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Quantifying the Impact of Chronic Stress on Racial Disparities in Cardiovascular Disease

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by

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DEDICATION

I dedicate this dissertation to my wonderful and loving parents, Prof. Alphonse and Dr. Stella Emechebe, who have been my biggest cheerleaders throughout my life. You are a constant source of inspiration, support and encouragement. You believed in me even in moments I struggled to believe in myself. Your fervent prayers throughout these years have significantly contributed to my achievement thus far. I am extremely honored to follow your footsteps in attaining a Ph.D. and blessed to have you as parents.

I dedicate this dissertation to my siblings: Chinedu, Uju, and Azubike. I am grateful for your support and prayers throughout this journey. I am fortunate to have older siblings to look up and aspire to; that is a gift I treasure.

I dedicate this dissertation to my lovely girlfriend (Kemi), close friends, cousins, and aunties. Thank you for your support and prayers.

Success is shared and you all played a part. Thank you!

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ABSTRACT

Background: Despite declining mortality in cardiovascular diseases (CVD), racial disparities between non-Hispanic Blacks (NHB) and to non-Hispanic Whites (NHW) persist. Although the prevalence of traditional risk factors of CVD such as hypertension, is higher in NHB compared to NHW, adjusting for this difference does not eliminate the disparity completely. This suggests other factors might explain the persisting disparities. Thus, the purpose of this dissertation is to quantify the impact of chronic stress in explaining the racial disparities in cardiovascular diseases (CVD). This dissertation contains three studies that addressed the following Specific Aims:

Specific aims:

1) To create and assess the reliability of various measures (definitions) of chronic stress and examine their incremental predictive benefit with incident cardiovascular disease.
2) To quantify the effect of chronic stress in explaining the racial disparities of incident CVD.
3) To identify chronic stress related metabolites associated with incident CVD

Methods: This dissertation leveraged data from the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. Heart SCORE is an ongoing, prospective cohort and community-based study of 2,000 community dwelling males and females, aged 45-75 years in Pittsburgh, Pennsylvania. Chronic stress was defined by counting the number of times an individual was exposed to various stressors such as perceived discrimination, financial difficulties, caregiving, job difficulties, and residing in a neighborhood with high depravity. This

measure was called the Cumulative Reported Chronic Stressors (CRCS). Chronic stress was also defined using perceived stress using Cohen's Perceived stress scale (PSS-4) and allostatic load (AL). Coronary heart disease- a type of cardiovascular disease- was as a composite outcome defined as the first occurrence of cardiac death, myocardial infarction or coronary revascularization. In Aim 1, reliability analyses using Cronbach Alpha, Weighted Kappa and Spearman correlations were used to assess the reliability of the derived measures of stress. Cox proportional hazard regression models were subsequently used with each measure of chronic stress to determine the incremental benefit in risk prediction with cardiovascular risk scores such as the Framingham risk score (FRS) and pooled cohort equation (PCE) risk score, when applicable. The increase in C-statistic, likelihood ratio test was used to determine predictive benefit and the net reclassification among events and non-events were used to provide a summary measure to quantify this effect. These analyses were repeated among various demographic subgroups. In Aim 2, marginal structural weighted Cox proportional hazards regression models were used to calculate the controlled direct effect of Race on CVD and the percentage of the disparity that would be eliminated. Finally, in Aim 3, multiple logistic and linear regression models were used to identify stress-related metabolites while controlling for multiplicity error using false discovery rate. Subsequently, ordinal and Cox proportional hazard regression models were used to identify stress related metabolites associated with ideal cardiovascular health and CVD.

Results: Among the 1,825 individuals who met the eligibility criteria in Aim 1, 17.3%, 20.1%, 31.4% of the population were classified as having high chronic stress according to CRCS, allostatic load and perceived stress, respectively. Allostatic load had the weakest agreement with the other measures of chronic stress (AL vs PSS, κ =0.02; AL vs CRCS, κ =0.11) while CRCS

and PSS had a slight agreement (κ =0.2). All measures of chronic stress did not improve CVD risk prediction in the overall population, however, CRCS improved the risk prediction among low-income Blacks (p=0.08). The net reclassification was 0.455 and -0.237 among low-income Blacks with and without CVD, respectively.

In Aim 2, the cumulative incidence of CVD was 5% among the 1,735 individuals who met the eligibility criteria. This resulted in an incidence rate of 5.07/1,000 individuals/year among non-Hispanic Blacks and 4.79/1,000 individuals/year among non-Hispanic Whites (incidence rate ratio: 1.04 (0.68, 1.59)). However, this was much higher among individuals aged 45 – 55 (4.29 (1.22, 15.06)). Among 1,443 individuals with complete data on all relevant study variables, the controlled direct effects using CRCS as the mediator were 1.45 (0.70 , 3.01) and 1.39 (0.64, 3) before and after adjusting for traditional risk factors of CVD. This equated in a 43% and 12.6% elimination of the racial disparity, respected. The effect of CRCS was largely driven by perceived discrimination which completely eliminated the disparity after adjusting for traditional risk factors. However, the effect of CRCS on the racial disparity was partially and completely attenuated when missing data were imputed before and after adjusting for traditional risk factors of CVD, respectively. Finally, results from Aim 3 identified 36 metabolites associated with chronic stress. Of these, 14 were associated with ideal cardiovascular health (ICH) and one was associated with incident CVD. However, this association was driven by its association with CVD among non-Hispanic Whites.

Conclusion: In conclusion, chronic stress, defined as a count of exposure to multiple stressors provided incremental predictive benefit beyond the Framingham risk score (FRS) and pooled cohort equation (PCE) among low-income Blacks. Chronic stress also plays a modest role as a mediator in the racial disparities in CVD. Finally, the results from this dissertation suggests other

biomarkers of chronic stress might exist and these biomarkers could elucidate the physiological relationship between chronic stress and CVD. All three conclusions need to be validated in a larger, biracial cohort. Future research should examine the drivers of the disparities and the impact of stress-related epigenetics in young NHB aged 45 – 55.

CHAPTER 1
INTRODUCTION

BACKGROUND

Cardiovascular disease (CVD) refers to diseases of the heart and blood vessels including vascular diseases of the brain [1] . It includes diseases such as coronary heart disease (CHD), stroke (ischemic and hemorrhagic), rheumatic heart disease, congenital heart disease, arrhythmias, cardiac myopathies such as heart failure and acute myocarditis, and deep vein thrombosis (DVT) [1]. It remains a leading cause of morbidity and mortality in the US and globally. In 2015, the WHO estimated 422.7 million people had at least one type of cardiovascular disease [2].

In the United States, cardiovascular disease affects about 92 million Americans and is responsible for approximately 836,000 deaths or 33% of mortality in 2017 [3]. Ischemic heart disease (IHD) or coronary heart disease (CHD), which is a broad term for various cardiovascular morbidities such as acute myocardial infarction (MI) and chronic stable angina is the leading cause of global CVD mortality [2]. Coronary heart disease accounts for 44% of CVD mortality [3], affects 15 million people [4] and was responsible for approximately 360,000 deaths in 2015 [3].

Despite the significant burden of CVD and CHD in the population, mortality rates have declined over the last four decades[5]. Specifically, the age-adjusted mortality rate of CHD decreased from 1,034 per 100,000 in 1968 to 327 per 100,000 in 2015 [6]. Although this represents a significant decrease in mortality rates, disparities exist in the decline and rate of mortality

between non-Hispanic Blacks (NHB) and non-Hispanic Whites (NHW). During the last four decades, the decline in mortality among NHW was at 2.4% per year compared to 2.2% per year among NHB. In absolute terms, the mortality rate of CVD among NHB was 396 per 100,000 in 2015 compared to 323 per 100,000 among NHW. The NHB to NHW mortality ratio is greater than 1 in 27 states in the country, with District of Colombia topping the list with 2.41[6]. These disparities are reflective of the burden of cardiovascular disease among NHB which place NHB at higher risk of CVD-related mortality, greater severity of CVD and earlier onset of CVD than NHW [7].

Inquests into the reasons behind these disparities have persisted for decades. Other research attributes the difference to a higher prevalence of risk factors for cardiovascular disease in African Americans such as hypertension, obesity, physical inactivity, and diabetes [8,9]. In addition, socioeconomic factors such as income, health system factors (access to medical care), perceived racism, and poor neighborhood conditions [4,6,10,11] that disproportionately affect African Americans, are posited to affect risk factors associated with cardiovascular and sub-clinical cardiovascular disease.

A plausible mechanism through which these non-biological or system-level risk factors impact CVD is chronic stress [12–14]. While numerous experimental and observational studies demonstrate an adverse effect of acute stress on cardiovascular disease, chronic stress such as job-strain with low control, caregiving stress, and social isolation are associated with an increase in the risk of cardiovascular disease [12,13,15,16]. These stressors elicit a physiological response that possibly influences the development of CHD through constant activation of the hypothalamic-pituitary-adrenal axis (HPA) and Sympathetic-Adrenal Medulla (SAM) pathway that results in

the proliferation of hormones such as the catecholamines and corticotrophin. This constant and prolonged adaptation to stressors adversely impacts various organs and leads to allostatic load [17].

Allostatic load is characterized by elevated biomarkers indicative of organ dysfunction across various systems such as cardiovascular, metabolic, inflammatory, and neuroendocrinology [18]. It is associated with numerous health outcomes including mortality [19], functional decline in the elderly [20], and chronic diseases including heart disease [21]. Furthermore, prolonged exposure to chronic stress elicits an inflammatory response, characterized by an increase of cytokines [22], that can contribute in the inflammatory process that results in atherosclerosis [23]. Last, exposure to chronic stress evokes a behavioral response associated with the uptake of unhealthy behaviors such as cigarette smoking, poor diet, and inadequate exercise [24–26].

Therefore, the evidence suggests that exposure to chronic stress elicits a biological and behavioral response that increases the susceptibility to cardiovascular disease. Given the chronic social disadvantage of NHB [27,28] it might be important to describe and quantify how chronic exposure to multiple social risk factors (stressors) and the response to these stressors (perceived stress and allostatic load) might explain the disparity in CVD outcomes between racial groups.

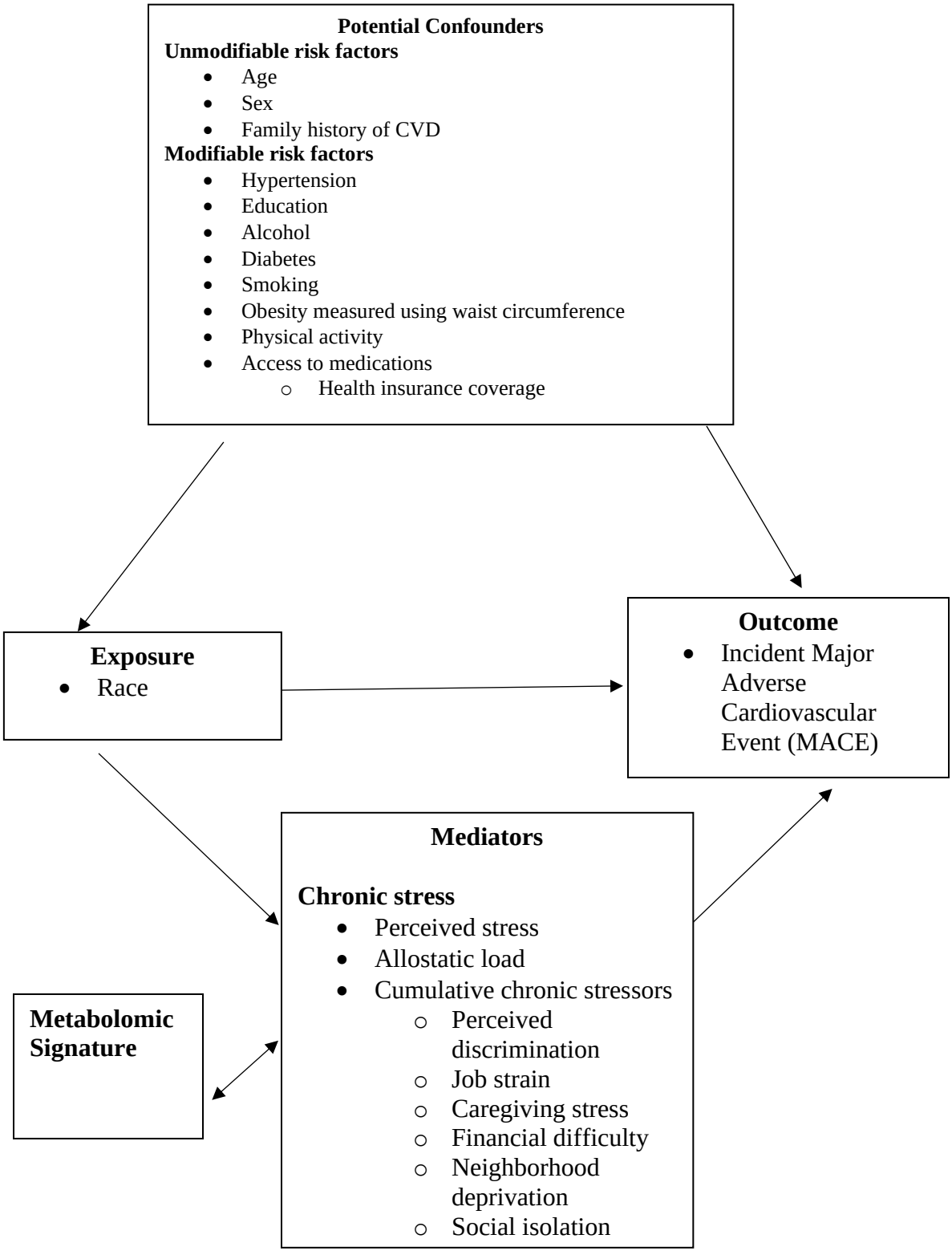

Figure 1: Proposed relationship between study variables

PURPOSE OF THE DISSERTATION

The primary purpose of this dissertation is to quantify the role of chronic stress in the racial disparity of cardiovascular disease. More specifically, this dissertation will determine the impact of exposure to chronic stressors such as perceived discrimination, financial difficulties, job stress, caregiving stress or living in a deprived neighborhood on cardiovascular diseases and perceived stress in explaining the worse outcomes in cardiovascular diseases (CVD) observed among non-Hispanic Blacks (NHB).

Numerous studies report higher exposure to and perception of chronic stress among NHB [28–30]. Furthermore, numerous studies have reported associations of stress with incident cardiovascular disease [14,31–35]. While studies have been inconsistent in the measurement of stress, the message is consistent: Stress is an independent risk factor of cardiovascular disease [13]. Therefore, this project is founded on the hypothesis that NHB are exposed to more chronic stressors across multiple domains (cumulative) compared to other racial groups, including NHW, and this differential exposure to stressors is a pathway that leads to excess risk of CVD observed in this population. Accordingly, the following questions emanated from the aforementioned hypothesis and will be answered across three specific aims of this dissertation:

Research Questions

1) Does the inclusion of measures of chronic stress result in an incremental benefit of predicting incident CVD compared to the Pooled Cohort Equation and Framingham risk score?
2) Do perceived chronic stress and cumulative exposure to multiple stressors mediate the excess risk in CVD among NHB
3) Are there metabolomic signatures associated with chronic stress and are these metabolites associated with CVD?

PUBLIC HEALTH SIGNIFICANCE: CLOSING THE GAP

The difference in life expectancy between NHB and NHW narrowed from 5.9 years in 1991 to 3.6 years in 2013 due to a reduction in mortality from cardiovascular disease, HIV and cancer [36]. Thus, continued effort in improving CVD outcomes among NHB could narrow this gap even further. Quantifying the role of chronic stress on racial disparities in cardiovascular outcomes could provide requisite evidence to increase funding for preventive CVD programs that target social determinants of health (SDoH). Furthermore, the results of this dissertation could provide compelling evidence to justify the continued inclusion of chronic stress management in the national prevention guidelines of cardiovascular disease and support efforts for the systematic collection of social stressors in electronic health records (EHR).

CHAPTER 2

LITERATURE REVIEW

CARDIOVASCULAR DISEASES

Cardiovascular disease refers to diseases of the heart and blood vessels. It remains a leading cause of morbidity and mortality in the US and globally. In 2015, the WHO estimated 422.7 million people have at least one type of cardiovascular disease [2]. In the United States, cardiovascular disease affects about 92 million Americans and is responsible for approximately 836,000 deaths or 33% of mortality in 2017 [3]. Coronary heart disease (CHD) accounts for 44% of the total mortality from CVD in the population and will be the primary focus in this dissertation.

Physiology of Coronary Heart Disease

Coronary heart disease is a disease of the coronary artery and the heart characterized by stenosis of the coronary arteries due to lipid deposits or atherosclerotic plaques [3]. The coronary artery is the blood vessel that supplies the heart with blood and is made up of three main layers: the adventitia, media, and the intima [37]. All three layers represent the outermost, middle and innermost layers of the coronary artery, respectively. The intima is essential in the pathogenesis of coronary artery disease because it's covered by the endothelium which is supported by the basement membrane and elastic lamina. The endothelial surface comes in direct contact with

blood and is responsible for the production of vasoactive substances such nitric oxide (NO), tissue plasminogen (tPA) and prostacyclin with potent vasodilatory and anti-thrombogenic functions [38,39]. The endothelial cells also ensure the blood remains liquid and prevents coagulation through the action of thrombomodulin, heparin and prostacyclin produced in the endothelium [39]. Thus, damage to the endothelium causes an imbalance that could lead to pre-thrombotic or pro-atherosclerotic changes. This underscores the importance of the vascular endothelium in the pathogenesis of coronary heart disease.

Atherosclerosis- A precursor to CHD

The pathogenesis of CHD begins with atherosclerosis. Atherosclerosis is a disease condition that results in the hardening and stiffening of the coronary artery due to accumulation of cholesterol laden plaques [40]. In addition to cholesterol, these plaques contain smooth muscle cells, calcium, inflammatory cells, and macrophages. The accumulation of these plaques in the intima of the coronary artery leads to narrowing of the artery, reduced blood supply, increase oxygen demand and acute coronary syndrome (myocardial infarction and angina) [40,41].

The atherosclerotic process begins with damage to the endothelial surface that increases vascular permeability, promotes coagulation, and inhibits the production of nitric oxide [41–44]. This damage is believed to be caused by risk factors of atherosclerosis (hypertension, smoking, and diabetes), inflammation, systemic infection, injury, or non-laminar or turbulent blood flow [40,41]. In response to the inhibition of nitric oxide (NO), proinflammatory cytokines (ex. IL-6) and adhesion molecules (such as vascular adhesion molecules (VCAM) and p-selectin) recruit immune cells like monocytes into the endothelial wall which mature into macrophages.

Macrophages further release cytokines, free O_2 radicals, proteases and complement factors that continue the inflammatory process and lead to further damage to the endothelium.

Hypercholesterolemia promotes the entrapment of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) in the endothelium that leads to their oxidation. Macrophages engulf these oxidized LDL particles to form foam cells which lead to a fatty streak. Fatty streaks in the arteries are believed to be indicative of early stages of an atherosclerotic process [42,44]. Macrophages also evoke an inflammatory response through recruitment of smooth muscle cells from the Media of the vascular endothelia. [23]. This entire process ends in the formation of atherosclerotic plaques. If the plaque is unstable, it can rupture and form an embolus which can lead to myocardial infarction or stroke. Plaque rupture is primarily responsible for myocardial infarction than stenosis or narrowing of the arteries [45]. Given the prominent role of local proinflammatory markers in the pathogenesis of atherosclerosis, researchers view atherosclerosis as a disease of inflammation rather than solely an accumulation of lipids [41].

Epidemiology of Coronary Heart Disease (CHD)

Coronary heart disease is a disease of the coronary arteries and the heart [1]. It includes conditions such as acute myocardial infarction, unstable angina, and stable angina. It is the leading cause of mortality from cardiovascular disease in the United States and globally [2]. Despite representing 26% of all cases of cardiovascular disease in the US, coronary heart disease represents 44 - 45.1% of all CVD related deaths in the US, making it a potent cause of mortality [3,46]. CHD was responsible for 360,000 deaths in 2015, making it the second cause of mortality behind cancer in the US and a major public health problem [3]. A recent study estimated that if all

CVD were eliminated, life expectancy in the US would increase by seven years[46]. It is therefore plausible that aggressive preventive efforts towards reducing the incidence of CHD could result in an increased life expectancy in the US.

Through concerted efforts in the medical and public health communities, the mortality rates from coronary heart disease are declining and have been declining for close to four decades due to early identification and intervention of individuals at risk of CHD[46,47]. The risk factors of coronary heart disease can be divided into two broad categories:

1) Unmodifiable risk factors
2) Modifiable risk factors

Unmodifiable risk factors include age, race/ethnicity, sex at birth, genetics and family history. Age and sex have long been linked with cardiovascular disease [48,49]. Although the leading cause of mortality among men and women above 65 years of age is CVD; the onset and severity of disease, however, is dependent on sex. Women are diagnosed with CVD at an age 10 years older than men and extends to 20 years for more severe forms of CVD [46]. This difference is attributed to vasodilatory effect of endogenous estrogens that ultimately confers some protection against CVD in premenopausal women [50]. However, administration of exogenous estrogen to premenopausal women for the prevention of CHD has been unsuccessful [50]. In 2017, the age-adjusted CHD mortality rates for men and women was 266.1 and 182.1 per 100,000, respectively [46].

Mounting evidence suggests the epidemiology of coronary heart disease differs by race and ethnicity. Non-Hispanic Blacks (NHB) have the highest rates in their respective sex groups. The mortality rates for men by race is 352.4, 267.8, and 192.4 per 100,000 for NHB, NHW, and Hispanics, respectively. Similarly, the mortality rates are 241.3, 182.1, and 131.7 per 100,000 for

NHBs, NHWs and Hispanic women, respectively[46]. This shows that NHB men have the highest rate than any sex-race group and NHB women have similar rates to NHW men but significantly higher rates than NHW women and Hispanic men and women.

Modifiable risk factors for heart disease can be further broken down into two categories: clinical and non-clinical risk factors. Clinical modifiable risk factors include high blood pressure (high blood pressure), high low density lipoprotein, remnant cholesterol, diabetes, and adiposity. Non-clinical modifiable risk factors include physical activity, depression, diet, stress and the built environment. These risk factors have been discussed extensively in numerous literature and are established risk factors of CHD. Thus, they will not be the emphasis of this dissertation.

Evidence of Racial Disparities in CVD

Despite national efforts to eliminate racial disparities in cardiovascular disease (CVD), these disparities persist [9]. Non-Hispanic Blacks (NHB) are more likely to be diagnosed with premature CVD- diagnosed with CHD <55 years of age, have 30% higher mortality rates than Non-Hispanic Whites (NHW) [7] and twice as likely to die from CVD compared to non-Hispanic whites [6]. These racial disparities are attributed to higher prevalence of traditional risk factors such as hypertension [51], diabetes, and obesity among African Americans [52,53]. The lifetime risk for developing atherosclerotic-related CVD (ASCVD) also varies by race. Among individuals aged 40 – 79, 42% of NHB men have more than 10% risk of developing ASCVD compared to 34% of NHW men. Similarly, 27.4% of NHB women compared to 16.7% NHW women have more than 10% risk of developing ASCVD [54]. Cardiovascular health, defined as the presence of seven health-related factors and behaviors such as blood pressure, cholesterol, hbA1c, BMI,

physical activity, diet, and smoking, is low among NHB. A 2018 study examined trends in ideal cardiovascular health among adults age 25 and over and concluded that NHB had the lowest prevalence of ideal cardiovascular health (15%) compared to Hispanics (25%) and NHWs (40%) over the 26-year observational period [55].

Racial disparities also exist among specific types of ASCVD. According to the annual update from the American Heart Association (AHA), the prevalence of coronary heart disease (CHD) is higher among NHB women compared to NHW women (5.7% vs. 5.1%). Conversely, NHW men have a higher prevalence of CHD compared to NHB men (7.7% vs. 7.3%). Despite the differences in prevalence, NHB men and women have higher incidence rates compared to NHW males and females (6.6 vs.3.8/1000 and 4.3 vs. 2.2/1000, respectively) [46]. Complications from first MI are worse among NHB than NHWs as NHB men and women are two times more likely to have a recurrent MI compared to NHW counterparts. Moreover, mortality rates from MI are highest for NHB males (150.6/100,000), followed by NHWs (137.5/100,000) and Hispanic men (98.4/100,000). Similar trends are observed among females with NHB women having the highest rates (89.4/100,000) and Hispanic women with the lowest rate (57.2/100,000) [46].

According to the CDC, 4.5% non-Hispanic Blacks reported to be diagnosed with stroke compared to 2.5% non-Hispanic Whites. NHB were second only to American Indians who had a reporting prevalence of 5.4% . However, data from 2006 – 2010 Behavioral Risk Factor Surveillance System (BRFSS) show that the trend in prevalence by NHWs and NHB have remained relatively constant over time (range: 2.2% - 2.4% for NHWs and 3.7% - 4.1% for NHBs). Age-adjusted incidence of first occurrence of stroke was higher among NHB compared to NHW and according to data from the REasons for Geographic and Racial Differences in Stroke (REGARDS) study, the black-white incidence ratio for stroke peaked at 4.02 among

individuals 45-55 years [56]. Similarly, mortality data shows that NHB have the highest age-adjusted mortality rate from stroke compared to other races, including NHWs. In 2014, 19 more NHB men died from stroke compared to NHWs men (56.5 per 100,000 vs 35.1 per 100,000) [46]

The reasons behind the disparities are mixed but largely center around higher prevalence of traditional risk factors among NHB compared to NHWs. Among the traditional risk factors, the higher prevalence of hypertension, diabetes and obesity among NHB appear to have the strongest evidence. The prevalence of high blood pressure (BP) among NHB men and women is higher than NHW men and women. Furthermore, NHB get diagnosed with hypertension at a younger age, are more likely to have non-dipping blood pressure, resistant BP, and higher ambulatory BP at nighttime compared to NHWs [46]. Surprisingly, the prevalence of traditional risk factors is not always higher among NHBs. For example, the prevalence of smoking is slightly higher among NHB men compared to NHW men (20.9% vs. 17.2%) but lower among NHB women compared to NHW women (13.3% vs. 16%) [57]. Similarly, NHB have lower total cholesterol levels (10.5%) compared to NHWs (13%) [58]. NHB have better low density lipoproteins (LDL), high density lipoprotein (HDL) and triglyceride profiles compared to NHWs [46].

Other explanations such as socioeconomic status and genetics have been suggested but do not account for the disparities. For example, Williams and Leavell, 2012 reported that NHB had higher CVD mortality compared to NHWs of similar education level. [7]. This suggests that poverty and measures of SES, although relevant to CVD outcomes, are not primary drivers in observed racial disparities between NHB and whites. Genetic disposition affects CVD outcomes, however, it is also unlikely to primarily account for racial disparities in CVD. A study of foreign-born, US-born blacks and NHWs concluded that foreign-born blacks had similar odds of stroke

to NHWs and lower odds compared to AA [59]. If genetics were a major driver, the relative odds between foreign-born and US-born blacks would be similar.

Given the evidence in the literature, it is apparent the reasons associated with the racial disparities are multifactorial and beyond traditional risk factors, poverty and genetics. Thus, discovering additional factors based on empirical evidence is necessary. The ideal putative risk factor would be more prevalent among NHB and associated with cardiovascular disease.

STRESS IN EPIDEMIOLOGICAL RESEARCH

Definition and Use of Stress in Epidemiological Research

Stress is a broad terminology used to describe the influence of external agents or noxious agents on the human physiological and biological systems [14,60]. It has also been described to occur whenever the psychosocial resources are insufficient to match the demand of the noxious agents [61]. The definition of stress has slowly evolved over time to differentiate stressors from the stress response. Stressors refer to the stimuli while the stress response refers to the individual's physiological response to the stressor and is dependent on the individual's perception or appraisal of the situation as a potential stressor [62].

The biological response to stress or stress response is of interest to the research community due to its potential etiological role in the pathogenesis of chronic diseases, specifically coronary heart disease [17,61,63]. The stress response is an adaptation of biological systems to negate effects of copious agents with the intention to restore and maintain homeostasis- a concept called allostasis [17,21,64]. It has been over seven decades since the first biological response to stress was described in the literature. In his seminal Letter to the Editor

titled “A Syndrome produced by Diverse Nocuous Agents”, Hans Selye described the biological response of lab rats to acute exposures such as cold, surgical injury, drugs as a protective effect to withstand the potential damage of the exposures[60]. The results from the paper had a provocative effect on the scientific community and was followed by an interest in the role of stress and disease. Following the work of Selye, psychiatrists Thomas Holmes and Richard Rahe who are credited with the creation of the social readjustment rating scale hypothesized that individuals who experienced more stressful events would require greater biological adaptation and excessive adaptation may result in poorer health outcomes [65]. They confirmed this hypothesis through empirical evidence that showed naval officers with more life changing experiences, cumulated over time, were more likely to have adverse health outcomes. Since then additional research have explored the role of stress in aging [66] and chronic diseases like coronary heart disease [13], cancer [67], and infection [68].

Types and Measures of Stress

Stress is an ubiquitous word that has been used in the literature to describe the exposure to obnoxious stimuli (stressors) or response to the stimuli (stress response). The Stress Network – a nonprofit organization interested in harmonizing measures of stress- summarized domains of stress and the characteristics of stressors [62]. Types of stress domains include:

1) Stressful life events and trauma
2) Financial strain
3) Job strain/stress
4) Discrimination
5) Caregiving stress

6) Loneliness
7) Environmental/neighborhood stress

As part of a global effort to unify terminologies used in stress research, the Stress Network summarized characteristics into main four (4) main categories:

1) Timescale: Acute, daily, life events, chronic
2) Life-period: In-utero, Childhood, Adulthood, Lifespan
3) Assessment window: Timeframe
4) Attributes: Life domains the stressor exists in.

These characteristics help in assessing how the stressor operates in association with disease. Furthermore, stress response can be divided into three categories:

1) Perceived stress: Measure of global response to stressors
2) Behavioral coping: poor diet, smoking, or diminished self-care
3) Physiological response: Allostatic load

Thus, a stressor could have an impact throughout the lifespan of an individual and exposure to this stressor could result in perceived, behavioral and/or physiological response . This provides a challenge in the measurement of stress. Numerous published research looking at chronic stressors and cardiovascular disease have measured stress in one domain. Thus, it would be important to examine the impact of cumulative exposure to multiple stressors across numerous domains on cardiovascular disease.

Numerous scientific theories and models are posited to explain the relationship between adverse social stressors and coronary heart disease. The following briefly introduces the theories and provide plausible explanations to the mechanism of stress-related health outcomes.

1) Social cognitive pathway model: Proposed by Jennifer Phillips and Williams Klein in 2010 [69], the Social Cognitive Pathway model attempts to explain the mechanism through which low SES leads to coronary heart disease (CHD). The Social Cognitive Pathway model assumes that an individual's perception of themselves and their surroundings may lead to low perceived self-efficacy, control, impede motivation to seek healthcare and mediate the association between low SES and CHD. However, the body of evidence that supports this theory have come from cross-sectional studies which are susceptible to reverse causality [69]. Also, few studies that have explicitly measured mediation have resulted in null findings.
2) Weathering hypothesis: Based on the premise that African Americans experience cumulative social disadvantage relative to other racial/ethnic groups which leads to 'weathering' [70]. The hypothesis was initially postulated to explain the disparities in birth outcomes between black and white mothers as well as the relative higher prevalence of teenage pregnancies among AA women compared to NHW women. However, the hypothesis can be extended to explain accelerated physiological aging among AA and the higher prevalence of early morbidity in the population [71]. The allostatic load for AA is higher across all ages than NHWs and may support the Weathering hypothesis [71]
3) Demand-Control and Effort-Reward imbalance models: These two models are a mainstay in occupational health and are often used to describe the impact of occupational stress on adverse health outcomes [72,73]. Demand-control postulates that individuals in a high demanding job with little control or resources to cope with the demands are susceptible to adverse health outcomes. Similarly, the effort-reward imbalance suggests that individuals

whose rewards are not commensurate to the effort they put in elicit a stressful reaction which could in turn lead to adverse health outcomes [74(p)].

PHYSIOLOGICAL RESPONSE TO STRESS IN RELATION TO CORONARY HEART DISEASE

The physiological response to acute stress is well known and characterized. Acute stressors perpetuate their effect through the activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS). This was tested in humans using the Trier Social Stress Test (TSST). The use of TSST enabled researchers observe the physiological response to acute stress under experimental conditions by subjecting participating subjects to mental tasks and public speaking. Results showed a marked increase in hormones of the HPA such as adrenocorticotropic hormone (ACTH) and cortisol [75].

Catecholamines are also implicated in the physiological response to acute stressors. In a recent study, catecholamine-adrenergic receptor complexes were hypothesized to lead to an increase in clotting factor VIII, fibrinogen, and D-dimer after infusion of norepinephrine that mimicked an acute stress response to human subjects [76]. These findings were supported by the attenuation of effect upon administration of phentolamine (adrenergic blocker) in the study and other studies in the literature that have shown thrombotic-stimulatory characteristics of norepinephrine [77,78]. Furthermore, circulating catecholamines suppress the parasympathetic nervous system leading to a decreased vagal tone and reduced activity by acetylcholine (ACH). Consequently, acetylcholine suppresses proinflammatory markers and a decrease in concentration could lead to an increase in proinflammatory markers [79]. Therefore, acute stressors

have the ability to affect CVD through activation of the HPA axis, increase in inflammation and promotion of coagulation. These processes are known to influence atherosclerosis [41,45,80].

Unlike acute stress, the physiological response to chronic stressors such as work-related stress or being a caregiver for a loved one with a debilitating condition [61] is less elucidated. Chronic stressors have been postulated to exert their impact by repeated activation of the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system [81]; ultimately resulting in allostatic load.

The hypothalamic-pituitary-adrenal axis is anatomically located in the central nervous system (CNS, i.e. hypothalamus and pituitary glands) and peripheral nervous system (PNS, i.e. adrenal glands). The principal role of the hypothalamus is to regulate homeostasis through the secretion of hormonal releasing factors that act on the pituitary gland which in turn produces hormones that affect biological organs in the peripheral nervous system.

Specifically, in response to stress, the hypothalamus secretes the corticotrophin releasing factor (CRF) which subsequently binds to its receptors in the anterior pituitary gland. The binding of the CRF leads to the production and release of adrenocorticotropic hormone (ACTH) into the blood. The hormone is absorbed by the zona fasciculata of the adrenal cortex in the adrenal gland which consequently causes the release of glucocorticoids into the blood. An important example of a glucocorticoid is cortisol. Excessive concentrations of glucocorticoids leads to the inactivation of the HPA axis through a negative feedback mechanism [82].

Glucocorticoids such as cortisol are a general name for hormonal steroids produced in the adrenal cortex that are involved in the production of glucose (gluconeogenesis), possess anti-inflammatory actions and regulate metabolism [83]. Thus, they are essential for the regulation of

key biological processes and protect the body from the harmful effects of stress. However, cortisol has a direct effect on the cardiovascular system by increasing sensitivity to circulating catecholamines. It also leads to lipolysis which could lead to an increase in circulating blood cholesterol. Thus, the continuous and excessive stimulation of the HPA axis can lead to the excessive production of cortisol which in turn could lead to the development of chronic diseases such as hypertension heart disease and diabetes [84–86].

Chronic stressors also elicit an inflammatory response by inducing cytokine production. These stress-induced cytokines stimulate expression of adhesion molecules like vascular cellular adhesion molecule (VCAM) in the intima of the artery, promote chemotaxis of leucocytes and monocytes into an atherogenic artery and increase the translocation of low-density lipoprotein into the arterial wall by increasing the expression of LDL receptors on endothelial cells in the artery [87]. These processes are crucial steps in the development of atherosclerosis, which is a causal risk factor of coronary heart disease. Cortisol is also implicated in the inflammatory process. This might sound counterintuitive for cortisol which has potent anti-inflammatory actions, however, preliminary evidence suggests cortisol promotes inflammation and inflammatory processes[88]. As described earlier, chronic stressors lead to repeated activation of the HPA which leads to prolonged secretion of cortisol. This could lead to downregulation of cortisol receptors leading to diminished anti-inflammatory action and potential increased inflammation. Also, increased secretion of cortisol may result in greater affinity for mineralocorticoid receptors which have proinflammatory actions [89]. Atherosclerosis- a causal risk factor of CHD- is a disease of inflammation.

EPIDEMIOLOGICAL EVIDENCE OF CHRONIC STRESS AS RISK FACTOR FOR CHD

The association between chronic stress and cardiovascular disease- especially coronary heart disease- has long been described in the literature. Numerous research have described the association between measures or correlates of stress and CHD using cross-sectional and prospective study designs.

The role of stress in CHD is complex and multifactorial [23,61]. Stress can have a direct effect in the pathogenesis of CHD by damaging the endothelium of the blood vessel. It could also have an indirect effect by promoting risk factors of CHD such as smoking and poor diet. The next few paragraphs will describe the major findings of the association between stress and MI in the literature and explore the potential mechanisms involved.

Chronic exposure to stress is measured in the literature using various measures such as work-related stress. Work-related stress has been defined as monotonous work, or jobs with high demand but low control (job strain) and low social support. These definitions are based off three models namely: job-strain model, effort and reward model, and organizational injustice model [90]. A recent meta-analysis conducted by Kivimaki et al[90] summarized the association between work-related stress and CHD using data from 14 prospective cohort studies. They concluded that work-related stress from organizational injustice contributed to approximately 50% excess risk of CHD among employees who experienced it (RR=1.47, 95% CI=1.12-1.95) [90]. Work-related stress from job strain and high effort with low rewards lost statistical significance after controlling for multiple risk factors. However, other research has shown mixed results between the association between job strain and coronary heart disease. Job strain did not lead to an increase the risk of CHD in a few studies [91–93] after controlling for potential confounders.

However, one cross-sectional study [94] and prospective cohort study [95] found associations between job strain and CHD. The prospective cohort study found an association between low-control at work and any CHD for men (HR: 1.43, 95% CI=1.15-1.78) but not women. When the data were restricted to non-fatal MI in the prospective cohort study, the association was attenuated and became non-statistically significant after controlling for other risk factors (HR: 1.30 95% CI=0.93-1.90).

Other measures of chronic stress such as marital stress, caregiving, and social isolation are associated with CHD. Marital stress in females- defined using the Stockholm marital stress scale (SMSS)- was determined to be a prognostic risk factor in the recurrence of acute myocardial infarction among a cohort of 279 Swedish females who were working or living together with a male partner [96]. The effect of marital stress resulted in a hazard ratio of 2.9 (95% CI 1.3-6.5). Lee at al studied the impact of taking care of a terminally ill spouse or parent as a proxy for psychosocial stress on incident CHD using data from the Nurses' Health Study [97]. CHD was defined as first MI or death from heart disease and results showed that women who spent at least 9 hours a week looking after a spouse were more likely to develop incident CHD (HR=1.82, 95% CI 1.08-3.05). A null association was found between caregiving for a parent and incident CHD. Social isolation and loneliness have been studied as proxies of chronic stress. A meta-analysis of prospective cohort studies in populations without prevalent CHD showed 50% increase in risk between social isolation and loneliness and incident CHD (RR=1.5; 95% CI= 1.2-1.9) [13].

The largest study till date that examined the association between stress and myocardial infarction is the INTERHEART study. The INTERHEART study was a global case-control study that investigated the risk factors of myocardial infarction using data from 29,972 study

participants (12,461 cases and 14,637 controls were used in the final analysis) from 52 countries in each continent [98]. Psychosocial factors, as a proxy for chronic stress were measured. A composite psychosocial index was derived by including subjects with work or home related stress, depression, major life events, and low control. The odds ratio of subjects with at least one psychosocial factor compared to subjects with none was 2.67 (99% CI: 2.21-3.22) after controlling for traditional risk factors. This represented a population attributable risk (PAR) of 32% in the population studied. Other examples include results from the Coronary Artery Risk Development in Young Adults (CARDIA) study which showed that work-related stress was associated with incident hypertension; a strong risk factor for acute myocardial infarction [99]. Other studies have operationalized the concept of job-strain differently but found similar findings[100,101].

Therefore, the impact of chronic stressors on CHD could be largely mediated by a behavioral response that promotes the incidence of traditional risk factors (diabetes, metabolic syndrome, and hypertension). However, stress might have a small direct effect on CHD by participating in the inflammatory process of atherosclerosis.

EVIDENCE OF RACIAL DISPARITIES IN EXPOSURE TO CHRONIC STRESSORS

African-Americans (AA) or Non-Hispanic Blacks are differentially exposed to economic, cultural and psychological stressors than other racial and ethnic groups in the US [7,102–104]. A study by Utsey and colleagues demonstrated a higher level of racism-related stress among African Americans compared to other ethnic groups and explained 16% of the variance associated of poor quality of life associated with racism-related stress [29]. The higher prevalence of these stressors among African Americans has been associated with poorer health outcomes. For example, a recent study conducted by investigators from the Jackson Heart study concluded

that individuals with moderate to high levels of financial stress had more than a two-fold increase in risk of incident coronary heart disease compared to individuals with low financial stress. This association was independent of traditional clinical risk factors of CHD, age, demographics and SES [105].

CHAPTER 3

SPECIFIC AIM 1

INTRODUCTION

Numerous studies link chronic stress with chronic diseases such as cardiovascular disease (CVD). Chronic stress is associated with risk factors of cardiovascular health [34,106,107], and cardiovascular outcomes like coronary heart disease (CHD) and stroke [13,108]. Despite the preponderance of evidence linking stress to CVD, the clinical utility of chronic stress in prognostic risk equations (RE) such as Framingham or Pooled Cohort risk equation remains unknown. This is especially important for African Americans who are differentially exposed to chronic stress and have worse CVD outcomes.

A possible reason could be the absence of a standardized, agreed upon measure of stress that adequately captures the chronicity and multidimensionality of stress. In her recent article, Epel et al advocated for the distinction between stressors and stress response when assessing the impact of stress on health [62]. This approach enables researchers to adequately assess which stressors are linked to the outcome of interest. Researchers use a variety of measures of stressors when examining chronic stress and CVD. For example, Steptoe and Kivimaki published a meta-analysis summarizing the evidence that established job strain and social isolation/loneliness as risk factors of CVD in a non-US population [109]. Within the United States, frequently studied

stressors on CVD include perceived discrimination [33,110,111] and disadvantaged neighborhoods [112,113]. These domains are frequently measured individually. Similarly, the response to stress is measured using hormones of the hypothalamic-pituitary adrenal (HPA) axis (such as cortisol, epinephrine and norepinephrine) and allostatic load. Perceived stress- a perception of lack of control over events happening in a person's life- is frequently examined with CVD [106,114]. However, studies examining the effect of multiple stressors across multiple domains are rare.

This is surprising given the viable plausibility of exposure to multiple chronic stressors over the life course of an individual. A recent study by Burroughs et al examined the impact of cumulative psychosocial stressors, including acute stressors on ideal cardiovascular health, in 25,062 older women concluded that black women had a 10-point higher cumulative stress score and worse ideal cardiovascular health (ICH)- a construct that confers protection from future CVD- compared to white women [107]. This difference in cumulative stress score also responsible for differences in ICH score by approximately 12.7%. However, the study was cross-sectional with a very low percentage of black women (1.76%). Another study found people with high chronic stress were less likely to achieve ideal cardiovascular health specifically due to high prevalence of smoking and fasting blood glucose [106].

Currently, there is a gap in the literature in assessing measures of chronic stress across multiple domains and CHD outcomes. Also, it is unknown whether these measures of stress have clinical utility in predicting future CHD events.

Therefore the objectives of this specific aim are as follows:

1) Create three measures of chronic stress according to the response to stress (Perceived Stress Score and Allostatic load) and exposure to the stressful stimuli (Cumulative Reported Chronic Stressors (CRCS))
2) Assess the relationship between measures of chronic stress using Spearman Correlations and weighted Kappa
3) Determine the incremental net benefit of including measures of chronic stress in risk prediction models for incident CHD.

METHODS

Recruitment and Data source

This project utilized extant data from the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. Heart SCORE is an ongoing prospective cohort and community-based study of 2,000 community dwelling individuals aged 45-75 years in Pittsburgh, Pennsylvania. The primary goal of the Heart SCORE study is to identify mechanisms that explain population differences in cardiovascular disease outcomes for the purposes of eliminating racial disparities in CVD [115]. Thus, the Heart SCORE population contains higher than average representation of African American participants (43%).

Recruitment of study participants began in 2003 and involved direct mailing of questionnaires, community and physician referrals, print and electronic media, and public service announcements. Data were collected at baseline and during annual follow-up visits on characteristics such as sociodemographic, clinical markers, medical history, psychosocial risk factors (depression, hostility, anger, anxiety, perceived stress, ongoing life events, and optimism)

and social network. To be eligible, participants had to be aged 45 – 74 years, have a life expectancy >5 years and be available for baseline or annual follow-up visits. Pregnancy or HIV status was not an exclusion criteria.

Study population and Eligibility Criteria

To answer the research questions associated with this study, individuals from the Heart SCORE study cohort with a prior history of coronary heart disease such as myocardial infarction (MI), percutaneous coronary intervention (PCI), cardiac catheterization or coronary artery bypass graft (CABG) (n=88). Also, individuals who did not report on race or identified with a race other than Black or White were excluded (n=51). Thus, the final sample size comprised of 1,861 individuals.

Study Outcomes

The primary study outcome was a composite outcome of incident major adverse cardiac events (MACE) defined as the first occurrence of myocardial infarction, cardiac death or any revascularization such as percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG). The timing and occurrence of the events were confirmed through medical records and adjudicated by medical experts.

To assess the impact on chronic stress on other cardiovascular diseases, an additional sensitivity analysis was performed by creating a composite CVD outcome that includes CHD (MI, revascularization or cardiac death) and ischemic stroke.

Study Variables

To examine the incremental benefit of including chronic stress in cardiovascular risk equations, three measures of chronic stress were created as follows:

Cumulative Reported Chronic Stressors (CRCS)

Six stressors frequently measured in the stress and cardiovascular literature were identified and used to calculative a cumulative stress score based of the presence or absence of each stressor. These stressors represent six domains such as financial stress [116,117], perceived discrimination [118], social isolation [119], neighborhood stress [112,113], caregiving stress [120], and job-related stress [99]. Social isolation was eliminated from the final derivation of the cumulative stress score due to poor correlation with other stressors and its removal improved the Cronbach alpha (0.49 to 0.55). Thus, the range of the cumulative chronic stress score was 0 – 5. Participants were subsequently classified into three (3) groups namely: None (0), moderate (1), and high exposure to CRCS (≥2) based on tertiles. Pearson correlations between each stressor was also measured. See table A4 for the definition, classification of each stressor, and correlation with each other.

Measures of Stress Response

The global perception of stress ("feeling of being stressed") in response to life stressors was measured using the 4-item Cohen's perceived stress scale [121] while the biological response was measured using allostatic load (AL). Perceived stress was measured using the 4-item Cohen's perceived stress scale (PSS-4; range: 0 – 16), a shorter version of the PSS-10. The PSS-4 is frequently used in epidemiological studies due to its brevity and has previously been validated in other studies [122]. Although concerns have been raised about the low reliability of the

PSS-4 instrument [123], the PSS-4 had acceptable reliability in our study population (α=0.79). Participants were subsequently divided into terciles according to the distribution of the PSS-4 score: Low (0-3), moderate (4-5), and high (≥6) perceived stress.

The second derived measure of the stress response was allostatic load (AL). The allostatic load was measured using 10 biomarkers across three domains: Inflammatory (hs-CRP and IL-6), metabolic (fasting blood glucose, waist-hip ratio, serum creatinine, and urine albumin) and cardiovascular (systolic blood pressure, diastolic blood pressure, triglycerides, and very low density lipoprotein). Individuals were classified as high or low risk for each biomarker if it fell outside or within the normal clinical range, respectively. Sex-specific clinical cutoffs were used for waist-hip ratio and serum creatinine. Individuals on anti-hypertensive and lipidemic agents with values within normal range were considered to be low risk. Each individual was subsequently assigned a score of 1 or 0 if they were classified as high or low risk, respectively. These scores were summed to get the allostatic load with a possible range of 0 – 10. This approach of deriving the allostatic load using clinical rather than empirical cutoffs is frequently used in the AL literature [124]. See table A3 for the clinical cutoffs of biomarkers used in computing the AL. The calculated Cronbach alpha for all biomarkers used in calculating the AL score was 0.6. For the purpose of assessing agreement between measures of chronic stress, participants were divided into three groups based on terciles of AL score: low (0 – 1), moderate (2 – 3), and high (≥4).

Cardiovascular Risk Scores

The 10-year Framingham risk score (FRS) or expected risk of CVD was calculated using the approach described by D'Agostino et al [125]. Similarly, the 10-year Pooled Cohort Equation (PCE) risk scores were computed using the equation described by Mutner et al [126].

Cardiovascular Health

An ideal cardiovascular health score was calculated for each individual as recommended by the American Heart Association (AHA) [127]. Participants were assigned a value of 0, 1, or 2 if the values of each component of the life simple 7 (LS7) was poor, intermediate or ideal. Finally, a total score was calculated by summing all values and categorizing individuals into poor, intermediate or ideal according to empirical cutoffs. See Table A13 for the operationalization of the ICH score.

Other Covariates

Information on other study variables were collected to compare the characteristics of individuals in each stress group. These included sociodemographic factors such as age, sex, race, income, insurance status, employment and educational attainment. Clinical factors such as systolic blood pressure (SBP; mm/hg), diastolic blood pressure, high density lipoprotein (HDL; mg/dl), history of hypertension, and history of diabetes were also included. Finally, anthropometric (waist circumference, waist-hip ratio, BMI), behavioral (current smoking status, self-reported quality of life), and psychosocial factors (ongoing life events (OLE), depressive symptoms measured using Center for Epidemiological Studies Depression Scale (CES-D), and optimism measured using Life Orientation Test (LOT), were included. OLE, depressive symptoms and optimism were included to assess concurrent and discriminant validity of the derived measures of chronic stress.

Statistical Analyses

The reliability of all instruments and derived scores (CRCS and AL) was calculated using Cronbach Alpha while the agreement between each measure of chronic stress was assessed using weighted Kappa statistics. Once constructed, the distribution of study covariates was examined by each measure of chronic stress and differences between groups within each measure of stress were assessed using Chi-square test of independence for categorical variables and ANOVA for continuous variables. Pearson and Spearman rank correlation tests were used to assess the correlations between each measure of stress and related constructs such as depressive symptoms and optimism.

Before conducting the regression analyses using Cox models, the proportional hazard assumption was tested using time dependent covariates by creating an interaction term of each measure of chronic stress and log of time. The resulting non-significant p-values confirmed the assumption was met. To determine if chronic stress added predictive information to cardiovascular risk equations, Cox proportional hazard regression models with calculated FRS scores were compared with models with FRS score and a measure of chronic stress using the test of negative two log likelihood ratio tests of nested models (-2LLR) [128]. A small p-value (<0.05) was indicative that the measure of chronic stress provided additional information to the model. Models were also compared using the Akaike information criterion (AIC) where smaller values were indicative of a better fit. Subsequently, Harrell concordance index (c-statistic) was calculated and compared with each model. In instances where the c-statistic appeared to improve with the addition of a measure of stress, the c-statistic was internally validated using 100 bootstraps to mitigate the effect of optimism arising from comparing predictive performance of two models with the same data [129,130]. Bootstrapping was conducted for the model with FRS or

PCE alone and the appropriate measure of chronic stress using logistic regression models. To quantify the incremental clinical benefit of the predictive measure of stress, the event and non-event Net Reclassification Index (NRI) were calculated [131]. To calculate the NRI, the 10-year predicted probabilities of having the event were calculated for individuals using the coefficients from a Cox model. These probabilities were subsequently categorized into four clinically significant groups: 0-<5%, 5-<10%, 10-<20%, and ≥20%. Subsequently, the NRI among those with the event was calculated as the difference between the probability of an upward reclassification and the probability of downward reclassification. Similarly, the NRI among subjects without the event was calculated by taking the difference of the probability of a downward reclassification and the probability of an upward reclassification. The NRI was only calculated in instances where the LR test was significant.

Sub-group analyses by age (45 – 55; 56 – 65; 66 – 75 years), sex (males and females), race (Blacks and Whites), income (<$20,000 vs ≥$20,000) were conducted. Further stratified analyses among mutually exclusive income and racial groups were also conducted. The cutoff of income was chosen because the average poverty level for a family of four between the years of recruitment (2003 – 2006) was $19,150 (aspe.hhs.gov). Furthermore, because the primary objective was assessing predictive performance, only complete data were used in all analyses.

RESULTS

The study population consisted of 67% females, had an average age of 58.8 (7.5), a high percentage of self-reported Blacks (43.5%), approximately 82% had an annual income ≥$20,000, were highly educated (81% had at least some college degree), 60% were employed, 76.3% had

private insurance while 6.4% had no insurance. Although 88.3% rated their quality of life was good or excellent, the prevalence of cardiovascular risk factors was particularly high. These include a history of hypertension (41%), history of smoking (52%), high cholesterol (24%), and physical inactivity (40.5%). Using the LS7 score, only 14% had ideal cardiovascular health. See table A1.

Using the three measures of chronic stress, 17.3%, 20.1%, 31.4% of the population were classified as having high chronic stress according to CRCS, allostatic load and perceived stress, respectively. There was congruence across all measures of stress when comparing people classified in high stress groups to moderate and low stress groups. Individuals in the high stress groups were generally younger, Black, female, less educated, lower income, depressed, and had poorer cardiovascular health compared to individuals in the moderate to low stress groups. These differences were statistically significant.

The validated instruments showed good reliability in the sample. The Cronbach alphas ranged from 0.67 for the OLE to 0.92 for the CES-D (see Table A2). Furthermore, the Cronbach alpha of variables used in creating the cumulative chronic stress score and allostatic load showed modest reliability (0.55 and 0.6, respectively). See Tables 3 and 4. Allostatic load had the weakest agreement with other three-level chronic stress measures (AL vs PSS, κ =0.02; AL vs CRCS, κ =0.11) while CRCS had a stronger agreement with PSS (κ=0.20). Last, all three measures of chronic stress were positively correlated with measures of depressive symptoms and negatively correlated with optimism, however, the association was stronger for CRCS and PSS. See tables 5a and 5b.

There were 93 events of CHD over a median follow-up time of 12.1 years. This equates to a cumulative incidence of approximately 5% or an incidence rate of 49 new cases of CHD per

10,000 individuals per year. A higher percentage of individuals with incident CHD were classified as being stressed compared to individuals without CHD (53.8% vs 51.7% for CRCS and 63.1% vs 55.9% for PSS). However, these differences did not reach statistical significance. Conversely, 70.8% of individuals with CHD were classified as having moderate to high allostatic load compared to 60.5% without CHD (p=0.04).

Cox proportional hazards regression models evaluating the incremental value of including measures of chronic stress resulted in null findings when using the total study population. This persisted for models stratified by sex, race, and income. When the population was stratified by race and income, CRCS provided incremental benefit to CVD prediction among low income Blacks. The c-statistic increased from 0.635 (FRS only) to 0.718 with a difference in Likelihood ratios of 4.9 approaching statistical significance, despite the small sample size (p=0.08). See Table A6. These results were confirmed using PCE for low income Blacks where the AUC increased from 0.625 to 0.703 after including CRCS (difference between LR=5.3, p=0.07). Internal validation of model performance using bootstrapping resulted in a c-statistic of 0.62 and 0.636 for model with FRS only and FRS plus CRCS, respectively. Among high income Whites, the inclusion of CRCS and FRS worsened the prediction of CVD. AUC decreased from 0.8 to 0.79 and paradoxically, the LR test was marginally statistically significant (LR=6.2, p=0.05). Among low income Whites, the inclusion of the PSS-4 improved the c-statistic from 0.8775 to 0.9249. However, internal validation using bootstrapping revealed these estimates were highly optimistic. After bootstrapping, the inclusion of PSS-4 reduced the c-statistic from 0.8789 to 0.8445.

The NRI_{event} for the model among low income blacks was 0.455 or 45.5% while the $NRI_{non\text{-}event}$ was -0.237 or -23.7%. These results were replicated using the PCE. Furthermore, the

NRI_{event} and $NRI_{non\text{-}event}$ among high income Whites was 0 and -2.9%, respectively. See tables 6 and 7.

DISCUSSION

The correlation and agreement between three derived measures of chronic stress was examined. These measures were created based on their role as stressors (cumulative chronic stressors) and response to stressors (perceived stress and allostatic load). All three measures had weak agreements and poor correlations with each other. However, perceived stress measured with the Cohen's perceived stress scale and the cumulative chronic stress score showed fair criterion validity with depressive symptoms and optimism. Allostatic load, a physiological measure of cumulative stress, had the weakest agreement with other measures of chronic stress, optimism and depressive symptoms.

Three measures of chronic stress were created to determine whether they provided additional predictive benefit in identifying people at risk of CHD. Although all three measures of chronic stress were positively associated with risk factors of cardiovascular health, only cumulative chronic stress provided incremental benefit to risk prediction with traditional risk factors among low income Blacks. The results showed a 45.5% and -23.7% (worse) net improvement in the reclassification of cardiovascular risk with the inclusion of CRCS in the model among those with and without the event, respectively. To the best of our knowledge, this contributes to the current knowledge base by examining the impact of chronic stress on cardiovascular risk prediction.

The income dependent effect of stress was previously reported in the literature. A prospective cohort study conducted by Redmond et al showed perceived stress measured using the PSS-4 was associated with increased risk of incident coronary heart disease among low income Blacks [35]. Another study showed that pooled cohort equations (PCE) performed better among individuals with social deprivation but overestimated individuals with less social deprivation [132].

Results also show that univariate associations between measures of chronic stress and CHD were statistically non-significant. This result was unexpected but isn't entirely surprising given that people in high stress groups were relatively younger than those in the lower stress groups. Age is the most important risk factor in CHD and analyses that fail to control for age will have similar findings. Upon stratification, the results are in the expected direction (result not shown). However, only PSS-4 reached statistical significance.

The weak agreement between measures of chronic stress was unexpected. While the results need to be replicated in a larger, representative cohort, the poor agreement is unlikely due to the chosen cutoffs in this study. If it were the case, we would expect stronger correlations between the raw, uncategorized measures, however, in congruence with the agreement scores, this was not the case. One explanation could be related to the difference in perception of stress and the presence of resilience factors among those exposed to multiple stressors. It is plausible that the detrimental effect of stress on health would only matter if people exposed to multiple stressors perceive their situation as stressful. In reality, this may not be the case due to resilience and personality traits. Thus, an individual exposed to multiple stressors may not perceive their situation as stressful while someone exposed to few stressors might and could explain the weak

linear correlation. Therefore, future research could consider defining chronic stress at the intersection of these two measures.

The agreement was weakest with allostatic load compared to other measures of stress. This is surprising because allostatic load represents the physiological response to stressors like neighborhood deprivation [133]. The lag between exposure to chronic stress and development of AL might explain this association and is recommended for future research.

Despite higher prevalence of perceived stress among Blacks and its known association with CHD, the inclusion of PSS-4 did not improve the performance of FRS among low income Blacks. This further underscores the need to validate the psychometric properties of the PSS-4 in minority populations, specifically in relation to CHD.

The implications of this finding has public health and clinical relevance. Given the susceptibility of exposure to multiple stressors, low income Blacks may benefit from enhanced screening for stressors during physician visits and promotion of interventions to offset the effect of stress such as provision of food markets in the community, access to food pantries, referrals to community organizations to address unmet social needs and increased health education materials to reduce cardiovascular risks. This messaging maybe timely with the clamor for surveilling social health and addressing unmet social needs [134]. Ensuring individuals with unmet social needs such as financial assistance, access to food, and medication assistance receive the necessary assistance may alleviate the stress experienced by these individuals [135,136].

This study was conducted using extant data and is limited by potential measurement error in the original dataset. Furthermore, the results of these analyses are generalizable to the source of the study population, Pittsburgh Pennsylvania.

There was limited sample size among low income Blacks. While results were further confirmed with bootstrapping analysis, the results need to be validated in a larger cohort of low income Blacks. In addition, the event rate in the study was low (5%) and may negatively impact statistical power. However, several studies examining risk factors in incident CHD have reported similar event rates .

Neighborhood deprivation was calculated by linking available addresses of study participants at enrollment to publicly available 2013 ADI. There are two noteworthy limitations. First, linkage was not feasible for approximately 12% of participants due to incomplete address. Second, it is likely an individual residing in a highly deprived neighborhood in 2003 might be misclassified as living in an affluent neighborhood in this analysis if the neighborhood underwent substantial gentrification. It is difficult to estimate this percentage. Last, it is also likely a participant might have changed residence over the course of the study, however, only 7.7% of the original population changed residence. Recent evidence suggests individuals classified as living in a highly deprived neighborhood are likely to move to a neighborhood of similar depravity [137]. Other research show that neighborhood deprivation measured at one time point is as predictive multiple measurements [138]

CONCLUSION

Three measures of chronic stress were weakly correlated with each other but were associated with poor ideal cardiovascular health and disproportionately affected Blacks compared to Whites. However, only cumulative measure of chronic stressors provided incremental predictive benefit to predicting CHD among low income Blacks. Further research is required to delineate the mechanisms driving this association in this subgroup.

CHAPTER 4

SPECIFIC AIM 2

INTRODUCTION

Despite declining mortality and morbidity rates in cardiovascular diseases (CVD), racial disparities persist. Non-Hispanic Blacks (NHB) experience higher incidence rates of coronary heart disease (CHD) (6.6 vs 3.8/1000), worse CVD-related mortality rates (150.6 vs 137.5/100,000) [2], experience stroke at a younger age (4:1 among 45 – 55 years old) and experience higher mortality from stroke (66.8 vs 47.2/1000,000) than Non-Hispanic Whites (NHW) [139]. These disparities in cardiovascular outcomes are associated with the disproportionate exposure to traditional risk factors. Numerous studies report higher prevalence of traditional or proximate risk factors including high blood pressure [51], diabetes [52], cigarette smoking among adult males [57], and adiposity [53] among African Americans relative to other racial groups in the US. Differences in socioeconomic status [140,141], medication adherence [142], and genetics [143] are postulated to be contribute to these disparities, however, they do not fully account for the excess cardiovascular mortality among NHB and other racial groups including NHW.

Stress is an independent risk factor of coronary heart disease [13]. Evidence from the literature suggests that the risk of incident CHD increases by 47% with work-related stress [90], 82% in women who look after a spouse for ≥ 9 hours per week [97], a 2.4 fold increase among

people with moderate or high financial stress [105], and 14% among men who experience discrimination [108]. However, the conclusion is not unequivocal. Some studies reported non-statistically significant associations of some measures of stress and incident CHD such as perceived stress [111,144], perceived racism [110], and job strain [92,145]. Nonetheless, results from a large body of research suggest chronic stress is associated with CVD [13,16,33,146]. Chronic stress is purported to influence the development of CVD risk factors such as hypertension, and promotes the indulgence in behaviors such as smoking and physical activity that subsequently lead to cardiovascular disease[24]. It could also accelerate the progress of age-related diseases such as CHD through alternative pathways by maintaining a chronic state of inflammation and shortening of telomeres [62].

In the United States, most studies assessing the association between stress and CVD typically involve stressors such as perceived discrimination [104,108,111] and neighborhood deprivation[112,113,147]. This approach is driven by a long standing hypothesis suggesting that NHB suffer worse health due to socioeconomic inequalities [141], plausibly leading to greater exposure to multiple stressors across multiple domains compared to other ethnic groups. Therefore, quantifying the role of chronic stress – measured by exposure to stressors across multiple domains- could potentially delineate the CVD disparities between African Americans and other ethnic groups.

Mediation allows the estimation of effects that explain the pathway through which an exposure affects an outcome. These effects are typically decomposed into total effects, direct effects (natural and controlled), and indirect effects (natural). Below are definitions of these effects.

Definition of effects [148–151]

1) Total Effects (TE): This represents the total effect of the exposure on the outcome, accounting for confounders between exposure and outcome and mediator and outcome. It can be decomposed further into natural direct and indirect effects.
2) Natural direct effect (NDE) represents the effect of the exposure on the outcome when the mediator is set to its natural value under the referent exposure value or in the absence of the exposure. The counterfactual notation for NDE is Y_1M_0-Y_0M_0 where M_0 is the value the mediator would assume in the absence of the exposure.
3) Natural indirect effect (NIE) represents the effect of the exposure on the outcome when the mediator is set to its natural value in the presence and absence of the exposure. Using the equation Y_1M_1-Y_1M_0 where X=1, we see to have a non-zero value, the exposure would have to change the mediator which then impacts the outcome. Thus, the NIE can simply be described as the effect of the exposure on the outcome due to the mediator. The relationship between the TE, NDE and NIE can be expressed by the equation: TE=NDE+NIE on the additive scale or TE=NDE*NIE on the multiplicative scale.
4) Controlled direct effect (CDE) represents the effect of the exposure on the outcome at a fixed level of the mediator, assuming exposure-outcome and mediator-outcome confounding. Counterfactual notations of CDE are Y_1xm-Y_1x^*m where M=m represents a fixed level of M, x=exposed and x^*=unexposed.
5) The proportion mediated quantifies the magnitude of total effects attributable to the indirect effect of the exposure. It is derived using natural direct and indirect effect. Proportion mediated is believed to be more suitable to answer etiological questions or describing the mechanisms an exposure affects an outcome.

6) The proportion eliminated quantifies the effect that will be eliminated if an intervention intervenes on the mediator. It is derived using controlled effects and total effects. This has more policy implications.

The estimation of these effects require the fulfilment of strong causal inference assumptions. These assumptions include

1) No confounding between exposure and outcome
2) No confounding between exposure and mediator
3) No confounding between mediator and outcome
4) Confounders of mediator and outcome should not be affected by the exposure

These assumptions cannot be tested using data, rather they rely on existing knowledge between the exposure and the outcome.

To estimate total effects, the first and second assumptions must be met. Assumptions 1 – 4 are required for estimating natural direct and indirect effects. Given race as the exposure in this study, assumption 4 will be difficult to meet because it's highly conceivable that most variables associated with stress are associated with race. However, this fourth assumption is not required to estimate controlled direct effects of race on CHD.

Other studies have examined the mediating effect of chronic stress in cardiovascular disease. However, these studies were cross-sectional and conducted in women only [107,152,153] or assessed the association treating stress using the difference method [154] with limited control of confounding.

Thus, the objective of this chapter is threefold: (1) to calculate the relative risk of CHD between non-Hispanic Blacks to non-Hispanic Whites, (2) the racial disparity that would remain

and (3) proportion eliminated after setting cumulative chronic stress to its baseline referent values will be calculated using data from an ongoing, biracial prospective cohort study of men and women.

METHODS

Recruitment and Data source

This project will utilize extant data from the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. Heart SCORE is an ongoing prospective cohort and community-based study of 2,000 community dwelling individuals aged 45-75 years in Pittsburgh, Pennsylvania. The primary goal of the Heart SCORE study is to identify mechanisms that explain population differences in cardiovascular disease outcomes for the purposes of eliminating racial disparities in CVD [115].

Recruitment of study participants began in 2003 and involved direct mailing of questionnaires, community and physician referrals, print and electronic media, and public service announcements. Data were collected at baseline and during annual follow-up visits on characteristics such as sociodemographic, clinical markers, medical history, psychosocial risk factors (depression, hostility, anger, anxiety, perceived stress, ongoing life events, and optimism) and social network. To be eligible, participants had to be aged 45 – 74 years, have a life expectancy >5 years and be available for baseline or annual follow-up visits. Pregnancy or HIV status was not an exclusion criteria.

Study population and Eligibility Criteria

To answer the research questions associated with this study, individuals from the Heart SCORE study cohort with a prior history of coronary heart disease such as myocardial infarction (MI) and coronary revascularization were excluded (n=88). Furthermore, individuals who did not report on race, identified with a race other than Black or White, or identified as Hispanics were excluded (n=177). Thus, the final sample size comprised of 1,735 Non-Hispanic Whites and Blacks.

Study Outcomes

The primary study outcome was a composite outcome of incident major adverse cardiac events (MACE) defined as the first occurrence of myocardial infarction, cardiac death or any revascularization such as percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG). The timing and occurrence of the events were confirmed through medical records and adjudicated by medical experts. Participants were censored if they didn't experience the study outcome as of April 2019. The time to censoring or experiencing an event was calculated as the difference between date of enrollment and censoring or event and expressed in days.

Study Variables

To quantify the role of chronic stress in the Black-White disparity in CVD outcomes, the following variables were utilized:

Exposure

The main exposure in this study was self-reported race. Participants were asked to select one race regardless of their ethnicity. Options followed the five categories of race recommended by the Office of Management and Budget (OMB) including American Indian or Native Alaskan, Asian, Black or African-American, Native Hawaiian or Pacific Islander, White or Other.

Mediators

Two measures of chronic stress were considered as mediators. The first was the cumulative chronic stressor and the perceived stress scale score as determined by the Cohen's PSS-4 due to their modest agreement witnessed in the previous chapter. These two measures were dichotomized to classify individuals as either having low or high stress to facilitate meaningful interpretations with policy implications. Individuals who lacked any chronic stressor as defined by the chronic stress score were classified as having low stress while those with at least one stressor were classified as having high stress. The components of these measures are described in chapters 3 of this dissertation.

Similarly, individuals with a PSS-4 score below 4 were categorized as having low stress while others with a score of 4 or higher were classified as having high stress. Although the initial approach was to model the trajectories of chronic stress over time, the post-hoc analysis showed the group membership at baseline appeared constant over six years of follow-up (figures 2.1 and 2.2). Thus, to obtain a parsimonious model, group classification at baseline was used in the analyses.

Covariates

Information on other study variables were collected to compare characteristics of individuals who self-identify as Black or White. These included sociodemographic factors such as age, sex, race, income, insurance status, employment and educational attainment. Clinical factors such as systolic blood pressure (SBP; mmhg), diastolic blood pressure, high density lipoprotein (HDL; mg/dl), history of stroke, history of hypertension, and history of diabetes were also included. Finally, anthropometric, behavioral, and psychosocial factors like waist circumference, BMI, current smoking status, and depressive symptoms measured using Center for Epidemiological Studies Depression Scale (CES-D).

A depiction of the hypothesized relationship between the exposure and the outcome is shown in figure 2.3.

Statistical Analyses

Univariate and Bivariate Analyses

The characteristics of the study population was summarized using counts and percentages for categorical variables and mean and standard deviation for continuous variables. Differences in the distribution of these characteristics by study groups (NHB and NHW) were tested using Pearson chi-square test and two independent sample t-test for categorical and continuous variables, respectively.

The cumulative incidence was calculated by taking the proportion of incident cases of MACE while the incidence rate was calculated by dividing the incident cases of MACE by the sum of follow-up time and expressed in person-years. These measures of incidence were calculated

among NHB and NHW. Furthermore, the cumulative incidence of MACE was examined by overall CRCS, its individual components, and the dichotomized measure of perceived stress.

Breslow-Day test was conducted to examine heterogeneity of incidence rate between NHB and NHW by age groups (45 – 55, 56 – 65, 66 – 74), sex (male and female) and income (≤$20,000, $20,001 to $80,000, > $80,000). Incidence rate ratios and their 95% confidence intervals were calculated by fitting a model with a Poisson distribution and a log link within each subgroup. Mediation analyses were not conducted in these subgroups.

A pair of subject-level stabilized IP weights were created by fitting two logistic regression models. The first regressed the binary stress variable on race, exposure-outcome confounders, and mediator-outcome confounders. The second, regressed race on exposure-outcome confounders only. These confounders are listed in Figure 2.3. The propensity scores from both models were used to generate weights as follows ($1/P_{stressed}$ and $1/1\text{-}P_{stressed}$ for those stressed and not stressed respectively) and ($1/P_{Black}$ and $1/1\text{-}P_{Black}$ for those self-identified as Black). To improve precision and mitigate occurrence of extreme weights, stabilized weights were produced by replacing the numerators with the mean of the proportion ($\hat{p}$) classified as stressed and Black, respectively ($SW_{stress}=\hat{p}_{stress}/ P_{stressed}$ or $\hat{p}_{stress}/ 1\text{-}P_{stressed}$; $SW_{race}=\hat{p}_{race}/ P_{Black}$ or $\hat{p}_{race} /1\text{-} P_{Black}$). Thus, the final subject-specific IP weights were calculated as $SW_{stress}*SW_{race}$.

Weighted Cox proportional hazard regression models were fit using Proc PHREG with the SW in the Weight statement. Components of the model include race, stress and the interaction term of race and stress. Using the CDE, the proportion eliminated will be calculated using the formula $\frac{Total\ effect-CDE}{Total\ effect-1}$ and expressed as percentages. Two total effects were calculated from models with and without adjustment of CHD risk factors. This approach allows

for the assessment of a direct and indirect effect of stress on CHD. The model without adjustment for risk factors of CHD included the following covariates: age, sex, income, education, insurance status, family history of pre-mature CHD, history of renal disease and depression. The second total effect will be calculated from a model that includes the aforementioned covariates and risk factors of CVD such as: smoking status, waist circumference, physical activity, systolic blood pressure, and total cholesterol.

The total and controlled direct effects were expressed as risk differences (RD) using a ten-year cutoff. Therefore, all subjects who experienced the event after 10 years were assigned a value of 0. Among those without the event, 9.7% and 25% were lost to follow-up before the 5^{th} and 10^{th} year. However, censoring was not accounted for in this analysis. The RD for total effect was calculated by fitting an unweighted linear model with robust standard errors containing race and exposure-outcome confounders as covariates and a binary outcome of CHD. Similarly, a weighted linear model with robust standard errors containing binary indicators for four groups: NHB + ≥1 stressor, NHB + no stressor, NHW + ≥1 stressor, and NHW + no stressor to calculate was fitted to determine the controlled direct effect. The coefficient of NHB + ≥1 stressor indicator represented the controlled direct effect.

A few sensitivity analyses were performed. First, participant's addresses taken at study entry were mapped to the 2013 area deprivation index (ADI) file- which is based off the 5-year summary file between 2009 and 2013-, it was important to assess the potential of misclassification. Although few participants changed addresses during the course of the study (7.7%), it's highly plausible that the neighborhoods would have undergone gentrification. To examine the robustness of the results, the analyses were repeated using a cumulative chronic stress score that was calculated without neighborhood deprivation.

Furthermore, the primary analysis was repeated using imputed data on missing variables. About 33% of the study population had missing data on at least one covariate and these data were imputed using multiple imputation with fully conditional specification (FCS). Variables used in the imputation model are outlined in table A12.2 located in the appendix. Five imputation datasets were created and were analyzed individually with the appropriate statistical method. Final results were subsequently pooled using SAS Proc MIANALYZE to get total effect, controlled direct effect and subsequent percentage eliminated.

RESULTS

A total of 1,735 were included in the final analyses. Of these, 42.7% were non-Hispanic Blacks, had an average age of 58.9 (7.5) years and a male to female ratio of 1:2. There were statistically significant differences in the distribution of sociodemographic, psychosocial, and cardiovascular risk factors between NHB and NHW. Compared to NHW, NHB were more likely to be younger (~ 43% vs 32% aged 45 – 55; p<.0001), females (70.6% vs 63.6%; p=0.002), have an annual income of $20,000 or less (28.3% vs 10.1%; p<.0001), receive Medicaid/other public insurance (5.3% vs 1.2%; p<.0001), and less likely to have a Bachelor's/Advanced degree (58.6% vs 35.4%; p<.0001). NHB were more likely to report fair/poor quality of life (19.9% vs 5.7%; p<.0001), higher perceived stress (4.6 vs 4.1; p<.0001), two or more chronic stressors (32.4% vs 5.8%; p<.0001) and higher depressive symptoms (7.5 vs 6.4; p<.0001). Similar statistically significant differences were seen across cardiovascular risk factors including smoking (13.7% vs 8.3%; p<.0001), waist-circumference (99cm vs 93cm;), higher BMI (32.1 vs 28.6; p<.0001), systolic blood pressure (141 vs 133; p<.0001) and lower

total cholesterol (206 vs 210; p=0.04). Only 6.8% of NHB were classified as having ideal cardiovascular health compare to 19% of NHW (p<.0001). See table A9.

The overall crude cumulative incidence of MACE was 5% (87/1735) and was similar among Blacks (5.13%) and Whites (4.93%). Among those with the event, revascularization procedures accounted for most of the cases (52.3%) followed by cardiac related death (27.6%) and myocardial infarction (19.5%). NHB were more likely to have MI (21 vs 18.4%) and cardiac-related death (34.2% vs 22.5%) but were less likely to undergo revascularization (44.7% vs 59.2%); however, these differences were not statistically significant (p=0.37). Parity was observed in the crude incidence rates. NHB had an incidence rate of 1.39 per 100,000 persons per day (5.07/1000/year) compared to 1.31 per 100,000 persons per day (4.8/1000/year). However, 21.1% and 20.4% of cases occurred within the first year of study entry for NHB and NHW, respectively.

Stratified analyses showed significant heterogeneity between groups by age at enrollment (p= 0.038). Although the incidence of CHD increased with age for both groups, the incidence was larger for younger NHB compared to NHW (3.97 vs 0.92 per 1,000 persons per year) aged 45 – 55 years and resulted in an incidence rate ratio (IRR) of 4.29 (95% C.I.=1.22, 15.06). The incidence rate per 1,000 persons per year was higher for NHW between ages 56 to 65 years (4.26 vs 5.52; IRR=0.77 , 95% C.I=0.38, 1.53) and marginally higher among NHB for people aged 66 to 74 (9.29 vs 8.67; IRR=0.99; 95% C.I.=0.49, 1.99). Although no statistical heterogeneity was observed by sex and income groups, stratified analyses revealed higher IRR for NHB compared to NHW in both sexes and lower income groups. See table A10 and A11.

Distribution of Study Outcome by Measures of Stress

Unexpectedly, the event rate in stress groups were low. No events were reported among people classified as having caregiving stress. Overall, the event rate of any CHD was lower among those classified as having job stress (3% vs 5.2%; p=0.65), financial stress (3.5% vs 5.2%; p=0.41), and living in a deprived neighborhood (4.5% vs 5.4%; p=0.69) compared to people without these stressors. However, the event rate of CHD was higher among those who perceived discrimination compared to those who did not perceive discrimination in their daily lives (5.8% vs 4.7%; p=0.012).

Using perceived stress, the event rate of CHD was higher in the group classified as having high stress (5.7 vs 4.3%). Assessing each CHD component, the event rate of cardiac-related mortality was highest among those who perceived discrimination (2.8 vs 0.8%) and perceived stress (1.9 vs 0.8%) compared to participants without these experiences. Similarly, the rate of myocardial infarction was highest among those with job-related stress (1.5 vs 0.9%) and perceived stress (1.3 vs 0.5%). See table A11.2.

To observe the racial disparity in CHD, two sets of analyses were conducted according to the measure of stress by utilizing a cumulative measure of chronic stressors (CRCS) and a binary indicator of perceived stress as measures of chronic stress, respectively.

Examining the mediating role of chronic stress in the racial disparity of CHD

The first measure of chronic stress considered is the CRCS. The total effect showed NHB had higher risk of CHD compared to Whites (HR=1.79, 95% C.I=1.05, 3.05) among 1,443 individuals with complete data. After conducting a weighted cox regression model using stabilized weights, the racial disparity in CHD that remained by setting the population stress-free

was 1.45 (95% C.I.=0.7, 3.01). This suggests that 43% of the racial disparity will be eliminated if NHB were not exposed to chronic stressors. To tease out the specific stressors that contributed the most to this difference, perceived discrimination or unfair treatment, difficulty at place of work, and taking care of a family member would reduce the racial disparity in CHD by 52.8%, 34.2% and 10.3%, respectively. However, the estimates for job strain and caregiving stress are unstable due to sparse data or near zero cells. Examination of financial stress and neighborhood deprivation showed an increase in racial disparity and resulted in negative percentage eliminated values. See table A12.1.

The total effect was recalculated by controlling for CV risk factors such as smoking, physical inactivity, systolic BP, total cholesterol, and waist circumference, in the model. Results showed that the racial disparity in incident CHD attenuated to 1.44 (95% CI=0.83, 2.51) among 1,378 individuals. The racial disparity that remained and not due to interaction nor mediation after setting the population stress-free was 1.39 (95% CI=0.64, 3.00). This suggests that the racial disparity between Blacks with similar sociodemographic and clinical characteristics as Whites would be eliminated by 12.6% if NHB and NHW were not exposed to chronic stressors. Examining individual stressors, setting the entire population to not experience discrimination or unfair treatment completely eliminated the disparity. This was closely followed by experiencing difficulty at work (97.7%) and caregiving stress (76.7%), however, the estimates may be unstable for reasons noted above. Similar to the model previously described, the racial disparity increased when examining financial stress and neighborhood deprivation. See table A12.1.

The second set of the analyses were conducted using perceived stress as a measure of chronic stress, revealed the controlled direct effect of race on incident CHD as 1.94 (95% CI= 0.82, 4.56) adjusting for a similar group of variables that yielded a total effect of 1.79. However,

when adjusting for the group of variables that yielded a total effect of race on CHD as 1.44, the controlled direct effect of race on incident CHD increased to 1.75 (0.72, 4.29). This was suggestive of some interaction between race and perceived stress and four mutually exclusive groups were created to further examine this effect. These groups included: NHB reporting high perception of stress, NHB reporting low perception of stress, NHW reporting high perception of stress, and NHW reporting low perception of stress. Compared to Whites with low perception of stress, Whites with high perceived stress had a 2.4-fold increase in risk of CHD (95% C.I.= 1.13, 5.22). This result was similar for NHB with high perceived stress (HR=2.5, 95% C.I= 1.12, 5.58)

Assessment using Risk Differences

In a model adjusting for variables other than CV related factors, 18.7 more cases of CHD occurred among NHB compared to NHW per 1,000 individuals. The disparity that remained if everyone was not stressed was 12.5 per 1,000 suggesting 6.2/1,000 Black people would be prevented from having CHD if the population was not stressed. Adjusting for CV related factors, the racial disparity attenuated to 10.6/1,000 and the CDE was 9.6/1,000. This suggests that 1/1,000 Black people would be prevented from having CHD if the population was not stressed. As observed with marginal hazard ratios, the CDE for perceived stress increased. See table A13.

Sensitivity Analyses

The analyses were repeated using CRCS without a measure of neighborhood deprivation. In the model that yielded a total effect of 1.79, the controlled direct effect of race on CHD was 1.59 (95% CI= 0.83, 3.07) resulting in a proportion eliminated of 24.8%. Furthermore, in the model that yielded a total effect of 1.44, the CDE increased to 1.48 (0.73, 2.98) and disparity was not attenuated.

The analyses were repeated using imputed data. The total effect without controlling for traditional risk factors of CVD using imputed data was a modest 1.25 (95% CI=0.78, 2.02) and the racial disparity that remained after setting the population to low chronic stress was 1.03 (95% CI=0.52, 2.01), representing an 88% reduction in the disparity. Similarly, a 36% and 56% reduction in the racial disparity was observed when perceived discrimination and perceived stress were set to their referent levels (low level of stress) while the disparity widened when neighborhood deprivation, financial stress, and caregiving stress were set to their referent levels. Interestingly, when traditional CVD risk factors were controlled, the total effect was 1.02 (95% CI=0.62, 1.67); suggestive of no racial disparity between NHB and NHW. Thus, mediation analyses were not conducted. See table A14.

DISCUSSION

This study examined the role of two measures of chronic stress in the racial disparity of CHD. Results suggest that a cumulative exposure to multiple stressors may act as a modest mediator and effect modifier in the racial disparity of CHD outcomes. The proportion of the racial disparity eliminated when both racial groups are set to low stress ranged from 12.6% in the presence of existing CVD risk factors to 43% in their absence. In absolute terms, this means that as many as 1 to 6.2 NHBs per 1,000 adults could be prevented from having CHD in the absence of chronic exposure to stress. These effects are modest and suggest exposure to multiple chronic stressors may play a modest role in the persistent disparities between NHB and NHW in CHD outcomes. Perceived stress, the other measure of chronic stress, did not explain the disparity between NHBs and NHWs.

The cumulative reported chronic stressors (CRCS) classified individuals based on exposure to any one of the following stressors: financial hardship, job difficulties, caregiving difficulties, living in a deprived neighborhood or perceived discrimination. The racial disparity between NHB and NHW that would be eliminated in the absence of chronic stressors was attenuated when considering a total effect of race on CHD from a model with and without adjustment for cardiovascular risk factors. This provides two pieces of evidence. First, chronic stress contributes to the racial disparity in CHD outcomes. Second, this contribution is independent of the indirect effect of stress on CHD that may occur through promoting behaviors associated with or leading to CHD risk factors like hypertension or adiposity and favors a plausible, albeit minimal, direct effect of stress on CHD. The second measure of chronic stress was measured using the Cohen's perceived stress scale (PSS). When CV risk factors were controlled in the model, the proportion of racial disparity eliminated resulted in a negative value because the CDE moved further away from the null. Stratified analyses suggests that individuals who report being stressed and have these risk factors are at increased risk of incident CHD. Therefore, these results are indicative of a dual role of stress- both as a mediator and effect modifier- in the racial disparities of CHD.

The analyses of individual stressors revealed the effect of the CRCS was driven primarily by perceived discrimination. Perceived discrimination eliminated the racial disparity that remained after accounting for SES and established risk factors of CVD. This underscores the importance of perceived discrimination as a potent stressor and a target for intervention. It could be that people who perceive to be discriminated report their actual experiences. This may prevent these individuals from accessing healthcare resources and lead to higher mortality. For example, an analysis conducted on a multiracial sample of women concluded women who report non-

racial discrimination were less likely to access cancer screening services [155]. Therefore it's plausible to conclude that people with these experiences may be less likely to interact with the healthcare system and less likely to benefit from preventive measures. In this study, persons who perceived to be discriminated were more likely to die of cardiac related mortality than those without this experience.

Perceived discrimination is also a known stressor associated with the uptake of unhealthy behaviors and adversely affect mental and physical health. It increases the likelihood of anxiety/depression [156], elevated cortisol [157], subclinical CVD [153], incident blood pressure [158], inflammation [159], and heart rate variability [160]. However, the association with cardiovascular endpoints in prospective studies remain equivocal [111]. A study conducted in multiracial cohort, authors reported everyday discrimination increased the risk of CVD among men and the association did not vary by race. It also did not matter if the discrimination was attributed to race [108]. Therefore, future research should examine how perceived discrimination differentially impacts NHBs in relation to CHD; especially in the uptake of cardioprotective behaviors and access to preventive healthcare services may provide some insights.

Various findings from this study are consistent with other studies. First, the distribution of characteristics by race is consistent with the literature that show NHB report higher exposure to chronic stressors and have worse cardiovascular health. Second, the overall incidence of coronary heart disease was marginally higher among NHB compared to NHW but significantly higher among younger NHB compared to NHW and attenuated in older groups [30,139]. Last, NHB experienced higher incidence of MI, were less likely to undergo revascularization procedures and experience higher cardiac-related mortality.

Few studies examining the mediating influence of chronic stress in explaining racial disparities in cardiovascular diseases have focused on cardiovascular health and resulted in mixed findings. In the cross-sectional study by Burrough et al, the authors concluded approximately 13% of racial disparities in ideal cardiovascular health were explained by a cumulative measure of psychosocial stress in an age-adjusted model[107]. Although the study population was restricted to older women, the true effect is may likely be larger given the methodological approach deployed by the authors [151]. Also, a 2018 study by Whitaker et al found that 27% of racial disparities in CV health behaviors were due to psychological risk factors, however, only 7% and 1% were linked specifically to racial discrimination and the chronic burden scale according to age- and sex-adjusted models [140]. The authors concluded that most of the racial disparities in CVH were related to differences in socioeconomic status rather than psychosocial factors. Despite adjusting for measures of SES, the effect size from this study is similar to the effect size reported by Whitaker et al.

Financial stress has previously been related to CHD risk factors and overall health. More recently, a study conducted in NHB showed financial stress increased risk of incident CHD except when depression was included in the model [105]. Therefore, it was unexpected to find the racial disparity widened when financial stress was examined individually especially despite bivariate analysis showing NHB reported more financial stress compared to NHW. This paradoxical finding requires more research.

Another unexpected finding was the increase in racial disparity related with neighborhood deprivation. Despite NHB living in more deprived neighborhoods than NHW, setting all participants to less deprived neighborhoods did not eliminate the racial disparity in CHD. Given established associations between neighborhood socioeconomic conditions and

cardiovascular disease [147], one possible explanation could be misclassification. Participant addresses collected at baseline between 2003 – 2006 were geocoded and linked to the 2013 Area Deprivation Index (ADI). Although the 2013 ADI utilizes the 2009-2013 data from the American Community Survey (ACS), it may not account for gentrification. Thus, a highly deprived neighborhood in 2005 could be classified as a lowly deprived neighborhood in 2010 with gentrification. This was further bolstered by an examination of events by deprivation. It showed participants in highly deprived neighborhoods were less likely to have the event. Sensitivity analyses which excluded neighborhood deprivation from CRCS showed some mediation by chronic stress albeit attenuated.

This study extends the literature by quantifying the contribution of chronic stressors that predispose NHB to worse CVD outcomes by conducting a formal mediation analyses using hard CVD endpoints rather than intermediate outcomes like cardiovascular health. Furthermore, these analyses are based off a prospective cohort study with a good ratio of Blacks to Whites and consists of males and females, although females are overrepresented. Last, CHD was ascertained using objective measures rather than self-report

The study is not without limitations. First, the cumulative incidence was low (5%) and may preclude the ability to calculate CDE and observe statistically significant associations with other effects. This event rate may be due to 68% of participants who were on anti-hypertensive therapy at some point during the study. However, other studies with similar definition of CHD reported similar event rates [35,105]. Second, the results were sensitive to missing data as the total effect from the complete case analysis was significantly higher than the estimate gotten from the imputed analysis. A descriptive analysis on the previously excluded individuals in the complete case analysis indicated a larger event rate of MACE/CHD among Whites compared to Blacks

(8.7 vs 2.1%). Thus, the risk of CHD among Whites might have been underestimated in the complete case analysis.

Although structural equation modelling (SEM) has the capability of determining direct and indirect effects, it usually requires assumptions of linearity and normality of all variables involved. Also, it assumes no confounding of all variables involved in the modeling, making these assumptions extremely susceptible to violations in biomedical research. SEM also lacks the ability to handle interactions. The difficulty in satisfying these assumptions and inability to handle interactions make SEM a less appealing alternative [151].

CONCLUSION

In a biracial sample of men and women, measures of chronic stress modestly explained the racial disparities in CHD outcomes and was largely driven by perceived discrimination. Future research should examine the mechanisms through which perceived discrimination negatively impacts NHBs in the context of CHD.

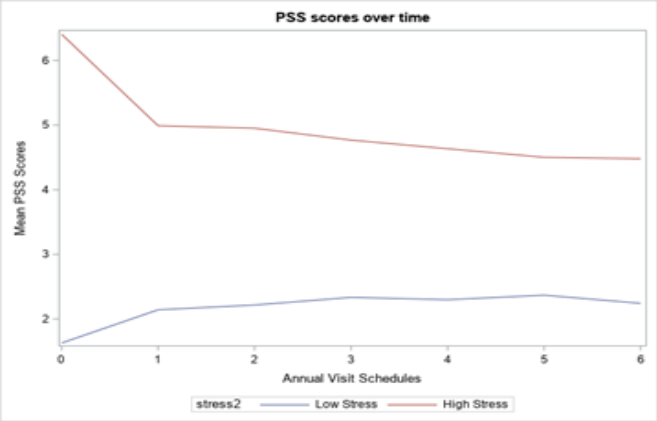

Figure 2.1: Average perceived stress score over six years of follow-up by baseline categorization.

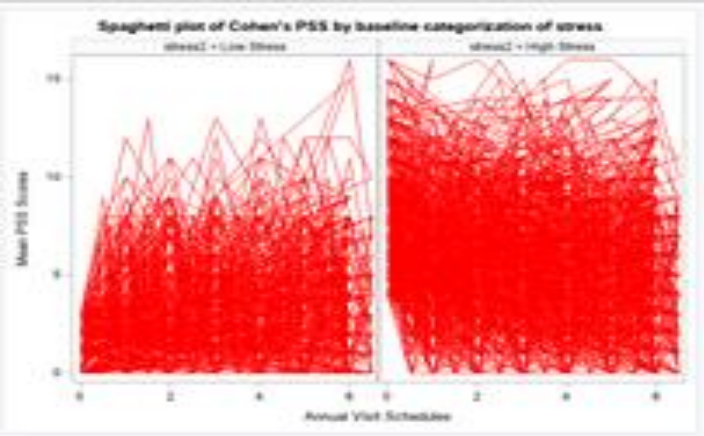

Figure 2.2: Spaghetti plots of perceived stress score over six years of follow-up.

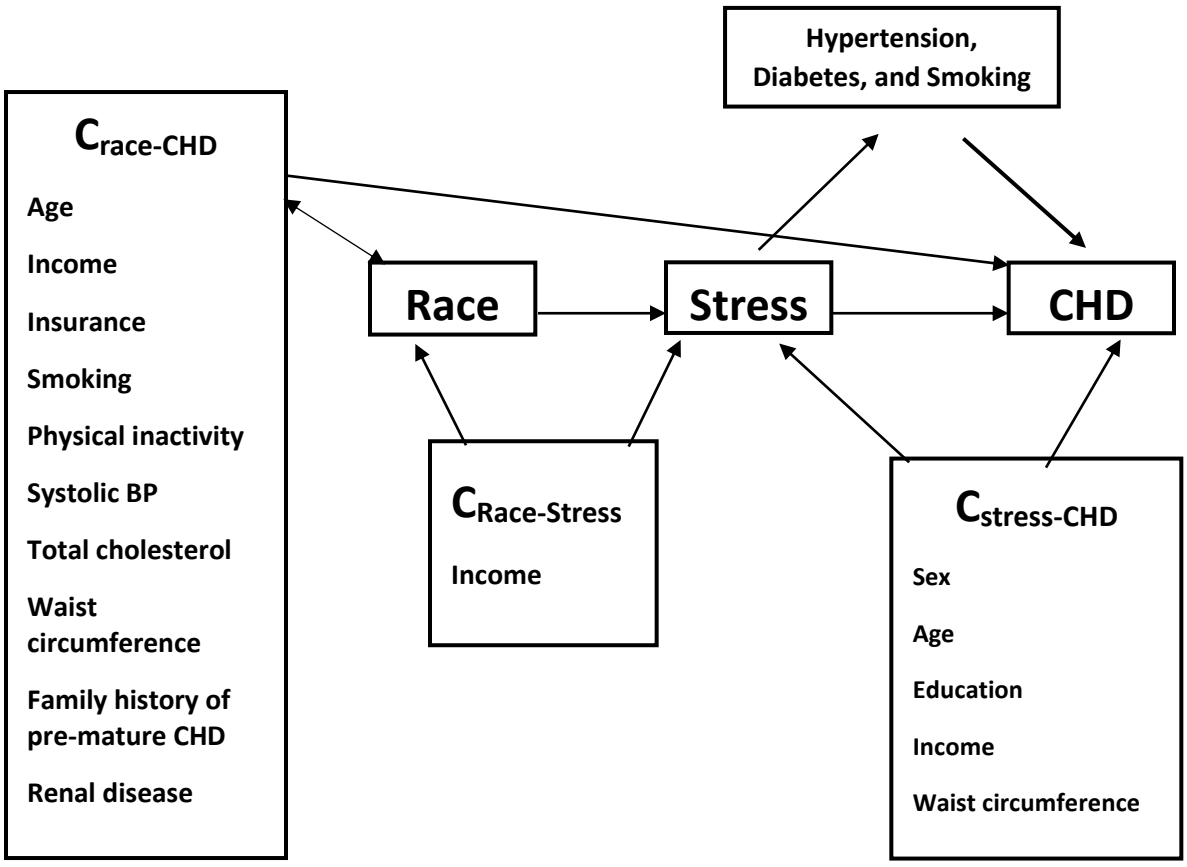

Figure 2.3: Relationship between Race, Stress and Coronary Heart Disease (CHD)

CHAPTER 5

SPECIFIC AIM 3

INTRODUCTION

Psychosocial stress is a risk factor of cardiovascular disease (CVD) [13,25,146,161]. Despite the consistent association of psychological and social stressors with cardiovascular disease (CVD), the precise mechanism through which chronic stress affects CVD remains elusive. Stress is purported to contribute to CVD through cortisol- the primary mediator of the hypothalamic-pituitary adrenal (HPA) axis [162]. However, the measurement of cortisol as a biomarker of chronic stress challenging due to its high binding capacity to plasma proteins and diurnal fluctuations [163,164]. Although hair cortisol presents a good alternative for measuring chronic stress, absence of standardized reporting and susceptibility to damage from chemicals like bleach impede its utility in epidemiological research [164].

Utilization of metabolomic data might facilitate the identification of biomarkers associated with the stress response. Metabolomics is a burgeoning field that permits the study of small molecules involved in biological processes, including chronic diseases such as CVD [165]. Adaptation of an epidemiological-based approach to metabolomic data may uncover metabolites linking chronic stress with CVD and unearth potential targets for prevention strategies. Previous application of metabolomic data to identify mediators of psychological conditions such as post-traumatic stress disorder (PTSD) [166], chronic stress in animals [167,168], depression [169] and

cardiovascular disease [165,170–173] are noted in the literature. However, to the best of my knowledge, no study has examined metabolomic data in relation to chronic stress in humans.

Ideal cardiovascular health (ICH) is a concept established and propagated by the American Heart Association (AHA) as a primordial prevention strategy to improve cardiovascular health at the population level [127]. It is defined as the simultaneous presence of seven health behaviors and factors. These health behaviors include abstinence from smoking within 12 months, ideal body mass index (BMI) of 18.5 -24.9 kg/m^2, physical activity at goal, and consumption of a cardiovascular health-friendly diet. Similarly, the health factors include abstinence from smoking within the previous 12 months, untreated total cholesterol <200 mg/dL, untreated blood pressure <120/<80 mm Hg, and absence of diabetes mellitus[127]. A cumulative score based on meeting each criteria can be calculated and is a prognostic factor for cardiovascular disease [174,175].

Therefore, the objectives of this exploratory analysis are threefold. First, the study will determine if there are differences in the metabolomic profile between individuals exposed to chronic stress. Second, the study will determine if these identified metabolites are associated with ideal cardiovascular health. Last, the identified metabolites will be examined for their association with the development of cardiovascular disease (CVD).

METHODS

Study Design and Data Source

This prospective cohort study uses extant data from the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. Heart SCORE is an ongoing prospective cohort and longitudinal study of 2,000 community dwelling individuals aged 45-75 years in Pittsburgh, Pennsylvania. The recruitment and objective of Heart SCORE study is described in previous chapters.

Study Population

To identify metabolites associated with stress and subsequent CVD, this study included individuals from the Heart SCORE study cohort without prior history of cardiovascular disease. Individuals who did not undergo a metabolomic analysis (n=80), had a history of MI or coronary revascularization (n=88), utilized corticosteroids 48 hours before study entry(n=118), did not identify as non-Hispanic Black or White (n=177), and had missing data on relevant variables (n=157) were excluded from the study. This resulted in a final sample size of 1,380 individuals for analysis.

Definition of Study Outcomes

The primary study outcome was ideal cardiovascular health. It was calculated by initially grouping participants into three groups and assigning a numerical value: ideal (2), intermediate (1), and poor (0) based on their self-reported values on seven factors and behaviors such as BMI, blood pressure, blood glucose, physical activity, diet, smoking, and blood glucose at baseline. Thus, the total ideal cardiovascular health score could range from 0 – 14; with higher scores denoting ideal cardiovascular health. Individuals were subsequently classified into three groups

based on this final score: ideal (assigned a value of 2; cutoff: 10-14), intermediate (assigned a value of 1; cutoff: 5-9) and poor (assigned a value of 0; cutoff: 0-4). The operationalization of the ICH is described in table A13 of the appendix.

The secondary study outcome was a composite outcome of incident major adverse cardiac events (MACE) defined as the first occurrence of myocardial infarction, cardiac death or any revascularization such as percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG). The timing and occurrence of the events were confirmed through medical records and adjudicated by medical experts. Participants were censored if they were lost to follow-up or didn't experience the study outcome as at April 2019. Follow-up time -censoring time or time to experiencing an event- was calculated as the difference in days between date of enrollment and censoring or event.

Definition of Chronic Stress

The study population was classified into high and low chronic stress as previously described in the preceding chapter of this dissertation. In brief, the cumulative reported chronic stress score sums the presence of stressors experienced by each individual. These include perceived discrimination, residing in a highly deprived neighborhood, experiencing ongoing financial difficulty, ongoing job difficulties and ongoing caregiving stress. Furthermore, chronic stress was also measured using the 4-item Cohen perceived stress scale (PSS) and was treated as a continuous variable.

Detection and Quantification of Metabolites

Plasma samples of study participants in the Heart SCORE study were collected at baseline and analyzed using an untargeted ultra-high performance liquid chromatography (UH-PLC) with mass spectrometry. This resulted in the identification of 1,228 metabolites consisting of 893 previously known and 335 unknown metabolites. Metabolites covered a broad array of biological classes including amino acids, carbohydrates, lipids, and xenobiotics. Extraction, identification, and quantification of metabolites was conducted by Metabolon®. Metabolon also assessed platform variability using a set of internal standards in the experimental and process samples. Results from their analysis showed 7% and 10% relative standard deviation (RSD) for internal and endogenous metabolites, respectively.

In this study, the metabolite data were cleaned before analysis and are noteworthy. First, 99 technical duplicates used to assess technical variability were excluded. Second, analysis was restricted to 893 named metabolites. Third, metabolites were excluded from the analysis if they were medications or metabolites of medications and had missing information on >50% of the study population [172,176,177]. These processes resulted in 718 metabolites available for analyses. Metabolites with missing data in the population were imputed by using half the minimum value of the metabolite in the study population [178,179] and all metabolites were subsequently log-transformed to eliminate skewness.

Other study variables

Information on other study variables were collected to account for confounding and assess relationships with identified metabolites. Variables were included based on their identification as established risk factors of cardiovascular disease and association with stress.

These included demographic factors such as age, sex, race, income, and educational attainment. Traditional risk factors of CVD such as systolic blood pressure, low density lipoprotein (*LDL; mg/dl*), current smoking status and history of diabetes were also included. Anthropometric and behavioral factors like waist circumference and depression were included. Measures of inflammation such as interleukin-6 (IL-6), highly sensitive C-reactive protein (hs-CRP), and intercellular adhesion molecules-1 (ICAM-1) were included. Finally, markers of the HPA axis such as serum cortisol, its metabolite cortisone, and dehydroepiandrosterone sulfate (DHEA-S) were included for analysis.

Statistical Analyses

The characteristics of the study population were described using mean, median, frequencies and percentages. Differences between stress groups were tested using Chi-square test of independence for categorical variables and two independent sample t-test or Wilcoxon rank test for continuous variables.

To identify metabolites independently associated with high stress, a two-part approach was taken. First, logistic regression models were created to assess the bivariate association between individual metabolites and chronic stress. The false discovery rate (FDR) was used to control the type I error rate and metabolites that met this threshold were deemed to be associated with stress. For stress measured using the Cohen PSS, simple linear regression was used to examine the association between metabolites and stress measured using PSS. All metabolites were checked for multicollinearity using a variance inflation factor (VIF) of 10 and non-collinear metabolites were included in a logistic regression model that sequentially adjusted for age, sex, socioeconomic factors (education and income), and race using Backward selection procedure.

Adjusted odds ratios and their corresponding 95% confidence intervals of metabolites in the final model were calculated. The metabolites from both models were assessed for their correlations with inflammatory markers and hormones of the HPA-axis before placed in an ordinal logistic regression model controlling for age, sex, and race to observe their relationship with ICH. The proportional odds assumption was assessed to ensure propriety of this modeling approach and ICH was modeled so that odds were cumulated over poor ICH. The final set of metabolites associated with ICH were chosen by performing backward selection procedure at an alpha level of 0.05. This approach was repeated for each component of the ICH. Analyses with total ICH score was further stratified by Race.

Principal component analysis was used to corroborate the results from the above analyses. All metabolites were assessed with PCA and components with an eigen value ≥1 were selected. These components were orthogonally rotated to maximize the variance and ensure zero correlation between components. Principal components (PC) were assessed with the cumulative measure of stress controlling for the FDR and were placed in various adjusted models as described above. To identify the underlying characteristic of each PC, multiple tests of correlations were conducted between each PC and 718 metabolites. Metabolites with a Pearson correlation coefficient of ≥0.4 with the PC were identified and collated.

Before conducting a Cox proportional hazards regression to examine the association between independent metabolites associated with cumulative stress and subsequent MACE, the proportional hazards assumption was tested using Schoenfeld residual plots and by assessing the statistical significance of the coefficient of an interaction term between each metabolite and logarithm of follow-up time. Metabolites that failed to meet this assumption were entered into

the final model as a time-dependent interaction term. The final Cox proportional regression model was created by having MACE as the outcome, the metabolites as predictors, and other study variables: sociodemographic and traditional risk factors of CHD as potential confounders. Results were further stratified by race. Hazard ratios and their 95% confidence intervals were calculated and reported. All analyses were conducted in SAS 9.4 and p-values less than 0.05 were considered statistically significant.

RESULTS

The study population consisted of 1,380 individuals with 63 events of MACE over a median (IQR) follow-up of 12 years (9.1 - 12.3 years), an average age of 58.7 (SD=7.4), 65.4% female and 56.8% white. About 30% of the study population resided in a deprived neighborhood, 46% earned an annual income $40,000 or less and approximately 18% at least had some college education. The mean perceived stress and ICH score were 4.3 (SD=3) and 7.1 (SD=2.2), respectively. See Table A14.

Compared to individuals classified as having low cumulative stress, individuals with high cumulative stress were younger (57.6 vs. 59.9), female (69% vs. 62%), Black (65% vs. 20%), income <$40,000 (57.2% vs 34.5%), current smokers (14.7% vs. 6.4%), had a history of diabetes (12% vs. 6.4%), and less likely to have ideal cardiovascular health (10.4% vs 18.4%). Individuals classified as having high cumulative stress also had higher waist circumference (97.3 cm vs 94.4 cm), higher depression scores (9.2 vs 4.5), and higher systolic blood pressure (138 vs 135). All differences were statistically significant. There were no statistically significant

differences between total cholesterol, low-density lipoproteins (LDL), dietary fat, dietary sodium, serum cortisol, serum cortisone and DHEA-S between the two stress groups.

Metabolic signature of cumulative chronic stressors

Before adjusting for demographic characteristics, 252 metabolites were statistically associated with high cumulative chronic stress after controlling for multiple testing. After eliminating highly collinear metabolites and adjusting for sociodemographic variables, 28 metabolites were associated with high cumulative chronic stress. See Table A15. These metabolites represented amino acids (n=3), lipids (n=13), Cofactors and vitamins (n=4), xenobiotics (n=7), and a partially characterized molecule. Amino acids were involved in histidine, leucine, and creatine metabolism. Others were involved in Vit A and nicotinamide metabolism (cofactors) and xanthine and benzoate metabolism for the xenobiotics. The association between these metabolites and stress was mixed. The lipid metabolites were involved in fatty acid metabolism, bile acid metabolism, androgenic steroids, ceramides, diacylglycerol and phospholipids. androstenediol- a metabolite of DHEA- were less likely to occur in people with high cumulative stress.

The top five metabolites with the highest odds [OR{95% CI}] of high cumulative stress include: tetradecanedioate (C14-DC) [2.28 {1.52-3.42}], glycosyl-N-palmitoyl-sphingosine (d18:1/16:0) [1.93 {1.14-3.28}], retinol (Vitamin A) [1.75 {1.03-2.95}], creatine [1.62 {1.13-2.31}], and 5-dodecenoylcarnitine (C12:1) [1.5 {1.11-2.03}]. Conversely, the top five metabolites with the lowest odds of high cumulative stress include: caproate (6:0) [0.53 {0.39-0.73}], hexadecanedioate (C16-DC), [0.55 {0.35-0.85}], laurate (12:0) [0.56 {0.4-0.78}], 3-

hydroxy-2-ethylpropionate [0.64 {0.41-0.99}], and androstenediol monosulfate [0.68 {0.56-0.84}]. See table A15.

Metabolomic signature of perceived stress

Thirty-nine metabolites were associated with chronic stress measured using the Cohen's perceived stress scale (PSS) from the univariate analysis. After adjusting for sociodemographic variables, eight metabolites were associated with perceived stress. These included lipids (n=4) and one each of amino acids, nucleotide, xenobiotics and metabolite associated with hemoglobin metabolism. Of these metabolites, only two (sphingomyelin (d18:1/18:1, d18:2/18:0) and 2-hydroxybehenate) were positively associated with perceived stress. See table A15.

The metabolites associated with CRCS and PSS are summarized in figure 3.1 at the super pathway level.

Principal component analysis

Results from the principal component analysis initially identified 128 principal components from the 718 metabolites in the study. Of these, three were associated with high cumulative chronic stress. Fourteen metabolites were strongly correlated with these components and shared a similar pattern with those identified above. They were largely related to lipid metabolites (n=12) involved in varying pathways, one xenobiotic and one amino acid. The lipid metabolites were either monohydroxy fatty acids or involved in Phosphatidylethanolamine (PE) and Phosphatidylcholine (PC) pathways. The amino acid is involved in histidine metabolism while the xenobiotic is a chemical.

The analysis was repeated using perceived stress. Four factors were identified and were strongly correlated with 12 metabolites representing lipids (n=8), amino acids (n=3) and xenobiotics (n=1). All lipid metabolites were involved in Phosphatidylinositol metabolism or monohydroxy fatty acids. Like with cumulative chronic stress, the amino acid is involved in histidine metabolism while the xenobiotic is a chemical. See table A16.

Metabolomic signature of chronic stress and markers of inflammation and the HPA axis

Overall, there were weak positive and negative correlations between markers of inflammation (Hs-CRP, IL-6, and sICAM) and HPA axis (serum cortisol, cortisone, and DHEA-S) with all 36 metabolites. However, 2-hydroxybehenate and glycosyl-N-palmitoyl-sphingosine (d18:1/16:0) were positively correlated with all markers while docosahexaenoylcholine was negatively correlated with all markers. See figure 3.2.

Metabolomic signature of stress and ICH

Table A17 summarizes the results of the relationship between metabolites associated with high cumulative stress and poor ICH. Among the 36 metabolites associated with cumulative chronic stress and perceived stress, 14 were associated with ICH. Of these, 10 were initially associated with cumulative chronic stress while four were associated with perceived stress. The association between the stress-related metabolites and ICH were in opposite directions in 6 of the 14 metabolites, i.e., these metabolites were positively (or negatively) associated with stress but negatively (or positively) associated with poor ICH. Stress-related metabolites associated with increased odds of poor ICH [OR (95% C.I.)] include **Lipids**: sphingomyelin (d18:1/18:1, d18:2/18:0) [3.34 (1.88-5.96)] , sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)

[1.84 (1.31-2.58)], 2-hydroxybehenate [1.65 (1.15-2.37)], laurate (12:0) [1.49 (1.10-2.02)], oleoyl-oleoyl-glycerol (18:1/18:1) [1.24 (1.09-1.41)], and androstenediol (3α, 17α) monosulfate [1.22 (1.01-1.47)]; **Vitamin A metabolites**: retinol [2.39 (1.50-3.81)] and **a uric acid metabolite**: 3,7-dimethylurate [1.20 (1.05-1.37)]. The stress-related metabolites associated with reduced odds of poor ICH include a **Vitamin A metabolite**: beta-cryptoxanthin [0.41 (0.34-0.48)]; **Lipids**: tetradecanedioate (C14-DC) [0.70 (0.57-0.87)] and caproate (6:0) [0.67 (0.50-0.90)]; **Nucleotide:** 3-aminoisobutyrate (BAIBA) [0.57 (0.44-0.74)]; and **Xanthines**: methyl-4-hydroxybenzoate sulfate [0.88 (0.80-0.96)], and 7-methylxanthine [0.83 (0.73-0.96)]. Stratified analyses revealed the associations were stronger in Whites than Blacks for sphingomyelin (d18:1/18:1, d18:2/18:0), BAIBA, laurate, androstenediol, retinol, and beta-cryptoxanthin.

Further examination of the association between the 14 metabolites and each component of ICH showed beta-cryptoxanthin was associated with a reduced odds of all components except ideal cholesterol. Similarly, sphingomyelin (d18:1/18:1, d18:2/18:0) and retinol were associated with five and four components of ICH, respectively. All but two metabolites were associated with BMI while only three metabolites were associated with blood pressure. See table A18.

Metabolomic signature of stress and Incident MACE

Of the 14 metabolites associated with ICH, only beta-cryptoxanthin was associated with MACE. Beta-cryptoxanthin, a Vit A metabolite, was associated with a 25% reduction in the risk of MACE after controlling for sociodemographic factors, systolic blood pressure, total cholesterol, and current smoking status [HR(95% C.I): 0.75 (0.59 - 0.97)]. There was a significant interaction effect with race. Upon stratification, the effect was stronger in Whites [0.6 (0.45 – 0.79)] and

was not associated with a non-statistically significant slight increase in risk of MACE in Blacks [1.01 (0.63 – 1.62)]. See table A19.

DISCUSSION

This metabolomic study examined the association between metabolites associated with chronic stress and cardiovascular disease over a median follow-up of 12 years. The results identified a combined 36 metabolites - involved in lipid, vitamin A, xanthine, amino acid metabolism-associated with two measures of chronic stress. Of these, 14 metabolites were associated with ideal cardiovascular health, however, only beta-cryptoxanthin was associated with a 25% reduction in the risk of incident MACE in the study. This effect was largely driven by the association in Non-Hispanic Whites.

The results contribute to the literature by identifying a metabolomic signature associated with cumulative chronic and perceived stress. Although further studies are needed to confirm the findings, the identification of these metabolites suggest the potential of identifying additional biomarkers associated with stress beyond cortisol and could delineate the mechanism through which stress impacts health. Cortisol is rightly heralded as the biomarker of stress in numerous studies, however difficulties in measurement and diurnal fluctuations have impaired its utility in assessing health outcomes in epidemiological studies. Given the weak correlation of identified metabolites with cortisol in these analyses, these metabolites could represent an alternative pathways of stress response. However, the results need to be validated in a separate cohort.

Human and animal studies have linked some identified metabolites in this study with increased morbidity such as stroke [180] [tetradecanedioate], uremic toxin [181] [N1-Methyl-2-pyridone-5-carboxamide], prostate cancer [182] [retinol] and depression [169,183] [retinol]. They have also been identified to be inversely associated with cholesterol [184] [BAIBA] , obesity [185] [beta-cryptoxanthin], cardiovascular disease [186] [beta-cryptoxanthin], anticancer activity [187] [umbelliferone and beta-cryptoxanthin], and anti-depressant activity [188] [creatine].

Chronic stress has a deleterious effect on numerous health outcomes and if these metabolites are truly representative of chronic stress, then it might explain the association of these metabolites with various morbidities. However, because the analyses did not control for these conditions, the observed association between these stress-related metabolites and ICH may be an artifact. Thus, these metabolites need to be validated in an independent cohort. Furthermore, an overwhelming number of metabolites were associated with the lipids biological pathway. This is not inconceivable given the lipolytic effect associated with the stress response. Stress is also implicated with unhealthy diet and individuals might indulge in high fat diet. Other biological pathways implicated include cofactors and vitamins, xenobiotics and require further research.

Of the 36 metabolites associated with measures of chronic stress, 3,7-dimethylurate (a uric acid metabolite), retinol (vitamin A), 2-hydroxybehenate, and sphingomyelin (d18:1/18:1, d18:2/18:0) were associated with an increase in stress and odds of poor cardiovascular health. The consistency in the direction of the associations may suggest these metabolites may be mediators in the effect of stress on CVD or increase the susceptibility of CHD in individuals with chronic stress. Although previous studies have not shown increase risk of vit A on the cardiovascular health, studies have shown an association with 2-hydroxybehenate and

sphingomyelin. Further analyses suggested this effect was driven by its association with high BMI. The positive association between Vit A and stress and cardiovascular health is unexpected given Vit A's anti-inflammatory effect. Conversely, beta-cryptoxanthin (vit A), 7-methylxanthine, 3-aminoisobutyrate, and caproate were negatively associated with stress and poor cardiovascular health. In this study, beta-cryptoxanthin was consistently associated with reduced odds of stress, reduced odds of poor ICH health, reduced odds of all components of ICH except cholesterol and reduced odds of incident MACE. Stratified analyses suggest this advantage exists only among Whites and not Blacks and might further explain the marginal favorable outcomes in CVD observed in Whites compared to Blacks.

This is the first study to examine the metabolomic implications associated with chronic stress in humans. The findings of this work provide preliminary evidence of other biomarkers associated with the stress response. The study is further strengthened by the large sample size and diverse demographic profile of the study population, thereby improving the generalizability of results.

There are few noteworthy limitations. First, the classification of individuals into high and low stress groups was based on an unvalidated scale with a modest internal consistency (α=0.55). This could potentially lead to misclassification of individuals in exposure groups. However, the potential bias was mitigated by classifying people as high stress only if they chose the highest response on each question. Furthermore, the PSS is a validated scale and a similar pattern of metabolites were identified. While the overall sample size was sufficient to determine metabolomic differences in stress [189], there were few events of MACE. The lack of sufficient events might have limited the statistical power to find additional statistical associations between

stress-related metabolites and incident CVD. Although this study examined the association of metabolites measured at a single timepoint with incident MACE, preliminary evidence suggests levels of metabolites remain stable over two years [190]. Last, as with many biomarkers, metabolites are involved in a myriad of complex biological processes and may lack some specificity to a particular phenotype or disease. Thus, caution is advised when interpreting these results.

CONCLUSION

This study examined the metabolomic profile of individuals with chronic stress and its subsequent association with cardiovascular health and incident cardiovascular disease. Although one stress-related metabolites was found to be associated with incident cardiovascular disease, this study contributes to the literature by identifying 36 metabolites associated with stress. Future studies in a larger, ethnically diverse population are needed to confirm the findings from this study.

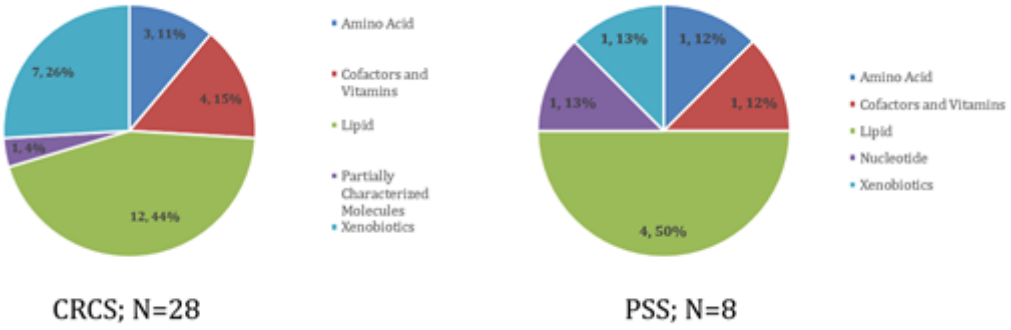

Figure 3.1: Super Pathway of Metabolites Associated with Chronic Stress

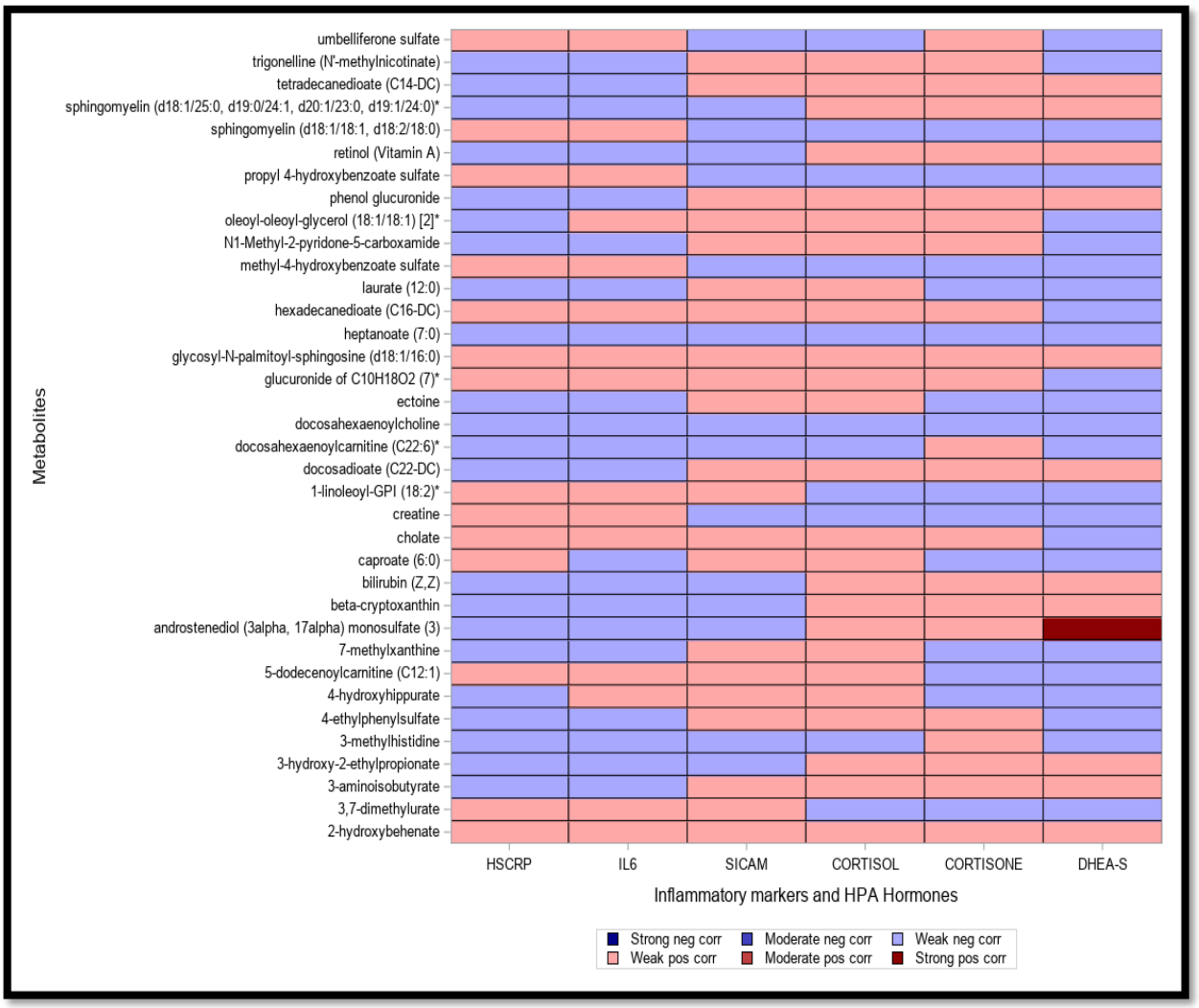

Figure 3.2: Pearson Correlations between Metabolites and Markers of Inflammation and HPA Axis

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

This dissertation quantified the impact of chronic stress in explaining the racial disparities observed in cardiovascular diseases (CVD) using data from an ongoing, prospective cohort study conducted in Pittsburgh, Pennsylvania.

First, chronic stress, defined as a count of exposure to multiple stressors was determined to provide incremental predictive benefit beyond the Framingham risk score (FRS) and pooled cohort equation (PCE) among low-income Blacks. In the second manuscript, the racial disparity that would remain if non-Hispanic Blacks (NHB) had similar exposure to chronic stress as non-Hispanic Whites (NHW) was calculated and subsequently used to determine the racial disparity that would be eliminated. Results showed that the racial disparity would be eliminated by 12.6% if NHBs had similar to exposure to chronic stress as NHW. However, this result was sensitive to missing data and both findings would need to be validated in a separate, larger cohort. Nonetheless, the results underscore the importance of chronic stress in identifying people at risk of future CVD.

The last manuscript leveraged metabolomic data to identify 36 metabolites associated with chronic stress. These metabolites were largely lipids and 14 were associated with ideal cardiovascular health (ICH). Of the 14 metabolites associated with ICH, only one metabolite (beta-cryptoxanthin) was associated with incident major adverse cardiac event (MACE)- a measure of CVD, although the association was present exclusively among non-Hispanic Whites.

This results suggest other biomarkers could serve as potential markers of chronic stress and explain the mechanisms stress influences CVD.

A challenge encountered in stress research is the feasibility and effectiveness of interventions focused on addressing chronic stress due to the pervasiveness of stress [191]. It is conceivable that all humans undergo stress at some point in their lifetime. However, the focus of this research is chronic stress and the most significant findings in this dissertation were attributable to exposure to multiple chronic stressors rather than the perception of stress. These chronic stressors are amenable to intervention. For example, since the 2015 Institute of Medicine report on the screening, and collection of unmet social needs such as financial assistance [192] was published, a growing body of literature have assessed the effectiveness of identifying and addressing patient's unmet social needs [134,193,194]. Furthermore, emerging research suggests meditation exercises may have a possible benefit on alleviating stress and cardiovascular disease [195,196] while improving resilience might prove to be effective from the deleterious effect of chronic stress [197]. Finally, the results from this dissertation reaffirms the current strategy of early intervention on traditional risk factors of CVD such as hypertension and diabetes in reducing the racial disparities in CVD. While numerous research strive to identify other reasons for these persistent racial disparities in CVD outcomes, it is imperative that ongoing efforts to intervene on traditional risk factors are equally encouraged.

The findings from the dissertation provided some insights into areas of further research. For example, when measuring chronic stress, it may be relevant to classify individuals who are exposed to chronic stressors and perceive their situation as stressful. This approach might minimize the discordance between exposure to chronic stress and perception of stress from resilient factors. Another recommendation for further research is the determination of drivers of

racial disparities of CVD in younger age groups (45 – 55), particularly the interplay of chronic stress and epigenetic markers. A model that captures early exposure to chronic stress may uncover the disproportionate high rate of CVD among NHB relative to NHW in this age group. For example, racial discrimination experienced in multiple settings was associated with decreased telomere length- an indicator of biological aging- in a small sample of African Americans with an average age of 39 [198]. Further application of epigenetics might further explain the increased racial disparity in this subgroup and provide intervention targets to narrow the racial gap in CVD outcomes.

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APPENDIX A: SUPPLEMENTAL TABLES AND IRB APPROVAL

Table A1: Distribution of Characteristics of Study Population by Measures of Chronic Stress

		Cumulative Chronic Stress			Allostatic Load			Perceived Stress		
Subject Characteristics	**Total N (%) (n=1861)**	**Low N (%) (n=908)**	**Moderate N (%) (n=632)**	**High N (%) (n=321)**	**Low N (%) (n=724)**	**Moderate N (%) (n=763)**	**High N (%) (n=374)**	**Low N (%) (n=805)**	**Moderate N (%) (n=451)**	**High N (%) (n=584)**
Age (years) , Mean (SD)	58.8 (7.5)	60.1 (7.4)	58.2 (7.4)	56.6 (7.2)	57.8 (7.3)	59.8 (7.7)	58.8 (6.9)	60.0 (7.3)	59.1 (7.6)	57.1 (7.1)
Waist-hip ratio , Mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Waist circumference (cm) , Mean (SD)	96.0 (15.2)	94.4 (14.3)	96.8 (16.1)	98.9 (15.5)	87.7 (12.0)	98.5 (13.9)	107 (14.7)	96.0 (15.1)	95.6 (15.6)	96.4 (15.1)
Body mass index , Mean (SD)	30.1 (6.4)	29.1 (5.9)	30.8 (6.6)	31.8 (6.6)	26.8 (4.2)	31.0 (6.3)	34.7 (6.7)	30.2 (6.4)	29.7 (6.1)	30.5 (6.6)
Total cholesterol , Mean (SD)	208 (41.6)	209 (39.3)	207 (42.9)	207 (45.4)	205 (39.7)	208 (42.0)	212 (44.1)	207 (41.5)	208 (42.4)	209 (41.5)
HDL cholesterol , Mean (SD)	55.7 (16.3)	55.4 (16.2)	56.1 (16.6)	55.6 (16.1)	58.5 (16.3)	55.3 (16.1)	50.9 (15.6)	55.4 (16.9)	56.1 (16.3)	55.9 (15.8)
Systolic BP , Mean (SD)	136 (19.9)	135 (18.4)	137 (21.2)	139 (20.8)	126 (14.1)	139 (18.6)	153 (18.8)	137 (19.4)	136 (19.4)	136 (20.8)
CESD score (>=16 items completed) , Mean (SD)	6.9 (8.0)	4.6 (5.3)	7.9 (8.3)	11.5 (10.8)	6.4 (7.3)	7.0 (8.4)	7.7 (8.4)	3.1 (4.1)	5.6 (5.1)	13.1 (10.0)
LOT score (optimism) , Mean (SD)	17.2 (4.0)	18.0 (3.5)	16.8 (4.1)	15.6 (4.5)	17.3 (4.0)	17.1 (4.0)	17.1 (3.9)	19.0 (3.1)	17.3 (3.1)	14.5 (4.2)
Race										
White	1051 (56.5)	727 (80.1)	263 (41.6)	61 (19.0)	488 (67.4)	425 (55.7)	138 (36.9)	474 (58.9)	269 (59.6)	300 (51.4)
Black	810 (43.5)	181 (19.9)	369 (58.4)	260 (81.0)	236 (32.6)	338 (44.3)	236 (63.1)	331 (41.1)	182 (40.4)	284 (48.6)

Table A1 (Continued)

Gender										
Male	615 (33.0)	329 (36.2)	189 (29.9)	97 (30.2)	277 (38.3)	239 (31.3)	99 (26.5)	286 (35.5)	155 (34.4)	169 (28.9)
Female	1246 (67.0)	579 (63.8)	443 (70.1)	224 (69.8)	447 (61.7)	524 (68.7)	275 (73.5)	519 (64.5)	296 (65.6)	415 (71.1)
Age (years)										
45 to 55	685 (36.8)	272 (30.0)	250 (39.6)	163 (50.8)	290 (40.1)	255 (33.4)	140 (37.4)	245 (30.4)	160 (35.5)	269 (46.1)
56 to 65	772 (41.5)	401 (44.2)	263 (41.6)	108 (33.6)	309 (42.7)	298 (39.1)	165 (44.1)	349 (43.4)	183 (40.6)	232 (39.7)
66 to 74	404 (21.7)	235 (25.9)	119 (18.8)	50 (15.6)	125 (17.3)	210 (27.5)	69 (18.4)	211 (26.2)	108 (23.9)	83 (14.2)
Annual income										
Less than $10,000	101 (6.0)	22 (2.7)	44 (7.7)	35 (11.7)	34 (5.2)	33 (4.8)	34 (10.0)	28 (3.8)	21 (5.0)	51 (9.8)
$10K to < $20K	205 (12.2)	67 (8.2)	80 (14.1)	58 (19.4)	60 (9.2)	87 (12.5)	58 (17.1)	80 (11.0)	55 (13.1)	69 (13.2)
$20K to < $40K	475 (28.2)	202 (24.7)	165 (29.0)	108 (36.1)	163 (25.0)	212 (30.5)	100 (29.5)	191 (26.2)	113 (26.8)	166 (31.9)
$40K to < $80K	564 (33.5)	276 (33.8)	209 (36.7)	79 (26.4)	226 (34.7)	238 (34.3)	100 (29.5)	248 (34.0)	138 (32.8)	173 (33.2)
$80,000 or more	340 (20.2)	250 (30.6)	71 (12.5)	19 (6.4)	169 (25.9)	124 (17.9)	47 (13.9)	182 (25.0)	94 (22.3)	62 (11.9)
How hard pay for basics										
Very hard	77 (4.2)	0 (0.0)	25 (4.0)	52 (16.3)	25 (3.5)	33 (4.4)	19 (5.1)	17 (2.1)	14 (3.1)	45 (7.8)
Somewhat hard	308 (16.7)	79 (8.8)	137 (22.0)	92 (28.8)	87 (12.1)	127 (16.8)	94 (25.3)	92 (11.5)	75 (16.7)	136 (23.4)
Not very hard at all	1460 (79.1)	823 (91.2)	462 (74.0)	175 (54.9)	605 (84.4)	597 (78.9)	258 (69.5)	688 (86.3)	361 (80.2)	399 (68.8)
Primary insurance										
Medicare	266 (14.3)	144 (15.9)	78 (12.4)	44 (13.7)	76 (10.5)	133 (17.5)	57 (15.2)	121 (15.1)	68 (15.1)	74 (12.7)
Medicaid/Other Public	54 (2.9)	11(1.2)	24 (3.8)	19 (5.9)	17 (2.4)	22 (2.9)	15 (4)	19 (2.4)	14 (3.1)	19 (3.3)
Private	1416 (76.3)	723 (79.9)	477 (75.8)	216 (67.3)	589 (81.7)	558 (73.4)	269 (71.9)	621 (77.4)	341 (75.6)	443 (76.0)
None/Self-pay	119 (6.4)	27 (3.0)	50 (7.9)	42 (13.1)	39 (5.4)	47 (6.2)	33 (8.8)	41 (5.1)	28 (6.2)	47 (8.1)
Education										

Table A1 (Continued)

Less than high school	36 (1.9)	10 (1.1)	24 (3.8)	2 (0.6)	9 (1.3)	17 (2.2)	10 (2.7)	15 (1.9)	6 (1.3)	15 (2.6)
High school diploma	314 (16.9)	140 (15.5)	108 (17.2)	66 (20.6)	100 (13.9)	140 (18.3)	74 (19.8)	121 (15.0)	72 (16.0)	118 (20.3)
Some college	599 (32.3)	227 (25.1)	228 (36.2)	144 (44.9)	207 (28.8)	250 (32.8)	142 (38.1)	241 (30.0)	134 (29.7)	217 (37.3)
Bachelor's degree	418 (22.5)	221 (24.4)	137 (21.8)	60 (18.7)	176 (24.4)	169 (22.1)	73 (19.6)	178 (22.1)	112 (24.8)	124 (21.3)
Advanced degree	489 (26.3)	308 (34.0)	132 (21.0)	49 (15.3)	228 (31.7)	187 (24.5)	74 (19.8)	249 (31.0)	127 (28.2)	108 (18.6)
Work status past 3 mo.										
Full-time	846 (45.6)	399 (44.1)	290 (46.1)	157 (48.9)	350 (48.5)	329 (43.2)	167 (44.8)	361 (44.9)	210 (46.6)	265 (45.6)
Part-time	274 (14.8)	139 (15.4)	99 (15.7)	36 (11.2)	118 (16.4)	108 (14.2)	48 (12.9)	117 (14.6)	71 (15.7)	83 (14.3)
Retired	497 (26.8)	292 (32.3)	143 (22.7)	62 (19.3)	169 (23.4)	232 (30.5)	96 (25.7)	263 (32.7)	120 (26.6)	112 (19.3)
Other	238 (12.8)	75 (8.3)	97 (15.4)	66 (20.6)	84 (11.7)	92 (12.1)	62 (16.6)	63 (7.8)	50 (11.1)	121 (20.8)
QOL: Health										
Excellent	303 (16.4)	208 (23.0)	74 (11.9)	21 (6.5)	168 (23.4)	107 (14.1)	28 (7.5)	190 (23.8)	62 (13.7)	50 (8.6)
Very good	673 (36.4)	388 (42.9)	204 (32.7)	81 (25.2)	296 (41.2)	278 (36.7)	99 (26.5)	319 (40.0)	182 (40.4)	162 (27.8)
Good	657 (35.5)	262 (29.0)	258 (41.3)	137 (42.7)	210 (29.2)	281 (37.1)	166 (44.5)	245 (30.7)	158 (35.0)	250 (43.0)
Fair	195 (10.5)	43 (4.8)	80 (12.8)	72 (22.4)	44 (6.1)	84 (11.1)	67 (18.0)	39 (4.9)	45 (10.0)	108 (18.6)
Poor	21 (1.1)	3 (0.3)	8 (1.3)	10 (3.1)	1 (0.1)	7 (0.9)	13 (3.5)	5 (0.6)	4 (0.9)	12 (2.1)
Current smoker										
No	1656 (89.2)	845 (93.3)	546 (86.8)	265 (82.6)	645 (89.3)	677 (89.0)	334 (89.5)	737 (91.7)	399 (88.7)	502 (86.3)
Yes	200 (10.8)	61 (6.7)	83 (13.2)	56 (17.4)	77 (10.7)	84 (11.0)	39 (10.5)	67 (8.3)	51 (11.3)	80 (13.7)
Framingham risk strata										
Low risk	1048 (57.2)	534 (59.6)	344 (55.5)	170 (53.8)	523 (73.0)	408 (54.4)	117 (32.0)	427 (54.1)	266 (59.5)	340 (59.1)
Intermediate risk	442 (24.1)	230 (25.7)	144 (23.2)	68 (21.5)	129 (18.0)	205 (27.3)	108 (29.5)	207 (26.2)	100 (22.4)	130 (22.6)
High risk	342 (18.7)	132 (14.7)	132 (21.3)	78 (24.7)	64 (8.9)	137 (18.3)	141 (38.5)	155 (19.6)	81 (18.1)	105 (18.3)

Table A1 (Continued)

Hx hypertension										
No	1097 (59.0)	591 (65.2)	346 (54.8)	160 (50.0)	564 (78.0)	412 (54.1)	121 (32.4)	465 (57.9)	279 (61.9)	340 (58.2)
Yes	761 (41.0)	316 (34.8)	285 (45.2)	160 (50.0)	159 (22.0)	350 (45.9)	252 (67.6)	338 (42.1)	172 (38.1)	244 (41.8)
ICH: BMI										
Poor	785 (42.6)	307 (34.1)	308 (49.4)	170 (53.1)	133 (18.5)	370 (48.9)	282 (76.4)	347 (43.6)	164 (36.7)	269 (46.5)
Intermediate	699 (37.9)	380 (42.3)	204 (32.7)	115 (35.9)	330 (46.0)	299 (39.6)	70 (19.0)	288 (36.2)	199 (44.5)	202 (34.9)
Ideal	358 (19.4)	212 (23.6)	111 (17.8)	35 (10.9)	254 (35.4)	87 (11.5)	17 (4.6)	161 (20.2)	84 (18.8)	108 (18.7)
ICH: Smoking										
Poor	188 (10.2)	48 (5.3)	80 (12.8)	60 (18.8)	73 (10.2)	77 (10.2)	38 (10.3)	60 (7.5)	49 (10.9)	76 (13.1)
Intermediate	772 (41.8)	361 (40.1)	267 (42.7)	144 (45.0)	273 (38.0)	345 (45.6)	154 (41.6)	348 (43.4)	188 (41.8)	232 (39.9)
Ideal	886 (48.0)	491 (54.6)	279 (44.6)	116 (36.3)	373 (51.9)	335 (44.3)	178 (48.1)	393 (49.1)	213 (47.3)	274 (47.1)
ICH: Physical Activity										
Poor	746 (40.5)	312 (34.7)	270 (43.3)	164 (51.4)	218 (30.5)	327 (43.3)	201 (54.2)	277 (34.6)	178 (39.8)	290 (50.0)
Intermediate	979 (53.2)	528 (58.8)	323 (51.8)	128 (40.1)	441 (61.8)	389 (51.5)	149 (40.2)	460 (57.4)	245 (54.8)	265 (45.7)
Ideal	115 (6.3)	58 (6.5)	30 (4.8)	27 (8.5)	55 (7.7)	39 (5.2)	21 (5.7)	64 (8.0)	24 (5.4)	25 (4.3)
ICH: Nutrition										
Poor	546 (29.8)	201 (22.3)	212 (34.5)	133 (41.8)	178 (25.0)	239 (31.9)	129 (34.8)	204 (25.7)	120 (26.6)	220 (38.0)
Intermediate	767 (41.8)	403 (44.8)	252 (41.0)	112 (35.2)	317 (44.5)	298 (39.8)	152 (41.0)	335 (42.1)	206 (45.7)	223 (38.5)
Ideal	520 (28.4)	296 (32.9)	151 (24.6)	73 (23.0)	218 (30.6)	212 (28.3)	90 (24.3)	256 (32.2)	125 (27.7)	136 (23.5)
ICH: Cholesterol										
Poor	451 (24.3)	223 (24.6)	157 (25.0)	71 (22.1)	169 (23.4)	193 (25.4)	89 (23.8)	193 (24.1)	120 (26.6)	134 (22.9)
Intermediate	946 (50.9)	498 (54.9)	289 (45.9)	159 (49.5)	363 (50.3)	396 (52.0)	187 (50.0)	422 (52.6)	219 (48.6)	296 (50.7)
Ideal	460 (24.8)	186 (20.5)	183 (29.1)	91 (28.3)	190 (26.3)	172 (22.6)	98 (26.2)	187 (23.3)	112 (24.8)	154 (26.4)

Table A1 (Continued)

ICH: Blood pressure										
Poor	842 (45.3)	379 (41.8)	304 (48.1)	159 (49.5)	114 (15.8)	407 (53.3)	321 (85.8)	380 (47.2)	190 (42.1)	270 (46.2)
Intermediate	738 (39.7)	385 (42.4)	235 (37.2)	118 (36.8)	405 (56.0)	286 (37.5)	47 (12.6)	314 (39.0)	199 (44.1)	211 (36.1)
Ideal	280 (15.1)	143 (15.8)	93 (14.7)	44 (13.7)	204 (28.2)	70 (9.2)	6 (1.6)	111 (13.8)	62 (13.7)	103 (17.6)
ICH: Blood Glucose										
Poor	136 (7.4)	51 (5.6)	51 (8.2)	34 (10.7)	7 (1.0)	39 (5.2)	90 (24.1)	63 (7.9)	25 (5.6)	46 (7.9)
Intermediate	514 (27.8)	264 (29.2)	155 (24.8)	95 (29.9)	167 (23.3)	236 (31.2)	111 (29.8)	237 (29.7)	113 (25.2)	161 (27.8)
Ideal	1196 (64.8)	589 (65.2)	418 (67.0)	189 (59.4)	543 (75.7)	481 (63.6)	172 (46.1)	497 (62.4)	311 (69.3)	373 (64.3)
ICH score overall										
Poor	219 (11.8)	71 (7.9)	76 (12.1)	72 (22.4)	16 (2.2)	97 (12.8)	106 (28.4)	86 (10.7)	46 (10.2)	86 (14.7)
Intermediate	1372 (74.1)	672 (74.3)	476 (76.0)	224 (69.8)	501 (69.6)	610 (80.5)	261 (70.0)	597 (74.3)	338 (74.9)	430 (73.6)
Ideal	260 (14.0)	161 (17.8)	74 (11.8)	25 (7.8)	203 (28.2)	51 (6.7)	6 (1.6)	120 (14.9)	67 (14.9)	68 (11.6)

ICH: Ideal cardiovascular health
Cumulative chronic stress: All p-values were less than 0.05 except for waist-hip ratio, total cholesterol, HDL, and ICH blood pressure
Allostatic load: All p-values were less than 0.05 except for total cholesterol, optimism, smoking, ICH smoking, and ICH cholesterol
Perceived stress score: All p-values were less than 0.05 except for waist-hip ratio, BMI, total cholesterol, HDL cholesterol, systolic blood pressure, Health insurance, Framingham Risk strata, History of Hypertension, ICH cholesterol, Blood Glucose, and overall ICH.

Table A2: Internal Reliability of Standardized Instruments in the Study Population

Instrument	Alpha	Sample size	Number of items
Cohen's Perceived Stress Scale (PSS-4)	0.786	1834	4
Everyday Discrimination Scale	0.869	1780	10
Ongoing life events (OLE)	0.671	1624	9
Life Orientation Test (Optimism; LOT)	0.787	1812	5
Center for Epidemiologic Studies-Depression (CES-D)	0.92	1784	20

Table A3: Derivation and Cronbach Alpha of Allostatic Load

Parameters	Domain	Sex-Specific	Clinically relevant cutoff	Males	Females
Highly sensitive C-Reactive Protein (mg/L)	Inflammatory	N	3		
Interleukin-6 (IL-6) (pg/ml)	Inflammatory	N	1.8		
Fasting Blood Glucose (mg/dl)	Metabolic	N	126		
Waist-Hip Ratio (cm)	Metabolic	Y		0.95	0.8
Serum Creatinine (mg/dl)	Metabolic	Y		1.2	1.1
Urine Albumin (mg/day)	Metabolic	N	30		
Systolic Blood Pressure (mmhg)	Cardiovascular	N	140		
Diastolic Blood Pressure (mmHg)	Cardiovascular	N	90		
Triglycerides (mg/dl)	Cardiovascular	N	200		
Very Low Density Lipoprotein (mg/dl)	Cardiovascular	N	30		
Cronbach Alpha					0.6

Table A4: Correlations of Components of the Cumulative Reported Chronic Stress Measure (CRCS)

Domain	Measurement	Validated Instrument	Identified as being stressed	Financial Strain 2	Perceived Discrimination	Job Strain	Caregiving Stress	Social Isolation	Neighborhood stress	
Financial strain 1	Ongoing financial difficulty	N	"Yes, very upsetting"	0.43	0.27	0.32	0.1	0.03	0.24	
Financial strain 2	Difficulty to pay for basics needs such as food, housing, medical needs and heating?	N	"Very Hard"		0.2	0.07	0.03	0.08	0.25	
Perceived discrimination	Perceived discrimination score	Y	Individuals with total score>=11			0.23	0.12	-0.001	0.24	
Job strain	Ongoing difficulties at work	N	"Yes, very upsetting"				0.07	-0.04	0.01	
Caregiving stress	Helping at least one sick, limited or frail family member or friend on a regular basis	N	"Yes, very upsetting"					-0.11	0.03	
Social Isolation**	Cohen's Social Network Scale	Y	Individuals with 4 or less interpersonal interactions							
Neighborhood Stress	Area Deprivation Index	Y	Individuals residing in the top 20% highly disadvantaged Neighborhoods in Pennsylvania					0.03		
Alpha without social isolation										0.55
Alpha Reliability with social isolation										0.49

** Social isolation was excluded due to negative impact on overall reliability

Table A5.1: Agreement Statistics between Measures of Chronic Stress

Measure of Chronic Stress	Weighted Kappa*	95% C.I.	Spearman Correlations**
Allostatic Load vs PSS-4	0.016	(-0.02, 0.052)	0.03
Allostatic Load vs Cumulative Chronic Stress score	0.11	(0.07, 0.14)	0.16
PSS-4 vs Cumulative Chronic Stress score	0.2	(0.17, 0.24)	0.30

* All measures of chronic stress are categorized into low, moderate, and high.
** Measures of chronic stress were assessed using their raw, uncategorized scores
PSS-4: Four-item Cohen's Perceived Stress Scale

Table A5.2: Pearson Correlations with Measures of Chronic Stress against Optimism and Depressive symptoms

Measure of Chronic Stress**	Depressive Symptoms	P-value	Optimism	P-value
Allostatic Load	0.06	0.006	-0.011	0.63
Cumulative Reported Chronic Stress	0.32	<.0001	-0.22	<.0001
PSS-4	0.53	<.0001	-0.49	<.0001

PSS-4: Four-item Cohen's Perceived Stress Scale (PSS-4)
**All measures of chronic stress are categorized into low, moderate, and high.
N=1,800 for all pair-wise correlations

Table A6: Incremental Predictive Performance of Measures of Chronic Stress

Model Parameters	Sample Size	"-2LLR"	AIC	C-statistic	Difference in 2LLR	Test of Difference	C-statistic Bootstrapping
FRS	1825	1158.061	1160.061	0.7665			
CRCS score (categorical)	1825	1204.47	1208.47	0.5231			
AL	1825	1201.252	1203.252	0.578			
Cohen PSS-4 (categorical)	1825	1204.031	1208.031	0.5363			
FRS + CRCS Score categorical)	1825	1154.645	1160.645	0.7562	3.416	0.181227886	
FRS + AL	1825	1157.84	1161.84	0.7697	0.221	0.638279023	
FRS + PSS-4 (categorical)	1825	1155.993	1161.993	0.757	2.068	0.355581785	
PCE	1825	1162.007	1164.007	0.7619			
PCE+CRCS Score(categorical)	1825	1159.298	1165.298	0.7542	2.709	0.2580763	
PCE + AL	1825	1161.79	1167.79	0.7647	0.217	0.641335337	
PCE + PSS-4 (categorical)	1825	1160.078	1166.078	0.7529	1.929	0.381173739	
FRS (Low Income Blacks)	205	111.097	113.097	0.6349			0.624
FRS (High Income Blacks)	523	259.117	261.117	0.7441			
FRS+CRCS Low Income Blacks	205	106.152	112.152	0.7184	4.945	0.084373661	0.636
FRS+CRCS Black High Income Blacks	523	258.707	264.707	0.7285	0.41	0.814647316	
FRS (Low Income Whites)	93	23.283	25.283	0.8775			0.8789
FRS (High Income Whites)	837	413.05	415.05	0.7994			
FRS+CRCS White Low income	93	22.704	122.861	0.8307	0.579	0.748637793	
FRS+CRCS High Income Whites	837	406.883	412.883	0.7855	6.167	0.04579868	
FRS+PSS-4 Low Income Whites	93	15.617	21.617	0.9249			0.8445
PCE (LIB)	205	112.036	114.036	0.6247	301.014		
PCE+CCS Score(LIB)	205	106.71	112.71	0.703	5.326	0.069738692	

CRCS: Cumulative Reported Chronic Stressors; PSS: Perceived Stress Score
FRS: Framingham risk score ; PCE: Pooled Cohort Equation risk score
-2LLR: Negative two log-likelihood ratio test; AIC: Akaike information criterion

Table A7.1: Net Reclassification Statistics Among Low-income Blacks using Framingham Risk Score (FRS) and Chronic Stress

	Among low-income Blacks without the event (n=194)				Among low-income Blacks with the event (n=11)			
	FRS + CRCS							
FRS only	0 – <5%	5 – <10%	10 – <20%	>20%	0 – <5%	5 – <10%	10 – <20%	>20%
0 – <5%	87	60	0	0	1	6	0	0
5 – <10%	19	15	7	0	1	1	1	0
10 – <20%	2	0	3	0	0	0	1	0
>20%	0	0	0	1	0	0	0	0
Net reclassification	-0.23				0.455			

CRCS: Cumulative Reported Chronic Stressors

Table A7.2: Net Reclassification Statistics Among Low-income Blacks using Pooled Cohort Equation (PCE) Risk Score and Chronic Stress

	Among low-income Blacks without the event (n=194)				Among low-income Blacks with the event (n=11)			
	PCE + CRCS							
PCE only	0 – <5%	5 – <10%	10 – <20%	>20%	0 – <5%	5 – <10%	10 – <20%	>20%
0 – <5%	79	58	0	0	1	6	0	0
5 – <10%	26	19	8	0	1	2	1	0
10 – <20%	1	0	3	0	0	0	0	0
>20%	0	0	0	0	0	0	0	0
Net reclassification	-0.20				0.545			

CRCS: Cumulative Reported Chronic Stressors

Table A7.3: Net Reclassification Statistics Among High-Income Whites using Framingham Risk Score (FRS) and Chronic Stress

	Among high-income Whites without the event (n=801)				Among high-income Whites with the event (n=36)			
	FRS + PSS-4							
FRS only	0 – <5%	5 – <10%	10 – <20%	>20%	0 – <5%	5 – <10%	10 – <20%	>20%
0 – <5%	681	17	0	0	15	2	0	0
5 – <10%	9	60	12	0	2	12	1	0
10 – <20%	2	0	13	6	0	0	3	0
>20%	0	0	0	1	1	0	0	0
Net reclassification	-0.03				0			

PSS-4: Perceived Stress Score

Formula for Net Reclassification

NRI event = P(up|event)−P(down|event)

NRI non-event= P(down|nonevent)−P(up|nonevent)

Table A8.1: Cox Proportional Hazards Regression of Measures of Chronic Stress with FRS against MACE

Variables	**Model 1***	**Model 2**	**Model 3**	**Model 4**
Number of events (events/total)	89/1825	89/1825	89/1825	89/1825
Cumulative Incidence	4.88	4.88	4.88	4.88
Framingham Risk Score	1.05 (1.04,1.06)	1.05 (1.04, 1.06)	1.05 (1.04,1.06)	1.05 (1.04,1.06)
Categorized Chronic Stress				
Low	Referent	Referent	Referent	Referent
Moderate	1.07 (0.68,1.68)	0.94 (0.59, 1.48)	NA	NA
High	0.73 (0.38,1.39)	0.56 (0.28, 1.09)	NA	NA
Categorized Allostatic Load				
Low	Referent	Referent	Referent	Referent
Moderate	1.53 (0.93,2.53)	NA	1.09 (0.65,1.82)	NA
High	1.73 (0.98,3.06)	NA	0.82 (0.44,1.53)	NA
Three level PSS-4 Score				
Low	Referent	Referent	Referent	Referent
Moderate	1.43 (0.86,2.38)	NA	NA	1.46 (0.88,2.44)
High	1.18 (0.71,1.95)	NA	NA	1.19 (0.72,1.99)

*Model 1 is the unadjusted model for all variables. NA: Variable was not in the model

Table A8.2: Cox Proportional Hazards Regression of CRCS with FRS against MACE among Low Income Blacks (n=205)

	Hazard Ratio (95% C.I.)	
Variables	**Model 1***	**Model 2**
Number of events (events/total)	11/205	11/205
Cumulative Incidence	5.37	5.37
Framingham Risk Score	1.03 (1.0, 1.07)	1.02 (0.99,1.06)
Categorized Chronic Stress Score		
Low	Referent	Referent
Moderate	3.41 (0.43, 26.9)	3.06 (0.38,24.53)
High	0.5 (0.03, 7.84)	0.50 (0.03,8.03)
C-Statistic	0.6349, 0.6754	0.7184
Negative 2 Log Likelihood Ratio	111.097, 107.462	106.152

*Model 1 is the unadjusted model for both variables

Table A8.3: Cox Proportional Hazards Regression of Measures of Chronic Stress and FRS against MACE defined using Stroke

	Hazard Ratio (95% C.I.)			
Variables	**Model 1***	**Model 2**	**Model 3**	**Model 4**
Number of events (events/total)	135/1825	135/1825	135/1825	135/1825
Cumulative Incidence	7.4	7.4	7.4	7.4
Framingham Risk Score	1.05 (1.04,1.06)	1.05 (1.04,1.06)	1.05 (1.04,1.06)	1.05 (1.04,1.06)
Categorized Chronic Stress Score				
Low	Referent	Referent	Referent	Referent
Moderate	0.93 (0.64,1.35)	0.93 (0.64,1.35)	NA	NA
High	0.67 (0.40,1.13)	0.67 (0.40,1.13)	NA	NA
Three level PSS-4 Score				
Low	Referent	Referent	Referent	Referent
Moderate	1.35 (0.90,2.04)	NA	NA	1.44 (0.96,2.17)
High	1.05 (0.70,1.58)	NA	NA	1.17 (0.78,1.75)
Categorized Allostatic Load				
Low	Referent	Referent	Referent	Referent
Moderate	1.83 (1.22,2.77)	NA	1.35 (0.89,2.06)	NA
High	2.05 (1.29,3.28)	NA	1.12 (0.68,1.84)	NA

*Model 1 is the unadjusted model for both variables. NA: Variable was not in the model

Table A8.4: Cox Proportional Hazards Regression CRCS with FRS against MACE defined using Stroke among Low Income Blacks (n=205)

	Hazard Ratio (95% C.I.)	
Variables	**Model 1***	**Model 2**
Number of events (events/total)	18/205	18/205
Percentage of events	8.78	8.78
Framingham Risk Score	1.03 (1, 1.06)	1.02 (0.99,1.05)
Categorized Chronic Stress Score		
Low	Referent	Referent
Moderate	1.79 (0.51, 6.23)	1.62 (0.46,5.71)
High	0.16 (0.02, 1.56)	0.17 (0.02,1.59)
C-Statistic	0.6443, 0.6859	0.7318
Negative 2 Log Likelihood Ratio	181.636, 174.637	172.641

*Model 1 is the unadjusted model for both variables

Table A9: Distribution of Baseline Characteristics by Race

Subject Characteristics	Total N (%) (n=1735)	Non-Hispanic Whites N (%) (n=994)	Non-Hispanic Blacks N (%) (n=741)	P-value
Age (years) , Mean (SD)	58.9 (7.5)	59.6 (7.4)	58.1 (7.5)	0
Waist-hip ratio , Mean (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.76
Waist circumference (cm) , Mean (SD)	95.9 (15.2)	93.6 (14.2)	99.0 (15.9)	0
Body mass index , Mean (SD)	30.1 (6.4)	28.6 (5.8)	32.1 (6.5)	0
Total cholesterol , Mean (SD)	208 (41.6)	210 (40.9)	206 (42.3)	0.04
HDL cholesterol , Mean (SD)	55.8 (16.3)	55.1 (16.1)	56.6 (16.6)	0.06
Systolic BP , Mean (SD)	136 (19.9)	133 (18.8)	141 (20.4)	0
Diastolic BP , Mean (SD)	80.8 (10.2)	79.1 (9.9)	83.0 (10.2)	0
Cohen stress score , Mean (SD)	4.3 (3.0)	4.1 (2.9)	4.6 (3.2)	0
Cumulative chronic stress score without social isolation , Mean (SD)	0.5 (0.7)	0.3 (0.6)	0.7 (0.7)	0
CESD score (>=16 items completed) , Mean (SD)	6.9 (7.9)	6.4 (7.4)	7.5 (8.5)	0
LOT score (optimism) , Mean (SD)	17.1 (4.0)	17.1 (4.1)	17.2 (4.0)	0.45
Gender				0
Male	580 (33.4)	362 (36.4)	218 (29.4)	
Female	1155 (66.6)	632 (63.6)	523 (70.6)	

Table A9 (Continued)

Age (years)				0
45 to 55	631 (36.4)	314 (31.6)	317 (42.8)	
56 to 65	720 (41.5)	443 (44.6)	277 (37.4)	
66 to 74	384 (22.1)	237 (23.8)	147 (19.8)	
Annual income				0
Less than $10,000	95 (6.1)	29 (3.3)	66 (9.7)	
$10K to < $20K	187 (11.9)	61 (6.8)	126 (18.6)	
$20K to < $40K	447 (28.5)	225 (25.3)	222 (32.7)	
$40K to < $80K	521 (33.2)	307 (34.5)	214 (31.5)	
$80,000 or more	320 (20.4)	269 (30.2)	51 (7.5)	
Financial stress				0
No	1558 (90.0)	921 (92.8)	637 (86.1)	
Yes	174 (10.0)	71 (7.2)	103 (13.9)	
Discrimination-related stress				0
No	1193 (70.3)	794 (80.9)	399 (55.7)	
Yes	504 (29.7)	187 (19.1)	317 (44.3)	
Caregiving stress				0.09
No	1630 (96.8)	943 (97.4)	687 (95.9)	

Table A9 (Continued)

Yes	54 (3.2)	25 (2.6)	29 (4.1)	
Job-related strain				0.13
No	1638 (96.0)	947 (96.6)	691 (95.2)	
Yes	68 (4.0)	33 (3.4)	35 (4.8)	
Primary insurance				0
Medicare	246 (14.2)	138 (13.9)	108 (14.6)	
Medicaid/Other public	51 (2.9)	12 (1.2)	39 (5.3)	
Private	1326 (76.7)	808 (81.5)	518 (70.2)	
None/self-pay	106 (6.1)	33 (3.3)	73 (9.9)	
Education				0
Less than HS	34 (2.0)	12 (1.2)	22 (3.0)	
HS diploma	293 (16.9)	154 (15.5)	139 (18.8)	
Some college	561 (32.4)	244 (24.6)	317 (42.8)	
Bachelor's degree	390 (22.5)	251 (25.3)	139 (18.8)	
Advanced degree	453 (26.2)	330 (33.3)	123 (16.6)	
Work status past 3 mo.				0
Full-time	783 (45.3)	432 (43.6)	351 (47.5)	

Table A9 (Continued)

Part-time	254 (14.7)	178 (18.0)	76 (10.3)	
Retired	472 (27.3)	273 (27.6)	199 (26.9)	
Other	220 (12.7)	107 (10.8)	113 (15.3)	
QOL: Health				0
Excellent	284 (16.5)	209 (21.2)	75 (10.2)	
Very good	632 (36.6)	430 (43.5)	202 (27.4)	
Good	606 (35.1)	293 (29.7)	313 (42.5)	
Fair	186 (10.8)	51 (5.2)	135 (18.3)	
Poor	17 (1.0)	5 (0.5)	12 (1.6)	
Current smoker				0
No	1548 (89.4)	910 (91.7)	638 (86.3)	
Yes	183 (10.6)	82 (8.3)	101 (13.7)	
Framingham risk strata at baseline				0
Low risk	976 (57.1)	606 (61.7)	370 (51.0)	
Intermediate risk	416 (24.4)	242 (24.6)	174 (24.0)	
High risk	316 (18.5)	134 (13.6)	182 (25.1)	
History of hypertension				0
No	1021 (58.9)	692 (69.8)	329 (44.5)	
Yes	711 (41.1)	300 (30.2)	411 (55.5)	

Table A10: Stratified Analyses of Demographic Characteristics Comparing Incidence of MACE in Non-Hispanic Blacks to Non-Hispanic Whites

	Black				White					
	Events/N	**Events**	**PY**	**IR**	**Events/N**	**Events**	**PY**	**IR**	**IRR (95% C.I.)**	**P-value**
Overall	38/741	38	7495	5.07	49/994	49	10226	4.79	1.04 (0.68, 1.59)	0.855
Age										
45 to 55	13/317	13	3276.2	3.97	3/314	3	3275.6	0.92	4.29 (1.22, 15.06)	0.023
56 to 65	12/277	12	2820.2	4.26	25/443	25	4527.6	5.52	0.77 (0.38, 1.53)	0.452
66 to 74	13/147	13	1399.5	9.29	21/237	21	2422.9	8.67	0.99 (0.49, 1.99)	0.996
Gender										
Female	18/523	18	5271.5	3.41	20/632	20	6569.8	3.04	1.09 (0.57, 2.06)	0.796
Male	20/218	20	2224.39	8.99	29/362	29	3656.4	7.93	1.14 (0.65, 2.02)	0.641
Income										
$20,000 or less	11/192	11	1962.2	5.61	3/90	3	920.4	3.26	1.72 (0.48, 6.16)	0.406
$20,001 to $80,000	23/436	23	4334.8	5.31	23/532	23	5591.3	4.11	1.22 (0.69, 2.18)	0.5
$80,000 or more	1/51	1	548.5	1.82	12/269	12	2660.3	4.51	0.44 (0.06, 3.38)	0.43

PY: Person-years; IR: Incidence rate per 1,000 PY; IRR: Incidence Rate Ratios; CI: 95% Confidence Intervals; MACE: Major Cardiac Adverse Events

Table A11.1: Distribution of Components of MACE by Race

	Race			
Characteristics	**White**	**Black**	**Total**	**P-value**
Cause				0.3684
Myocardial Infarction	9 (18.4)	8 (21.1)	17 (19.5)	
Revascularization	29 (59.2)	17 (44.7)	46 (52.9)	
Cardiac Death	11 (22.4)	13 (34.2)	24 (27.6)	
P-values are from a Chi-square test for categorical variables				

MACE: Major Cardiac Adverse Events

Table A11.2: Association between Components of Cumulative Reported Chronic Stressors by Components of MACE

	Caregiving stress			**Job-related strain**			**Financial Stress**			**Discrimination-related stress**			**Neighborhood Deprivation within PA**		
Characteristics	**No**	**Yes**	**P-value**	**No**	**Yes**	**P-value**	**No**	**Yes**	**P-value**	**No**	**Yes**	**P-value**	**No**	**Yes**	**P-value**
Cause			0.4027			0.6508			0.4106			0.0124			0.687
No event	1546 (94.9)	54 (100)		1554 (94.9)	66 (97.1)		1477 (94.8)	168 (96.6)		1136 (95.2)	475 (94.3)		1005 (94.6)	442 (95.5)	
Myocardial Infarction	16 (1)	0		15 (0.9)	1 (1.5)		15 (1)	2 (1.2)		11 (0.9)	5 (1)		10 (0.9)	5 (1.1)	
Revascularization	44 (2.7)	0		45 (2.8)	1 (1.5)		42 (2.7)	4 (2.3)		36 (3)	10 (2)		32 (3)	9 (1.9)	
Cardiac death	24 (1.5)	0		24 (1.5)	0		24 (1.5)	0		10 (0.8)	14 (2.8)		15 (1.4)	7 (1.5)	
P-values are from a Chi-square test for categorical variables															

MACE: Major Cardiac Adverse Events

Table A12.1: Total and Controlled Direct Effects of Race on MACE with Chronic Stress as a Mediator

	Before controlling for traditional risk factors of CHD						**After controlling for traditional risk factors of CHD**				
Study Measures	**Sample size**	**Total Effect (95% C.I)**	**Sample Size**	**Controlled Direct Effect (95% C.I.)**	**Percent Eliminated (%)**		**Sample Size**	**Total Effect (95% C.I)**	**Sample Size**	**Controlled Direct Effect (95% C.I.)**	**Percent Eliminated (%)**
CRCS	1,443	1.79 (1.05 , 3.05)	1,443	1.45 (0.70 , 3.01)	43		1,378	1.44 (0.83 , 2.51)	1,378	1.39 (0.64, 3)	12.6
CRCS (without n/hood deprivation)	1,443	1.79 (1.05 , 3.05)	1,443	1.59 (0.83 , 3.07)	34		1,378	1.44 (0.83, 2.51	1,378	1.48 (0.73, 2.98)	-7.7
CRCS (with imputation)	1,735	1.25 (0.78, 2.02)	1,735	1.03 (0.52, 2.01)	88		1,735	1.02 (0.62, 1.67)	_	NA	NA
Individual components of CRCS											
Financial difficulty	1,443	1.79 (1.05 , 3.05)	1,443	1.89 (1.12 , 3.19)	-12.8		1,378	1.44 (0.83 , 2.51)	1,378	1.69 (0.96, 2.95)	-54.9
Discrimination	1,443	1.79 (1.05 , 3.05)	1,424	1.37 (0.74 , 2.56)	52.8		1,378	1.44 (0.83 , 2.51)	1,359	0.90 (0.49, 1.68)	121.7
Caregiving	1,443	1.79 (1.05 , 3.05)	1,410	1.71 (1.03 , 2.85)	10.3		1,378	1.44 (0.83 , 2.51)	1,346	1.10 (0.66, 1.85)	76.7
Job stress	1,443	1.79 (1.05 , 3.05)	1,428	1.52 (0.91 , 2.55)	34.2		1,378	1.44 (0.83 , 2.51)	1,363	1.01 (0.60, 1.71)	97.7
Deprivation	1,443	1.79 (1.05 , 3.05)	1,270	2.17 (1.22 , 3.86)	-48.1		1,378	1.44 (0.83 , 2.51)	1,215	1.55 (0.86, 2.78)	-24.2
PSS	1,443	1.79 (1.05 , 3.05)	1,441	1.94 (0.82, 4.56)	-19		1,378	1.44 (0.83 , 2.51)	1,376	1.75 (0.72, 4.29)	-69.7

** The estimates for job stress and caregiving stress are unstable due to sparse data or near zero cells
CRCS: Cumulative Reported Chronic Stressors; PSS: Perceived Stress Score

Table A12.2: Variables used in Multiple Imputation Analysis

Variable	**% missing**	**Imputed**	**Imputation model**
Age	0	No	NA
Sex	0	No	NA
Race	0	No	NA
Systolic Blood Pressure	0	No	NA
Education	0.23	Yes	Age, Sex, and Race
Employment status	0.35	Yes	Age, Sex, Race, and imputed Education
Insurance status	0.35	Yes	Age, Sex, Race, imputed Education, and imputed employment status
Renal disease	0.46	Yes	Age, Sex, Race, and systolic blood pressure
Self-rated quality of life (QoL)	0.58	Yes	Age, Sex, Race, imputed Education, imputed employment status, and imputed insurance
Cohen PSS	0.98	Yes	Age, Sex, Race, and imputed QoL
CESD	1.1	Yes	Age, Sex, Race, imputed QoL, and imputed Cohen PSS
Family hx of premature CAD	6.92	Yes	Age, Sex, Race, and systolic blood pressure
Income	9.51	Yes	Age, Sex, Race, imputed Education, imputed employment status, imputed insurance, and imputed QoL

Table A13: Derivation of the Ideal Cardiovascular Health (ICH) Score

Life Simple 7 (LS7) Component	**Poor (0)**	**Intermediate (1)**	**Ideal (2)**
BMI (kg/m^2)	>=30	25 - <30	<25
Smoking	Current smoker	Past smoker	Never smoked
Physical Activity	Mild or sedentary	Moderate	Strenuous
Nutrition (based on daily consumption of fruits and vegetables, only)	<2	2 - <=4	>=4
Cholesterol	>=240	200 - <240 or <200 and on treatment	<200 and no treatment
Systolic and diastolic blood pressure	>=140 or >=90	<120 and <80 and treated or systolic BP >=120 and <140 or diastolic BP >=80 and <90	<120 and <80 without treatment
Fasting Blood Glucose	>=126	100 to 126 or <100 with treatment	<100 and without treatment
Total possible score	0	7	14
Final ICH group	<=4	5 to 9	10 or higher

Table A14: Distribution of Baseline Characteristics by Chronic Stress

Subject Characteristics	Total N (%) (n=1380)	No Chronic Stress N (%) (n=669)	High Chronic Stress N (%) (n=711)	P-value
Age (years) , Mean (SD)	58.7 (7.4)	59.9 (7.3)	57.6 (7.3)	0.00
Cohen stress score , Mean (SD)	4.3 (3.0)	3.5 (2.4)	5.2 (3.3)	0.00
Waist circumference (cm) , Mean (SD)	95.9 (14.9)	94.4 (14.0)	97.3 (15.4)	0.00
Body mass index , Mean (SD)	30.1 (6.3)	29.1 (6.0)	31.1 (6.4)	0.00
Systolic BP , Mean (SD)	136 (19.5)	135 (18.2)	138 (20.5)	0.00
Total cholesterol (mg/dL) , Mean (SD)	208 (41.1)	208 (38.2)	208 (43.7)	0.76
LDL cholesterol (mg/dL) , Mean (SD)	143 (36.4)	144 (33.9)	142 (38.6)	0.34
Triglycerides (mg/dL) , Mean (SD)	122 (75.9)	123 (72.2)	121 (79.3)	0.65
VLDL cholesterol (mg/dL) , Mean (SD)	13.3 (9.4)	13.6 (8.9)	13.0 (9.8)	0.18
ICH Score , Mean (SD)	7.1 (2.2)	7.5 (2.2)	6.7 (2.1)	0.00
CESD score (>=16 items completed) , Mean (SD)	6.9 (7.9)	4.5 (5.0)	9.2 (9.4)	0.00
Cholesterol (mg) , Mean (SD)	124 (97.0)	116 (83.8)	131 (108)	0.00
Total dietary monounsaturated fat (gm) , Mean (SD)	8.6 (4.7)	8.4 (4.4)	8.9 (5.0)	0.06
Total dietary polyunsaturated fat (gm) , Mean (SD)	2.3 (1.0)	2.2 (0.9)	2.3 (1.1)	0.05
Total dietary saturated fat (gm) , Mean (SD)	9.1 (4.9)	8.9 (4.6)	9.2 (5.1)	0.30

Table A14 (Continued)

Total dietary fat (gm) , Mean (SD)	22.5 (11.6)	22.0 (10.8)	23.0 (12.3)	0.11
Trans dietary fats (gm) , Mean (SD)	1.3 (0.7)	1.3 (0.7)	1.3 (0.8)	0.80
Dietary protein (gm) , Mean (SD)	32.8 (16.6)	32.5 (15.2)	33.1 (17.8)	0.56
Dietary sodium (mg) , Mean (SD)	459 (218)	453 (199)	465 (234)	0.34
Gender				0.00
Male	478 (34.6)	257 (38.4)	221 (31.1)	
Female	902 (65.4)	412 (61.6)	490 (68.9)	
Race				0.00
White	783 (56.7)	533 (79.7)	250 (35.2)	
Black	597 (43.3)	136 (20.3)	461 (64.8)	
Annual income				0.00
Less than $10,000	76 (5.5)	16 (2.4)	60 (8.4)	
$10K to < $20K	167 (12.1)	53 (7.9)	114 (16.0)	
$20K to < $40K	396 (28.7)	163 (24.4)	233 (32.8)	
$40K to < $80K	468 (33.9)	234 (35.0)	234 (32.9)	
$80,000 or more	273 (19.8)	203 (30.3)	70 (9.8)	
Education attainment				0.00
At least High School	247 (17.9)	106 (15.8)	141 (19.8)	
Some college	452 (32.8)	158 (23.6)	294 (41.4)	
Bachelor's or Advanced degree	681 (49.3)	405 (60.5)	276 (38.8)	
Current smoker				0.00
No	1230 (89.3)	625 (93.6)	605 (85.3)	
Yes	147 (10.7)	43 (6.4)	104 (14.7)	
History of diabetes				0.00

Table A14 (Continued)

No	1247 (90.7)	624 (93.6)	623 (88.0)	
Yes	128 (9.3)	43 (6.4)	85 (12.0)	
ICH score in 3 categories				0.00
Poor	160 (11.6)	55 (8.2)	105 (14.8)	
Intermediate	1021 (74.1)	491 (73.4)	530 (74.8)	
Ideal	197 (14.3)	123 (18.4)	74 (10.4)	
Eat stanol or sterol products				0.12
Less than once per week	394 (74.5)	205 (71.9)	189 (77.5)	
Once per week	26 (4.9)	11 (3.9)	15 (6.1)	
2-4 times per week	58 (11.0)	39 (13.7)	19 (7.8)	
Nearly daily or daily	46 (8.7)	28 (9.8)	18 (7.4)	

Table A15: Metabolites Associated with Cumulative Chronic Stressors and Perceived Stress

BIOCHEMICAL	SUPER_PATHWAY	SUB_PATHWAY	Odds Ratio {95% CI}
Tetradecanedioate (C14-DC)	Lipid	Fatty Acid, Dicarboxylate	[2.28 {1.52-3.42}]
Glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	Lipid	Ceramides	[1.93 {1.14-3.28}]
Retinol (Vitamin A)	Cofactors and Vitamins	Vitamin A Metabolism	[1.75 {1.03-2.95}]
Creatine	Amino Acid	Creatine Metabolism	[1.62 {1.13-2.31}]
5-dodecenoylcarnitine (C12:1)	Lipid	Fatty Acid Metabolism(Acyl Carnitine)	[1.5 {1.11-2.03}]
1-linoleoyl-GPI (18:2)*	Lipid	Lysophospholipid	[1.45 {1.01-2.08}]
4-hydroxyhippurate	Xenobiotics	Benzoate Metabolism	[1.35 {1.12-1.62}]
N1-Methyl-2-pyridone-5-carboxamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	[1.33 {1.01-1.75}]
Trigonelline (N'-methylnicotinate)	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	[1.17 {1.02-1.35}]
3,7-dimethylurate	Xenobiotics	Xanthine Metabolism	[1.17 {1.01-1.35}]
Methyl-4-hydroxybenzoate sulfate	Xenobiotics	Benzoate Metabolism	[1.15 {1.01-1.3}]
Glucuronide of C10H18O2 (7)*	Partially Characterized Molecules	Partially Characterized Molecules	[1.1 {1-1.21}]
Umbelliferone sulfate	Xenobiotics	Food Component/Plant	[0.92 {0.84-0.99}]
Cholate	Lipid	Primary Bile Acid Metabolism	[0.91 {0.83-0.99}]
Docosahexaenoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)	[0.87 {0.78-0.97}]
Docosahexaenoylcarnitine (C22:6)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)	[0.86 {0.76-0.99}]
4-ethylphenylsulfate	Xenobiotics	Benzoate Metabolism	[0.86 {0.77-0.96}]
Propyl 4-hydroxybenzoate sulfate	Xenobiotics	Benzoate Metabolism	[0.85 {0.77-0.93}]
7-methylxanthine	Xenobiotics	Xanthine Metabolism	[0.84 {0.72-0.98}]
Oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	Lipid	Diacylglycerol	[0.82 {0.7-0.95}]
3-methylhistidine	Amino Acid	Histidine Metabolism	[0.78 {0.7-0.87}]
Beta-cryptoxanthin	Cofactors and Vitamins	Vitamin A Metabolism	[0.72 {0.6-0.86}]
Docosadioate (C22-DC)	Lipid	Fatty Acid, Dicarboxylate	[0.71 {0.56-0.91}]
Androstenediol (3alpha, 17alpha) monosulfate (3)	Lipid	Androgenic Steroids	[0.68 {0.56-0.84}]
3-hydroxy-2-ethylpropionate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	[0.64 {0.41-0.99}]
Laurate (12:0)	Lipid	Medium Chain Fatty Acid	[0.56 {0.4-0.78}]
Hexadecanedioate (C16-DC)	Lipid	Fatty Acid, Dicarboxylate	[0.55 {0.35-0.85}]

Table A15 (Continued)

Caproate (6:0)	Lipid	Medium Chain Fatty Acid	[0.53 {0.39-0.73}]

BIOCHEMICAL	**SUPER_PATHWAY**	**SUB_PATHWAY**	**β(SE); P-value**
Sphingomyelin (d18:1/18:1, d18:2/18:0)	Lipid	Sphingolipid Metabolism	1 (0.35); 0.004
2-hydroxybehenate	Lipid	Fatty Acid, Monohydroxy	0.55 (0.22); 0.013
Phenol glucuronide	Amino Acid	Tyrosine Metabolism	-0.12 (0.05); 0.02
Ectoine	Xenobiotics	Chemical	-0.15 (0.06); 0.015
Bilirubin (Z,Z)	Cofactors and Vitamins	Hemoglobin and Porphyrin Metabolism	-0.19 (0.09); 0.03
Heptanoate (7:0)	Lipid	Medium Chain Fatty Acid	-0.23 (0.08); 0.004
3-aminoisobutyrate	Nucleotide	Pyrimidine Metabolism, Thymine containing	-0.39 (0.15); 0.01
Sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*	Lipid	Sphingolipid Metabolism	-0.44 (0.2); 0.03

Odds Ratios represent effect size for metabolites associated with the Cumulative Reported Chronic Stress (CRCS) while β(SE) represent effects for metabolites associated with perceived stress
CI: confidence interval; PSS: perceived stress score; CRCS: cumulative chronic stress score
* The metabolite has not been confirmed based on a standard but there is high confidence in its identity

Table A16: Metabolites with the Largest Correlation Coefficients with Principal Components Associated with Chronic Stress

BIOCHEMICAL	**SUPER_PATHWAY**	**SUB_PATHWAY**
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Lipid	Fatty Acid, Dicarboxylate
docosahexaenoate (DHA; 22:6n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
docosahexaenoylcarnitine (C22:6)*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)
eicosapentaenoate (EPA; 20:5n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)
1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*	Lipid	Phosphatidylcholine (PC)
hydroxy-CMPF*	Xenobiotics	Chemical
1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	Lipid	Phosphatidylcholine (PC)
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	Lipid	Phosphatidylethanolamine (PE)
1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	Lipid	Phosphatidylcholine (PC)
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)*	Lipid	Phosphatidylethanolamine (PE)
2-hydroxyarachidate*	Lipid	Fatty Acid, Monohydroxy
2-hydroxybehenate	Lipid	Fatty Acid, Monohydroxy
2-hydroxynervonate*	Lipid	Fatty Acid, Monohydroxy
N-acetylhistidine	Amino Acid	Histidine Metabolism

* The metabolite has not been confirmed based on a standard but there is high confidence in its identity

Table A17: Ordinal Regression Showing Relationship of Stress-related Metabolites and Ideal Cardiovascular Health (ICH) Overall and by Race

Variables	ICH score in 3 categories (n=1378)	ICH score in 3 categories: Whites (n=783)	ICH score in 3 categories: Blacks (n=595)
Age	1.02 (1.00-1.04)	1.04 (1.02-1.07)	1.00 (0.97-1.03)
Sex			
Female	Referent	Referent	Referent
Male	1.10 (0.76-1.61)	1.36 (0.81-2.29)	0.90 (0.51-1.58)
Education			
Bachelor's or Advanced degree	Referent	Referent	Referent
At least High School	1.12 (0.76-1.64)	1.38 (0.82-2.31)	0.76 (0.41-1.41)
Some college	1.25 (0.92-1.70)	1.12 (0.74-1.71)	1.25 (0.78-1.99)
Income			
$80,000 or more	Referent	Referent	Referent
$10K to < $20K	1.20 (0.72-2.01)	1.09 (0.51-2.34)	1.06 (0.42-2.70)
$20K to < $40K	1.45 (0.97-2.16)	1.40 (0.86-2.28)	1.28 (0.55-2.98)
$40K to < $80K	1.24 (0.86-1.78)	1.34 (0.88-2.06)	0.90 (0.40-2.07)
Less than $10,000	1.50 (0.79-2.86)	1.87 (0.66-5.30)	1.32 (0.47-3.76)
Race			
White	Referent	Referent	Referent
Black	3.21 (2.23-4.60)	NA	NA
7-methylxanthine	0.83 (0.73-0.96)	0.77 (0.63-0.94)	0.90 (0.74-1.10)
beta-cryptoxanthin	0.41 (0.34-0.48)	0.37 (0.30-0.47)	0.44 (0.34-0.59)
laurate (12:0)	1.49 (1.10-2.02)	2.54 (1.63-3.95)	0.86 (0.56-1.34)
tetradecanedioate (C14-DC)	0.70 (0.57-0.87)	0.62 (0.45-0.84)	0.80 (0.58-1.10)
androstenediol (3alpha, 17alpha) monosulfate (3)	1.22 (1.01-1.47)	1.34 (1.03-1.74)	1.09 (0.82-1.45)
methyl-4-hydroxybenzoate sulfate	0.88 (0.80-0.96)	0.89 (0.79-0.99)	0.88 (0.76-1.02)
oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	1.24 (1.09-1.41)	1.32 (1.10-1.59)	1.17 (0.97-1.42)
3,7-dimethylurate	1.20 (1.05-1.37)	1.31 (1.08-1.57)	1.07 (0.88-1.30)
retinol (Vitamin A)	2.39 (1.50-3.81)	2.98 (1.51-5.86)	2.03 (1.04-3.98)

Table A17 (Continued)

caproate (6:0)	0.67 (0.50-0.90)	0.71 (0.49-1.04)	0.54 (0.33-0.89)
sphingomyelin (d18:1/18:1, d18:2/18:0)	3.34 (1.88-5.96)	4.84 (2.12-11.06)	2.42 (1.04-5.62)
3-aminoisobutyrate	0.57 (0.44-0.74)	0.38 (0.24-0.60)	0.73 (0.53-1.00)
sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)*	1.84 (1.31-2.58)	1.99 (1.26-3.13)	1.64 (0.97-2.79)
2-hydroxybehenate	1.65 (1.15-2.37)	1.72 (1.06-2.80)	1.70 (0.97-2.97)

* The metabolite has not been confirmed based on a standard but there is high confidence in its identity

Table A18: Ordinal Regression of Stress-related Metabolites and Components of Ideal Cardiovascular Health (ICH)

Variables	ICH BP (n=1380)	ICH BMI (n=1371)	ICH SMOKE (n=1374)	ICH CHOLESTEROL (n=1378)	ICH Diet (n=1371)	ICH PA (n=1370)	ICH GLUCOSE (n=1370)
3-aminoisobutyrate	0.81 (0.66-0.99)	0.66 (0.54-0.82)	NA	0.75 (0.61-0.92)	NA	0.75 (0.61-0.93)	0.65 (0.52-0.82)
beta-cryptoxanthin	0.74 (0.66-0.83)	0.50 (0.43-0.57)	0.69 (0.61-0.78)	1.14 (1.00-1.30)	0.48 (0.42-0.55)	0.72 (0.63-0.81)	0.72 (0.63-0.83)
Retinol (Vitamin A)	1.77 (1.22-2.55)	0.63 (0.43-0.93)	1.49 (1.02-2.17)	3.43 (2.30-5.12)	NA	NA	NA
sphingomyelin (d18:1/18:1, d18:2/18:0)	NA	4.22 (2.60-6.85)	0.42 (0.27-0.66)	4.70 (2.91-7.61)	1.84 (1.19-2.84)	2.32 (1.46-3.69)	NA
sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0)	NA	1.60 (1.20-2.11)	NA	2.62 (1.97-3.47)	NA	NA	NA
laurate (12:0)	NA	1.33 (1.03-1.72)	NA	NA	1.32 (1.06-1.65)	NA	NA
7-methylxanthine	NA	0.80 (0.71-0.91)	NA	NA	NA	NA	0.77 (0.68-0.87)
tetradecanedioate (C14-DC)	NA	0.73 (0.60-0.87)	0.71 (0.61-0.84)	NA	NA	NA	NA
3,7-dimethylurate	NA	1.32 (1.18-1.48)	NA	NA	NA	NA	1.21 (1.08-1.37)
caproate (6:0)	NA	0.43 (0.33-0.55)	NA	1.42 (1.11-1.82)	0.68 (0.54-0.87)	NA	0.62 (0.48-0.82)
methyl-4-hydroxybenzoate sulfate	NA	0.87 (0.81-0.94)	NA	0.92 (0.86-0.99)	NA	NA	0.92 (0.85-0.99)
2-hydroxybehenate	NA	NA	1.41 (1.05-1.90)	1.93 (1.43-2.60)	NA	NA	1.78 (1.29-2.47)
oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	NA	NA	1.13 (1.01-1.27)	1.15 (1.03-1.28)	NA	NA	1.20 (1.05-1.36)

PA: Physical activity; BMI: Body Mass Index; BP: Blood Pressure; NA: Variable not in the model

* The metabolite has not been confirmed based on a standard but there is high confidence in its identity

Table A19: Cox Proportional Hazards Regression of Beta-Cryptoxanthin and Incident MACE

Variables	Model 1 (n=1380)	Model 2 (n=1380)	Model 3 (n=1377)	Model 4 (Whites) (n=783)	Model 5 (Blacks) (n=597)
Beta-Cryptoxanthin	0.77 (0.61,0.97)	0.75 (0.59,0.95)	0.75 (0.59,0.97)	0.60 (0.45,0.80)	1.01 (0.63,1.62)
Age (years)	NA	1.06 (1.02,1.10)	1.05 (1.02,1.10)	1.09 (1.03,1.15)	1.04 (0.99,1.10)
Gender					
Female		Referent	Referent	Referent	Referent
Male		3.40 (2.01,5.74)	3.22 (1.86,5.58)	4.28 (1.82,10.05)	2.87 (1.39,5.95)
Education attainment					
Bachelor's or Advanced degree		Referent	Referent	Referent	Referent
At least High School		2.03 (1.02,4.02)	2.04 (1.03,4.05)	2.73 (1.06,7.08)	1.34 (0.50,3.56)
Some college		1.44 (0.74,2.80)	1.38 (0.71,2.70)	2.00 (0.75,5.33)	1.04 (0.43,2.55)
Annual income					
$80,000 or more		Referent	Referent	Referent	Referent
$10K to < $20K		0.68 (0.24, 1.94)	0.66 (0.23, 1.87)	0.51 (0.1, 2.67)	2.14 (0.23, 20.24)
$20K to < $40K		0.77 (0.33, 1.78)	0.74 (0.32, 1.73)	0.47 (0.16, 1.38)	2.45 (0.29, 21.04)
$40K to < $80K		1.05 (0.49, 2.22)	1.05 (0.49, 2.22)	0.59 (0.23, 1.5)	2.94 (0.37, 23.68)
Less than $10,000		0.93 (0.27, 3.16)	0.92 (0.27, 3.15)	1.01 (0.12, 8.55)	2.65 (0.25, 28.07)
Race					
White		Referent	Referent	Referent	Referent
Black		1.84 (1.07,3.17)	1.68 (0.96,2.93)	NA	NA
Systolic BP		NA	1.01 (1.00,1.03)	1 (0.98, 1.02)	1.02 (1, 1.04)
Total cholesterol		NA	1.00 (1.00,1.01)	1 (0.99, 1.01)	1 (0.99, 1.01)
Current smoker					
No		Referent	Referent	Referent	Referent
Yes		NA	1.22 (0.57,2.64)	1.25 (0.38, 4.07)	1.39 (0.49, 3.92)

NA= Variable not in model

UNIVERSITY OF SOUTH FLORIDA

APPROVAL

February 26, 2020

Kevin Kip
12901 Bruce B. Downs Blvd.,
MDC 22
Tampa, FL 33612-4766

Dear Dr. Kevin Kip:

On 2/20/2020, the IRB reviewed and approved the following protocol:

Application Type:	Modification / Update
IRB ID:	Pro00002213_MOD000002
Review Type:	Expedited
Title:	Secondary Data Analyses of the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) Limited Dataset
Funding:	Name: State of Pennsylvania
IND, IDE, or HDE:	None

The modifications, as described by the study team below, have been approved:

I am Co-Major Advisor for Nandozie Emechebe, a Ph.D. student in Epidemiology in the College of Public Health. Nnadozie has previously conducted secondary data analyses with the Heart SCORE data.

For his dissertation, NNadozie seeks to construct a social deprivation index score based on participant address at the time of entry into the study.

Therefore, we seek to request and gain approval for this additional data.

These data will be provided by the University of Pittsburgh in accordance with the previously Data Use Agreement on file.

The exact data fields to be requested and used in the analysis are as follows:

Address (street address of participant)

City (city of participant)

Date_Contact_Info (date of enrollment and address determination)

Form_Name (form name)

IDNUM (deidentified ID number)

Postal_Code (postal code of participant)

State (state of residence of participant)

The data are completely deidentified without identifiers.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

Tabassum Tasnim
IRB Research Compliance Administrator