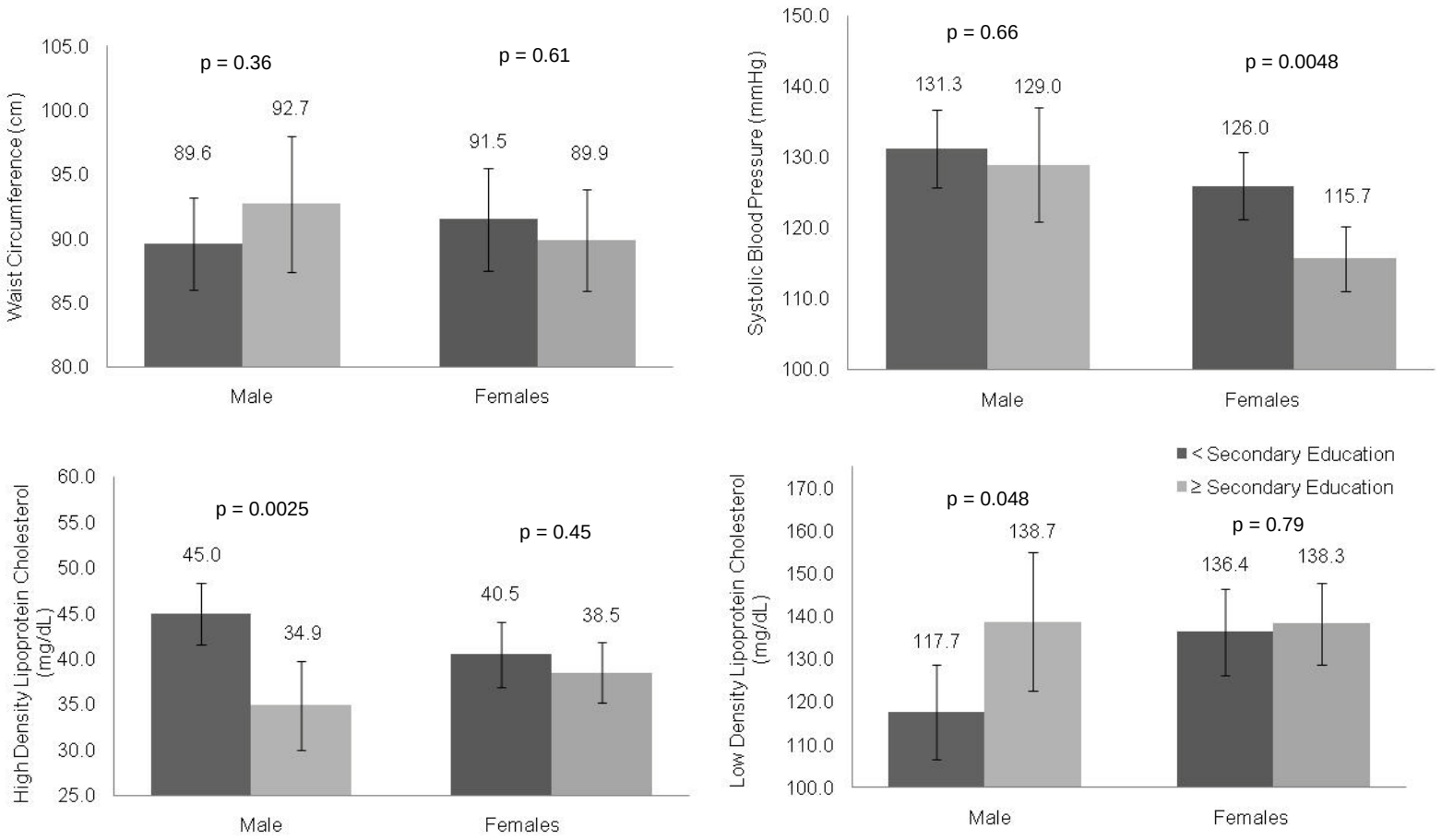

Figure 4.2 Age adjusted means for cardiovascular risk factors by education level for males and females.

Adjusted for age.

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5.0 AUTONOMIC AND INFLAMMATORY PREDICTORS OF SUBCLINICAL CARDIOVASCULAR DISEASE: FINDINGS FROM THE STUDY TO SLOW ADVERSE VASCULAR EFFECTS

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5.1 ABSTRACT

Background: Autonomic nervous system (ANS) activity is known to modulate inflammation. Tonic and reactivity measures of sympathetic and parasympathetic activity, determined by heart rate variability (HRV), and C-reactive protein (CRP) have been associated cross-sectionally and prospectively with subclinical and incident cardiovascular disease (CVD). This analysis aimed to determine which CVD risk factors predicted HRV, CRP and aortic pulse wave velocity (PWV), a validated measure of arterial stiffening. Also determined was how HRV and CRP together predicted aortic PWV. Methods: Indicators of tonic HRV and HRV reactivity, in response to standard autonomic challenges, were obtained with continuous heart rate and respiratory rate recordings in 240 moderately overweight and obese young adult men and women from the study to Slow Adverse Vascular Effects (SAVE). Results: Tonic sympathetic cardiac activity was positively associated with aortic PWV, while parasympathetic reactivity to a deep breathing challenge was inversely associated with aortic PWV, even after adjustment for CVD risk factors (β = 36.3, p-value = 0.029 and β = -35.1, p-value = 0.0085, respectively). Cardiac parasympathetic activity during deep breathing was inversely associated with CRP (β = -0.208, p-value = 0.0059), and CRP was positively associated with aortic PWV (β = 19.9, p-value = 0.057) in a multivariable model, but was attenuated after adjusting for parasympathetic reactivity during deep breathing (β = 14.5, p-value = 0.17). Conclusions: In overweight and obese young adults, tonic cardiac sympathetic activity and parasympathetic reactivity were independent predictors of arterial stiffening. The significant findings observed for indicators of HRV, but not CRP, may be explained by autonomic anti-inflammatory mechanisms linking HRV and CRP.

5.2 INTRODUCTION

Heart rate variability (HRV) and the inflammatory biomediator, C-reactive protein (CRP), are known predictors of future cardiovascular events [1-8]. Measures of HRV provide an indirect index of autonomic nervous system (ANS) activity, which is instrumental for regulating peripheral levels of inflammatory biomediators [9]. Moreover, emerging evidence has linked dysregulated activity in the sympathetic and parasympathetic divisions of the ANS with increased risk for inflammatory diseases, including coronary atherosclerosis [2]. Aortic pulse wave velocity (PWV) is a validated measure of subclinical cardiovascular disease (CVD), and is a known predictor of myocardial infarction, stroke and CVD mortality [10-12]. The associations between HRV and PWV and between CRP and PWV have been reported, yet prior findings are conflicting and the two autonomic and inflammatory predictors of PWV, HRV and CRP, have not been evaluated together.

More precisely, few studies have assessed the association between HRV and PWV. In young Japanese adult men, an HRV indicator of tonic sympathetic activity was positively associated with brachial ankle PWV; however, no association was observed for tonic parasympathetic activity [13]. Further, HRV indicators and carotid femoral PWV were not associated in UK adult men [14]. Additionally, in a case-control study of East Asian men and women, no significant HRV-brachial ankle PWV associations were found in the control group [15]. Critically, no study evaluated the relative predictive roles of tonic sympathetic and parasympathetic HRV indicator variables in the same models of PWV.

CRP is a marker of general inflammation and a robust predictor of incident CVD; however, findings regarding whether CRP is an independent predictor of PWV are mixed [6]. In diverse cohorts of adult men and women, positive associations between CRP and PWV have been reported, with adjustment for traditional CVD risk factors [16-23]. Other studies

have also observed an attenuation of association estimates after adjustment for similar factors [24-26]. In one report, a significant CRP-brachial ankle PWV association was observed only in men [27].

An inverse association between indicators of HRV and CRP has been reported in several studies, suggesting decreased HRV is associated with peripheral inflammation. In the CARDIA study, CRP was inversely associated in multivariable models with both low- and high-frequency HRV measures, which are thought to reflect sympathetic (LF-HRV) and parasympathetic (HF-HRV) levels of cardiac control, respectively [28]. HRV indicators of tonic sympathetic activity have also been inversely associated with CRP in several other populations of men and women [29-31]. Cross-sectionally, HF-HRV indicators of tonic parasympathetic activity have also been inversely associated with CRP; however, recent longitudinal findings suggest a possible direct relationship between HF-HRV → CRP [32, 33]. In a large population of men and women from Germany, a significant inverse association between tonic parasympathetic activity and CRP was documented after adjustment for tonic sympathetic activity [34]. Only one of the mentioned studies used a measure of HRV reactivity, as opposed to tonic activity, in response to a relaxation exercise, and no study has evaluated HRV reactivity to standard autonomic challenges [32].

Increased autonomically-mediated cardiovascular reactivity (blood pressure and heart rate changes) in response to stressors have shown to predict subclinical and incident CVD [35, 36]. Furthermore, cardiac parasympathetic reactivity in response to an impromptu speech challenge, was independently predicative of coronary calcification in the Healthy Women's Study [37]. Hence, measures of HRV reactivity in addition to tonic HRV may provide additional insight into the mechanisms by which the ANS regulates inflammation and inflammatory diseases.

The classic method for analyzing HRV is limited because the LF-HRV component is affected by both sympathetic and parasympathetic outflow, whereas the HF-HRV component is a closer marker of parasympathetic (vagal) outflow [38]. Because respiratory rate strongly determines parasympathetic cardiac outflow, it can be used to improve the validity of the LF-HRV component as an index of autonomic activity [39]. Specifically, deriving HF-HRV spectral power estimates from bandwidths centered at respiratory spectral peak may allow for a better partitioning of sympathetic and parasympathetic contributions to LF- and HF-HRV estimates [40]. Mixed reports in previous literature regarding HRV indicators may be due to the limitation of the LF-HRV as an indicator of sympathetic outflow.

Three studies to date have evaluated the relationship between HRV and PWV in healthy adults, and only one of these included females. Furthermore, no study has assessed HRV-PWV associations in conjunction with CRP. The research supporting the association between HRV reactivity and aortic calcification warrants further investigation into other markers of subclinical atherosclerosis. Using a measure of sympathetic and parasympathetic outflow that adjusts for respiratory rate may improve the predictability of the LF-HRV estimates. Using both measures of sympathetic and parasympathetic outflow simultaneously and including HRV reactivity to a Valsalva (index of sympathetic reactivity) and deep breathing (index of parasympathetic reactivity) challenge may provide further evidence into how indicators of HRV relate to early atherosclerotic changes.

Accordingly, the purpose of this report was to determine which CVD risk factors predicted HRV, CRP and aortic PWV in a population of moderately overweight and obese young adult men and women from the study to Slow Adverse Vascular Effects (SAVE). Additional aims were to examine the predictive effects of HRV and CRP together on aortic PWV, and finally, how reactivity measures of HRV to standard autonomic challenges, in addition to tonic measures of HRV, relate to aortic PWV.

5.3 METHODS

5.3.1 Study Population

The study to Slow Adverse Vascular Effects (SAVE) is a randomized clinical trial designed to test the impact of a dietary and an activity intervention on arterial stiffening. A total of 349 overweight to obese (BMI 25 - 39.9 kg/m^2) men and women from Allegheny County, Pennsylvania, between the ages of 20 and 45 years, were randomized. To be eligible for the SAVE study participants had to have a blood pressure < 140/90 mmHg and a fasting glucose < 126 mg/dL. Potential participants were ineligible if they had a known history of weight loss surgery, CVD, inflammatory disease or a condition where salt restriction would be harmful. Anyone who was pregnant/nursing, on lipid lowering medication or on vasoactive medication was ineligible for the study. The University of Pittsburgh institutional review board approved research protocols for the SAVE study. All participants provided written informed consent prior to enrollment.

The goal of the intervention was a 10% weight reduction and an increase in activity of 150 - 200 minutes per week. In addition to the combined effects of weight loss and increased activity, the effect of a 50% sodium reduction on arterial stiffening was observed. This study has two treatment arms: weight loss, increased physical activity and normal sodium intake versus weight loss, increased physical activity and 50% reduction in sodium intake. The following measures of vascular health were assessed at baseline, 6, 12 and 24 months: aortic and peripheral pulse wave velocity (PWV), carotid intima media thickness (IMT), carotid adventitial diameter (AD) and endothelial function. The primary outcome of the SAVE study was aortic PWV following 6 months of intervention.

An ancillary study to the parent study, SAVE, assessed heart rate variability (HRV) at baseline and at the 6 month follow up visit. Of the 349 randomized participants, 300 were available for this baseline analysis. A final sample size of 240 participants was used because 60 participants had missing HRV and aortic PWV data. Participants with missing data had significantly higher leptin levels and higher household income compared to those included in this analysis. All other variables were equally distributed.

5.3.2 Heart Rate Variability

HRV was measured using an ANSAR monitor (ANX-3.0, ANSAR Group Inc, Philadelphia, Pennsylvania), which provides for continuous and noninvasive measurements of electrocardiogram signals (for HRV assessment) and bioimpedance plethysmography signals (for respiratory rate variability assessment; RRV), respectively. Testing began with attachment of electrocardiogram electrodes in a modified Lead-II configuration to the participant's chest, along with a blood pressure cuff to the left arm. Participants were asked to sit with their feet flat on the floor and refrain from sudden movements or talking. Resting measures at a normal breathing rate were taken for 5 minutes followed by deep breathing (6 breaths per minute) for 1 minute. Participants returned to their resting rate for 1 minute, performed Valsalva challenge for 1.5 minutes and returned to resting again for 2 minutes. To finish, participants remained in a standing position for 5 minutes.

A spectral analysis of the HRV and RRV was generated using ANSAR software. The low-frequency area (LFa) was centered on the HRV spectrum from 0.04 - 0.10 Hz, which is taken to reflect sympathetic cardiac activity. From the spectral analysis of the RRV, the frequency of the peak mode was defined as the fundamental respiratory frequency (FRF). A 0.12 Hz wide window from the HRV spectrum was centered at the FRF

and was used to generate the respiratory frequency area (RFa), which is taken to reflect parasympathetic cardiac activity [39-42]. During low FRF, the RFa shifts into the low-frequency bandwidth. The area under the spectral curve centered on the FRF is computed as RFa. The remaining area under the spectral curve in the low-frequency bandwidth is computed as LFa.

Four measures of HRV were identified for subsequent analyses: (1) LFa and (2) RFa during the initial 5 minute resting period (corresponding to tonic sympathetic and parasympathetic activity levels, respectively) and (3) LFa and (4) RFa during the Valsalva and deep breathing challenge, respectively (corresponding to sympathetic reactivity to the Valsalva maneuver, and to parasympathetic reactivity to deep breathing, respectively). Reproducibility analyses between 3 technologists were conducted on 30 participants (10 for each pair). Between technologist reproducibility intraclass correlation coefficients (ICC) ranged from 0.61 to 0.93 for the four HRV measures.

5.3.3 Pulse Wave Velocity

Aortic PWV was measured using an automatic waveform analyzer (Colin, Cardio Vascular Profiling System, VP1000, Omron Healthcare Co, Komaki, Japan). Methodology has been previously published [43]. Once pulse waves, electrocardiogram and phonocardiogram were stable, pulse waves were captured for 10 seconds. A second 10 second recording was obtained and the two runs were averaged. The technologist measured the distance from the suprasternal notch to the umbilicus and from the umbilicus to the femoral artery (ΔL_{hf}). The Colin machine estimated the time for the blood to travel from the heart to the femoral artery by summing the interval between the second heart sound and the carotid dicrotic notch (ΔT_{hc}) plus the interval between the foot of the carotid pulse to the femoral pulse

(ΔT_{cf}). The heart femoral PWV, measure of aortic PWV, was calculated as (ΔL_{hf}) / (ΔT_{hc} + ΔT_{cf}). Within-technologist ICC was 0.88 for heart femoral PWV.

5.3.4 Covariates

Demographic factors such as age, martial status, alcohol use and smoking were obtained through questionnaires at the baseline visit. Participants were asked their highest level of education completed. Race and ethnicity were self-defined. Blood pressure was collected by a standard sphygmomanometer in the right arm of participants after a 5 minute rest period. A total of three pressures were collected and the last two were averaged. Participants were weighted in light clothing and without shoes. Waist circumference was collected to the nearest 0.1 cm at the narrowest portion of the torso in each participant. Fasting blood samples were collected and analyzed at Heinz Laboratory (University of Pittsburgh, Pittsburgh, Pennsylvania). Serum glucose was quantitatively determined by an enzymic reaction as previously described [44]. HDL-c (mg/dL) was determined using the enzymatic method of Allain *et al.* and LDL-c (mg/dL) was estimated with the Friedewald equation [45, 46]. CRP was quantified with an enzyme-linked immunoassay (Alpha Diagnostic International, Inc). Finally, leptin, adiponectin and ghrelin were determined using radioimmunoassay kits (Linco Research, Inc).

5.3.5 Statistical Analysis

Baseline visit data from the SAVE study was used in this report. Continuous variable distributions were assessed for normality. Natural log transformations were performed to approximate a normal distribution when needed. Insulin, CRP, ghrelin, and the four HRV

measures were naturally log transformed for regression analyses; however, medians (IQR) are presented in Table 1. All other continuous variables were analyzed without transformation. Categorical variables were collapsed into the following cutoffs to maintain adequate sample size in each category: education was dichotomized into ≤ secondary education versus tertiary education, household income was divided into ≤ $75,000 versus > $75,000, marital status was categorized into married versus divorced/separated, widowed or single, smoking status into ever smokers versus never smokers and alcohol into at least one drink per month versus no alcohol. Lastly, black race was compared to non-black race to assess the impact of black race on HRV, CRP and aortic PWV.

Univariate linear regression was performed with CVD risk factors as predictors of HRV, CRP and aortic PWV. LFa during Valsalva and RFa during deep breathing were evaluated in bivariate and multivariable regression models adjusted for LFa at rest and RFa at rest, respectively. Model variance inflation factors ≤ 2.0 were verified to reduce the risk of multicollinearity. Stepwise linear regression was utilized to determine which CVD risk factors remained independent predictors for HRV, CRP and aortic PWV. Weight was chosen in multivariable models because it explained more of the variation compared to BMI. Models repeated using BMI showed similar results to those including waist circumference. Statistical analyses were completed in SAS 9.1 and two sided p-values ≤ 0.05 were considered statically significant.

5.4 RESULTS

Complete characteristics for the 240 SAVE participants used in this analysis are reported in Table 1. The average age was 38 years, with fifty-six male participants (23%) and 38 (16%)

defining themselves as black. Thirty-six percent had ever smoked and 50% drank one or more alcoholic beverages per month. The average BMI was 32.8 kg/m^2. Participants were normotensive (114/73 mmHg) and normoglycemic (97 mg/dL). The median CRP was 2.4 mg/dL and ranged from 1.3 to 5.5 mg/dL. Average mean aortic PWV was 818 cm/sec.

In univariate linear regression models (data available on request), age was inversely associated with LFa and RFa at rest, and continued to be an independent predictor in multivariable models (Table 2). Weight was directly related to LFa at rest and LFa during Valsalva and inversely to RFa at rest and RFa during deep breathing; however, after adjusting for age and male gender, weight was not independently related to LFa at rest. Using stepwise regression, LFa at rest and RFa at rest were independent predictors of LFa during Valsalva and RFa during deep breathing, respectively. CRP was only a significant predictor of RFa during deep breathing.

Independent predictors of CRP included adiponectin, leptin and RFa during deep breathing (Table 3). RFa during deep breathing was significant unadjusted and adjusted for RFa at rest.

LFa at rest, RFa at rest and LFa during Valsalva (unadjusted and adjusted for RFa at rest) were not associated with aortic PWV in univariate and bivariate analyses, whereas RFa during deep breathing, unadjusted and adjusted for RFa at rest, significantly predicted aortic PWV (data available on request). LFa at rest and RFa during deep breathing (unadjusted and adjusted for RFa at rest) were independent predictors of aortic PWV in multivariable regression (Table 4). LFa at rest and RFa during deep breathing (adjusted for RFa at rest) remained significant predictors after adjustment for heart rate, LFa during Valsalva and remaining CVD risk factors: weight, LDL-c, insulin, adiponectin, leptin, ghrelin, alcohol, male gender, smoking, education, marital status and household income (data available on request). CRP was not a significant predictor of aortic PWV in univariate

analysis (β = 14.4 p-value = 0.21). After adjustment for age, SBP, height, HDL-c, black race, LFa at rest and RFa at rest, CRP was borderline directly associated with aortic PWV (β = 19.9, p-value = 0.057), but was attenuated after adjusting for RFa during deep breathing (β = 14.5, p-value = 0.17).

5.5 DISCUSSION

Indicators of HRV independently predicted aortic PWV in a population of overweight and obese young adults. Tonic sympathetic cardiac activity, as reflected by resting LFa in this study, was positively associated with aortic PWV, while parasympathetic reactivity to deep breathing, as reflected by RFa, was inversely associated with aortic PWV, even after adjustment for CVD risk factors. The significant association with tonic sympathetic cardiac activity accords with findings from *Nakao M et al*., who observed a positive association with the low-frequency/high-frequency ratio and brachial ankle PWV in a multivariable model [13]. No association was observed for tonic parasympathetic cardiac activity in the *Nakao* population and in SAVE participants. However, the SAVE study included a measure of parasympathetic reactivity during deep breathing, which was significantly associated with aortic PWV, but tonic and reactivity measures of HRV cannot be compared directly. This is an important finding in that parasympathetic cardiac reactivity, as opposed to tonic parasympathetic activity, may be a better predictor of subclinical CVD. Moreover, parasympathetic reactivity measures may reflect a homeostatic ability to adapt to environmental challenges and stressors and potentially compensate for hypersympathetic outflow [47].

Additionally, the *Nakao* population of men, were 24 to 39 years in age, which is similar to the SAVE study participants. On the other hand, no females were included, the mean BMI was much lower compared to SAVE participants and arterial stiffening was assessed using brachial ankle PWV, a measure of peripheral stiffening, whereas the SAVE study used aortic PWV, a measure of central stiffening. Despite these differences, similar results were found for HRV measures of sympathetic activity. This is in contrast to the two additional studies reporting negative results for the association between HRV and PWV. The first, used carotid femoral PWV, a measure of central stiffening, among a population of UK men; however, the age range, 40 to 60 years, exceeded that of the SAVE study [14]. The second found no significant correlation with brachial ankle PWV, again a measure of peripheral stiffening, among the normal controls in a case-control study. A potential reason for the lack of association may be the measure of sympathetic cardiac activity used (QT interval variability), which has been shown to only moderately correlated with low-frequency HRV, which is not a pure measure of sympathetic outflow [15, 48]. Also, a very small sample size (n=23) was used, and only 4 females were included. The lack of females in all these studies may partially account for the differences in reported findings [49]. Besides the study design dissimilarities in HRV and PWV measures, the multivariable models did not incorporate separate measures of sympathetic and parasympathetic cardiac activity simultaneously.

The two branches of the ANS regulate target organ activity concurrently, and therefore evaluating activity in one branch without the other may yield an incomplete picture of ANS control. The health status of the ANS is likely to reflect an overall autonomic regulatory capacity and flexibility to adapt to change; however, understanding of these processes as they relates to cardiovascular health is evolving [50]. Both measures of sympathetic and parasympathetic cardiac control were predictive of prior myocardial

infarction in separate models; however, an overall measure of HRV regulation (summation of sympathetic and parasympathetic activation) was inversely related to prior myocardial infarction even after adjusting for sympathetic and parasympathetic control. This suggests that not only does each branch of the ANS affect health outcomes, but also the overall ability of the ANS to adapt to change adds valuable information regarding disease processes. Examining both tonic sympathetic and parasympathetic activity, in addition to reactivity, in the SAVE study demonstrated that both branches predict arterial stiffening adjusting for each other. Furthermore, tonic sympathetic activity only became an independent predictor of aortic PWV once differences in parasympathetic reactivity were accounted for, suggesting the relationship between the two branches may offer additional insight into mechanisms of disease.

Another significant finding in this report was the association between HRV and CRP. Cardiac parasympathetic activity during deep breathing, as reflected by RFa, was inversely associated with CRP, however tonic parasympathetic at rest was not a significant predictor. These findings suggest HRV reactivity may also be a better predictor of inflammation than tonic HRV. On the other hand, *Nolan et al*, *Thayer et al. and Singh et al.* observed an inverse association between tonic parasympathetic cardiac activity measures and CRP [32, 33, 34]. Differences in the health status of the three populations compared to SAVE participants may account for these conflicting findings. SAVE participants were relatively healthy; no history of hypertension, diabetes or CVD, whereas *Thayer* and *Nolan* included participants with known CVD and all three studies included participants who were on anti-hypertensive and anti-diabetic medications*. Nolan* used a measure of parasympathetic reactivity during a self guided relaxation technique, however, the model depicted tonic parasympathetic activity and CRP predicting parasympathetic reactivity, whereas the SAVE study evaluated how reactivity adjusted for tonic basal activity predicted CRP. *Thayer* additionally adjusted

tonic sympathetic activity for urinary norepinephrine (NE) in a multivariable model, however, NE was not a significant independent predictor of CRP, which is similar to the SAVE study in that tonic sympathetic activity at rest was not a significant predictor. Conversely, tonic sympathetic activity has been shown to be inversely associated with CRP among several other populations of men and women [29-31]. These studies, however, included older adults compared to the SAVE study, and used measures of sympathetic activity (LF-HRV and SDNN) that may actually reflect both tonic sympathetic and parasympathetic activity. These conflicting results suggest that the association between measures of HRV and CRP is complex and factors such as age, health status of the study population and methodology used to capture HRV may affect the findings. Nevertheless, overall, the literature and this report indicate a connection between HRV and CRP.

CRP was positively associated with aortic PWV, but was attenuated once parasympathetic reactivity during deep breathing was added to the model. This is consistent with the studies showing a non-significant relationship between CRP and aortic PWV once adjusted for CVD risk factors, however, none of these studies adjusted for HRV [24-26]. Given the significant association between parasympathetic reactivity during deep breathing and CRP, it is plausible that the variation in aortic PWV explained by CRP is accounted for by parasympathetic reactivity. There is evidence suggesting a causal pathway linking HRV → CRP → CVD via cholinergic anti-inflammatory mechanisms [51]. Higher cardiac parasympathetic activity during paced respiration, was shown to be associated with lower cytokine production (IL6 and TNFα) in adult men and women from the Adult Health and Behavior project, supporting the relationship between parasympathetic activity and inflammatory competence [52]. Additionally, the direct association observed in longitudinal analysis of HRV and CRP also support the cholinergic anti-inflammatory

mechanisms; those with higher CRP had higher tonic parasympathetic activity a year later [33].

This analysis is unique because tonic and reactivity HRV measures of sympathetic and parasympathetic function were assessed and considered together with CRP in models predicting an indicator of subclinical CVD, aortic PWV. Tonic levels and reactivity differences between individuals likely convey different information regarding the status of the ANS. It is plausible that changes in reactivity due to age, weight or other CVD risk factors may precede changes to tonic levels or visa versa. The lack of association with LFa, reflecting sympathetic cardiac activity, during the Valsalva challenge may be due insufficient power given the large standard deviation of the HRV measures. Further research into the role of tonic versus reactivity measures and how they relate to subclinical CVD need to be assessed in longitudinal studies and larger samples sizes. Because HRV, CRP and aortic PWV are measured at the same time point, the mechanisms linking HRV and aortic PWV via CRP should be interpreted cautiously. It is plausible that changes in blood flow return to the heart due to arterial stiffness could lead to changes in cardiac output and subsequently changes to autonomic outflow; however, adjustment for heart rate did not attenuate the associations between HRV and aortic PWV in this report (data available on request). Unfortunately, the relatively small sample size of black race and males prohibits the comparison of differences between groups. Further research needs to evaluate racial and gender differences in the observed associations. Lastly, a measure of physical activity at baseline was not available for this analysis, which is known to affect HRV; therefore residual confounding could account for some of the variability observed [53].

Analysis of the 6 month follow up visit data will be evaluated once it becomes available. Findings from this report provide empirical basis to test whether dietary changes impact subclinical disease via autonomic-inflammatory pathways. The effects of weight

reduction and increased physical activity on HRV, CRP and aortic PWV will be assessed. In addition, the effect of sodium reduction on HRV, CRP and aortic PWV will be determined. Evaluation of the changes in HRV and CRP and how they affect the observed associations seen in this report will be the primary focus.

In conclusion a measure of tonic cardiac sympathetic activity and parasympathetic reactivity were independent predictors of arterial stiffening, as measured by aortic PWV. CRP is a known marker of general inflammation and a predictor of clinical and subclinical CVD. However, this analysis suggests the variability in aortic PWV explained by CRP may be explained, in part, by autonomic anti-inflammatory mechanisms linking HRV and CRP. This report is significant because therapeutic approaches targeted at autonomic activity may be more successful at reducing CVD compared to those aimed at CRP, which may only be part of the causal pathway.

5.6 TABLES FOR CHAPTER FIVE

Table 5.1 Participant characteristics

Characteristic	**Total** *(n=240)*
Age (years)	38.1 (6.0)
Males (%, n)	23.3 (56)
Blacks (%, n)	15.8 (38)
Tertiary Education (%, n)	69.2 (166)
Income <$75,000 (%, n)	57.0 (134)
Married (%, n)	60.0 (144)
Ever smoke (%, n)	35.8 (86)
Alcohol > once/month (%, n)	50.0 (120)
BMI (kg/m^2)	32.8 (3.8)
Waist Circumference (cm)	100.2 (11.2)
Weight (kg)	92.0 (15.1)
Height (cm)	167.2 (8.5)
SBP (mmHg)	113.5 (10.7)
DBP (mmHg)	73.1 (8.5)
LDL (mg/dL)	122.5 (32.8)
HDL (mg/dL)	52.1 (13.0)
Glucose (mg/dL)	97.4 (8.1)
Insulin (μU/mL) *	12.4 (9.4, 18.2)
CRP (mg/dL) *	2.4 (1.3, 5.5)
Leptin (mg/dL)	24.5 (12.3)
Ghrelin (mg/dL) *	657 (539, 879)
Adiponectin (mg/dL)	11.9 (5.7)
Mean Aortic PWV (cm/sec)	818 (188.9)
LFa at Rest (bpm^2) *	1.8 (1.1, 3.5)
LFa during Valsalva (bpm^2) *	53.2 (29.5, 92.1)
RFa at Rest (bpm^2) *	1.8 (1.0, 3.2)
RFa during Deep Breathing (bpm^2) *	25.7 (13.5, 47.4)

Values presented as means (SD) or % (n) where indicated.
* Non-normal distribution; values are presented as medians (IQR)

Table 5.2 Independent predictors of heart rate variability measures in multivariable linear regression models

HRV Measure Predictors	**Beta Estimate**	**p-Value**	**95% Confidence Interval**
LFa at Rest *			
Age	-0.035	<0.0001	-0.052, -0.019
Male gender	0.268	0.025	0.034, 0.502
LFa during Valsalva *			
Weight	0.009	0.015	0.002, 0.016
Adiponectin	-0.023	0.015	-0.041, -0.005
LFa at Rest *	0.237	0.0003	0.111, 0.362
RFa at Rest *			
Age	-0.031	0.0016	-0.050, -0.012
Weight	-0.011	0.0041	-0.019, -0.004
RFa during Deep Breathing *			
Weight	-0.007	0.042	-0.014, -0.0003
CRP *	-0.149	0.0028	-0.247, -0.052
RFa at Rest *	0.353	<0.0001	0.237, 0.469

* Non-normal distribution; measure natural log transformed

Table 5.3 Independent predictors of natural logarithm of C-reactive protein in a multivariable linear regression model

Predictors	Beta Estimate	p-Value	95% Confidence Interval
Adiponectin	-0.044	<0.0001	-0.066, -0.023
Leptin	0.033	<0.0001	0.023, 0.043
RFa during Deep breathing *	-0.208	0.0059	-0.355, -0.060
RFa at Rest *	0.033	0.66	-0.114, 0.180

* Non-normal distribution; measure natural log transformed

Table 5.4 Independent predictors of mean aortic pulse wave velocity in a multivariable linear regression model

Variable	Beta Estimate	p-Value	95% Confidence Interval
Age	10.9	<0.0001	7.1, 14.6
SBP	2.0	0.062	-0.1, 4.1
Height	3.1	0.028	0.3, 5.8
HDL	-2.2	0.015	-3.9, -0.4
Black race	77.4	0.014	15.8, 139.0
LFa at Rest *	36.3	0.029	3.7, 68.9
RFa during Deep Breathing *	-35.1	0.0085	-61.1, -9.0
RFa at Rest *	-1.8	0.91	-31.5, 28.0

* Non-normal distribution; measure natural log transformed

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6.0 GENERAL DISCUSSION

6.1 SUMMARY OF FINDINGS

This dissertation aimed to evaluate novel risk factor associations for subclinical CVD in three distinct populations. A summary of the findings for chapters 3, 4 and 5 are presented below.

In chapter 3, the protective effect of HDL-c for subclinical CVD appeared to diminish in late perimenopausal and postmenopausal (LP/Post) women compared to premenopausal and early perimenopausal (Pre/EP) women. Specifically, HDL-c was protective against aortic calcification, carotid plaque and intima medial thickness among the Pre/EP women, but directly associated with subclinical CVD among the LP/Post women. Furthermore, in a subcohort of women, the concentration of small HDL-c particles was higher in LP/Post compared to Pre/EP women. These findings suggest that lipid profile changes with the menopausal transition may in part explain the increased risk of CVD seen after menopause.

In chapter 4, the protective effect of education for adventitial and lumen diameter was evident only among females from an Afro-Caribbean population. In addition, females with higher education had lower SBP than those with lower education, and higher educated males had higher LDL-c and lower HDL-c compared to lower educated males. These educational differences in CVD risk factors for males and females provide potential mechanisms for the education-subclinical CVD relationship observed on the island of Tobago.

In chapter 5, measures of heart rate variability (HRV) predicted subclinical atherosclerosis in a population of overweight and obese young adults. Specifically, tonic cardiac sympathetic activity and parasympathetic reactivity were independent predictors of aortic pulse wave velocity. The effect of C-reactive protein (CRP) on arterial stiffening is potentially explained by the autonomic anti-inflammatory mechanisms linking HRV and CRP. Therapeutic approaches targeted at autonomic activity may aid in the reduction of CVD.

6.2 PUBLIC HEALTH SIGNIFICANCE

Myocardial infarctions and strokes are the leading causes of death worldwide. Both men and women are at increased risk for CVD due to rising rates of obesity, smoking, diabetes, hypertension and hyperlipidemia. Understanding how these risk factors impact early atherosclerosis is imperative to the prevention and early detection of CVD. Findings from this dissertation emphasize the need to potentially change the way in which CVD risk is assessed in various populations. Specifically, changes in lipid profiles associated with the menopausal transition suggest the need to evaluate HDL-c and HDL particle size as women transition through menopause. High levels of HDL-c may not confer the same protection for premenopausal and postmenopausal women. Furthermore, the protective effects of education on CVD risk factors and early atherosclerosis differ for Afro-Caribbean males and females. Opportunities to advise this population on healthy lifestyle choices in a clinical setting may be missed if assumptions are made that males with higher education have lower CVD risk. Lastly, indicators of heart rate variability independently predicted early atherosclerosis. Heart rate variability, a noninvasive indicator of increased CVD risk, is a plausible option for widespread clinical application. These findings emphasize the need to

critically evaluate CVD risk on an individual level. In addition, applying current and new risk stratification methods support the important public health objective of reducing the global burden of CVD morbidity and mortality through prevention and early detection of CVD.

6.3 FUTURE RESEARCH

Findings from this dissertation offer novel CVD risk factor associations for early atherosclerotic changes. Using these associations to identify people at increased risk is only part of the challenge. Further research is warranted for primary and secondary prevention methods targeted at CVD risk.

Lipid profile changes during the menopausal transition may lead to increased CVD risk due to a shift in HDL particle size. Conventional behavioral modifications, such as smoking cessation, weight reduction, physical activity and moderate alcohol consumption have shown to increase HDL-c [101]. However, the HDL particle subfraction that increases differs for diet and physical activity [69, 102]. Physical activity is more effective at increasing large HDL particles than hypocaloric and low fat diets, which may initially reduce this subfraction. The pharmacologic increases in HDL-c with niacin, fibrates and statins also vary by particle size [69]. Niacin has proven to be the most effective agent at increasing HDL-c, with substantial increases in large HDL particles and minimal changes in small HDL particles, whereas fibrates have shown the opposite, an increase in small and decrease in large HDL particles. Research on how women respond to these therapies during the menopausal transition is needed. Tailoring behavioral modification strategies and pharmacologic therapy to target an increase in the large HDL particle subfraction may prove to slow the burden of CVD among postmenopausal women.

The lack of a protective effect from education against early atherosclerotic changes among Afro-Caribbean males is a cause for concern. A community-based approach for promoting CVD risk factor reduction involving dietary, physical activity and smoking cessation campaigns across community centers, clinics, schools and public places could potentially create a network of support. This network may help improve the likelihood that both males and females, across several age ranges, make healthy lifestyle choices. Previous community-based prospective studies have shown to be successful at increasing fruit and vegetable consumption and regular exercise, while reducing cholesterol, smoking and hypertension [103-105]. An ongoing study, involving 15005 individuals in Iran, aims to reduce the high burden of hyperlipidemia, hypertension, diabetes and smoking. The effect of primary prevention strategies (public nutrition classes, information pamphlets, religion meeting lectures and a school-based lifestyle modification program) and secondary prevention strategies (dieticians and smoking clinics) will be monitored over a 20 year period [106]. Such community-based strategies are lacking in developing countries of African descent; therefore, further research into these methods may be successful at reducing CVD burden for Afro-Caribbean males.

According to recent reports, despite the robustness of CRP as an indicator for CVD burden and events, it is not a causal determinate of CVD and including it in risk stratification does not improve prediction. Consequently, therapies targeted at CRP reduction may not be beneficial at reducing CVD [96, 107]. The autonomic nervous system modulates inflammation via the cholinergic anti-inflammatory pathway. Therapeutic approaches aimed at autonomic activity, including behavioral and pharmacologic methodologies, may be a more successful therapeutic strategy than CRP [108]. Omega-3-fatty acid consumption, stress reduction through mediation and aerobic conditioning have all shown to increase parasympathetic activity and therefore may reduce risk factors and CVD [109-111].

In conclusion, future research focusing on primary and secondary prevention measures in relation to novel mechanisms for CVD risk may further aid in the reduction of the global burden and economic cost of CVD.

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