THE UNIVERSITY OF CHICAGO

BIOLOGICAL ASPECTS OF HEALTH: VALIDATING, ANALYZING, AND PROFILING CYTOKINES IN OLDER U.S. ADULTS

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE DIVISION OF THE SOCIAL SCIENCES

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF COMPARATIVE HUMAN DEVELOPMENT

BY

KRISTINA L. PAGEL

CHICAGO, ILLINOIS

MARCH 2020

TABLE OF CONTENTS

ABSTRACT

Increases in life expectancy and advances in medical fields have led to an upsurge in the number of people that live into old age. This and the corresponding increase in age-related diseases has not yet been met with an appropriate amount of research. Little is known about the biological, psychological, and social changes that accompany aging. The National Social Life, Health, and Aging Project (NSHAP) was initiated in 2005 to examine the relationship between health, social support, and sexuality in aging Americans. In addition to survey data, this study collects several biomarkers indicative of health. Using this data, the aim of my dissertation is extensive. First, to internally validate the NSHAP data, I show that sex based discrepancies in diagnosis and treatment of heart disease extend into older age. I then reveal that these discrepancies are not accompanied by differences in traditional biomarkers of inflammation. Then, I discuss how we developed and validated our measurement of cytokines, which are protein molecules with interactive and cascade effects that regulate immune processes, to examine health status by means of immune functionality. The sheer number of cytokines and their numerous possible interactions makes studying their roles in health necessarily complex. Further, little is known about the distribution of individual cytokines or cytokine profiles among healthy older adults. In 2010-11, NSHAP introduced an innovative protocol to measure 22 cytokines. I describe the univariate and joint distributions of cytokines among U.S. older adults, multivariate methods for analyzing multiple analytes within individuals that address both challenging measurement issues and possibly non-linear and discrete relationships, and use these methods to study the association between inflammatory profiles and health conditions related to immune processes. Ultimately, I show that cytokine levels are a reliable and specific indicator of immune function and health status in a population-based study of older adults.

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

To my Ph.D. advisors, Martha K. McClintock & Guanglei Hong.

To my dissertation committee, Elbert Huang and Linda Waite, and others at The University of Chicago; especially Joscelyn Hoffmann, Hannah You, and Philip Schumm.

To my dad.

To my mom and Tucker, for supporting me the entire way.

To my son, Asher, the reason.

Chapter 1: Introduction and Dissertation Overview

During the past century, there has been a marked reduction in age-specific human mortality: people are living longer now than ever before. From 1910-2010, the life expectancy for a United States man has increased from 48.4 to 75.9 years and from 51.8 to 80.7 years for a woman (World Bank, 2008). Society has taken extreme measures in hopes of extending human life expectancy, and has been obviously successful in such efforts, but have not yet adequately addressed disease morbidity in older ages. With increased age comes a rise in the number of associated age-related diseases that affect the physical body, but there are also aspects of one's psychology and social life involved in healthy aging. Much research is needed to understand how the biological, psychological, and social changes that accompany age are interconnected. As an example, this proposal will focus on inflammatory mechanisms and psychosocial factors of heart disease.

The general approach of this dissertation is to use survey data to help clarify findings in experimental and clinical research. This will allow me to establish my findings as naturally occurring relationships in the aging U.S. population. However, it will not allow for the verification of causal relationships. I will be able to assess how psychological and social factors relate to mental and physical health, but I will not be able, nor will I attempt, to discern the source of such diseases. I will focus on the very real issue of sex differences in both physical and mental health during aging. Various factors, ranging from the biological to the psychological and social, give reason to expect that there are important sex differences to be examined that can help us understand heart disease.

Heart Disease

Definition of CVD by the AHA: Cardiovascular Disease - For data on hospitalizations, physician office visits, and mortality, CVD is defined according to ICD codes given in Chapter 27 of the present document. This definition includes all diseases of the circulatory system, as well as congenital CVD. Unless so specified, an estimate for total CVD does not include congenital CVD. Prevalence of CVD includes people with hypertension, HD, stroke, PAD, and diseases of the veins.

As I discuss in Chapter 3, heart disease is a serious concern in the U.S. In the past, research and treatments have been focused on middle aged men (Shirato & Swan, 2010), which may partly explain why there are higher prevalence rates for men than women of the same ages. This becomes even more complex when taking into account that some have shown rates of traditional risk factors for heart disease are higher in women than in men (DeVon, Ryan, Ochs, and Shapiro, 2008; Go et al., 2012). The differences extend even into the way that patients describe symptoms to their doctors, with women and elderly presenting those that may be atypical compared to symptoms commonly noted in middle aged men (DeVon et al., 2008). Surely this adds to the similar sex based discrepancies in frequency of cardiovascular disease treatments; as women have less diagnostic tests run and receive fewer therapeutic procedures, including lifestyle counseling and surgical procedures (Ayanian & Epstein, 1991; Gulati et al., 2012; Go et al., 2012). There has been a large push in the past 15 years to fill this void and raise awareness of women's heart disease. However, a need still exists for a more comprehensive diagnostic and treatment routine with guidelines that are perhaps more flexible, or at least suitable for men and women.

These guidelines must also be appropriate for older aged adults. In the year 2014, adults aged 65-84 years accounted for 15% of the total population, but this proportion is estimated to increase to approximately 24% by the year 2060 (Colby & Ortman, 2015). This will definitely lead to a population-based increase in the incidence of cardiovascular disease, as it is the leading cause of death among men and women over 65 years old (Go et al., 2012). Development of inclusive diagnostic and treatment standards will only be possible by understanding the complexities of heart disease origin and its progression, which incorporates inflammatory mechanisms and psychosocial factors.

Inflammatory Mechanisms. Atherosclerosis (thickening of artery walls) is the most common cause of cardiovascular disease (Pearson, et al., 2003). Atherosclerosis can be described as an inflammatory response to injury (Pearson, et al., 2003), so there has been a large amount of research focused on finding biomarkers related to cardiovascular disease. Early studies have concentrated on C-reactive protein (CRP), which is a protein produced by the liver that increases throughout the body in response to inflammation. The American Heart Association and the Centers for Disease Control (CDC) have published categories of cardiovascular risk based on serum levels of CRP (Smith et al., 2004), as increased levels of CRP are related to higher incidence of heart disease. However, this biomarker indirectly indicates risk and cannot distinguish cardiovascular disease from any other source of inflammation.

There is knowledge to be gained by studying other inflammatory molecules, such as cytokines, which are protein molecules in the immune system that are vast in number and function. For example, the production of CRP is induced by interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), and is modified by transforming growth factor beta (TGF-β) (Taylor, 1990).

One cytokine in particular, monocyte chemoattractant protein (MCP) -1, is produced by macrophages, monocytes, smooth muscle cells, and endothelial cells within atherosclerotic plaques and promotes atherosclerosis when at increased levels (de Lemos, et al., 2007). Other cytokines, such as interferon gamma (IFN-g), are associated with processes that lead to atherosclerotic plaque rupture (Libby, Ridker, & Maseri, 2002), which can in turn cause a heart attack. Along with the established categorization of CRP, the measurement of cytokines offers a more precise method of examining cardiovascular by means of the complex immune system. There is a need to standardize cytokine assays and guidelines as none currently exist. This is especially the case when considering that there are no set protocols for collecting cytokines outside of laboratory or clinical settings (see Chapter 5).

Psychological Factors. Another aspect that must be considered is that of mental health; specifically, depression may be involved in heart disease as it is related to immune function. A review of the literature provides evidence for a robust causal relationship between inflammatory markers, namely CRP and IL-6, and depression (Valkanova, Ebmeier, & Allan, 2013). Perhaps accounting for this complex mechanistic network will help understand discrepancies found in diagnosis and treatment of cardiovascular disease.

Dissertation Overview

The research questions I address will build off of each other, but are all related to the mechanisms behind inflammation and cardiovascular health. In Chapter 2, I describe the research project and sample my data come from, and go into detail on each of the measures used in my analyses. Chapter 3 examines the prevalence of heart disease in men and women included in this sample and compares it to other nationally representative data, which serves as an internal validation for the NSHAP sample and cardiovascular measures. In Chapter 4, I explore whether

the health disparities in diagnosis and treatment can be explained by biomarkers of inflammation. Lastly, Chapter 5 is a report on validation studies for the NSHAP biomeasures used in this dissertation, specifically for blood collection, assays, and statistical techniques. This chapter is especially important because there currently is no standard for analyzing data for multiple inflammatory analytes in survey research.

References

Colby, S. L., & Ortman, J. M. (2015). Projections of the Size and Composition of the US Population: 2014 to 2060. *US Census Bureau, Ed*, 25-1143.

Costanzo, E. S., Lutgendorf, S. K., Sood, A. K., Anderson, B., Sorosky, J., & Lubaroff, D. M. (2005). Psychosocial factors and interleukin-6 among women with advanced ovarian cancer. *Cancer*, *104*(2), 305-313.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., ... & Stroke, S. S. (2013). Heart disease and stroke statistics--2013 update: A report from the American Heart Association. *Circulation*, *127*(1), e6.

Lewis, M. A., & Rook, K. S. (1999). Social control in personal relationships: Impact on health behaviors and psychological distress. *Health Psychology*, *18*(1), 63-71.

Libby, P., Ridker, P. M., & Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation*, *105*(9), 1135-1143.

Loucks, E. B., Sullivan, L. M., D'Agostino Sr, R. B., Larson, M. G., Berkman, L. F., & Benjamin, E. J. (2006). Social networks and inflammatory markers in the Framingham Heart Study. *Journal of Biosocial Science*, *38*(06), 835-842.

Teo, A. R., Choi, H., & Valenstein, M. (2013). Social relationships and depression: Ten-year follow-up from a nationally representative study. *PloS one*, *8*(4), e62396.

Valkanova, V., Ebmeier, K. P., & Allan, C. L. (2013). CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. *Journal of Affective Disorders*, *150*(3), 736-744.

Wang, H. X., Mittleman, M. A., & Orth-Gomer, K. (2005). Influence of social support on progression of coronary artery disease in women. *Social Science & Medicine*, *60*(3), 599-607.

Chapter 2: General Methods

The National Social Life, Health, and Aging Project (NSHAP), a study initiated in 2005 by a team of investigators at the University of Chicago, working with the National Opinion Research Center (NORC) at the University of Chicago, is designed to be representative of and yield conclusions about US older adults (ages 57-85) living in their homes. Broadly, the aim of this survey is to examine the relationship between health, social support, and sexuality in aging Americans.

NSHAP uses non-medically trained field interviewers to conduct in-home assessments; collecting health, social, and behavioral surveys, as well as biomeasures. The range of information collected is extensive and includes measures of health (physical, cognitive, emotional, behavioral, sexual, medication usage, health service usage), psychological attributes (attitudes, values, quality of life), chronic disease (diabetes, hypertension, arthritis, cardiovascular), social networks (marriage, intimate, friend), isolation (objective and subjective), and biomeasures (weight, waist circumference, blood pressure, smell, vision, blood, urine, vaginal swabs, saliva).

Study Design

The study is set up as a longitudinal design, and has so far completed three waves of data collection. Wave 1 of NSHAP was conducted between July 2005 and March 2006 with 3,005 completed interviews on adults aged 57 to 85. Data from Wave 2 of NSHAP was collected from 2010-2011 and included 2,261 of the original Wave 1 participants as well as interviews from 955 of their cohabiting spouses and romantic partners as well as 161 Wave 1 Non-Interviewed Respondents, resulting in a total of 3,377 respondents. Detailed description of the collection protocol is described in Jaszczak, et al. (2014). All participants provided informed consent and

procedures were performed as approved by the institutional review board of the University of Chicago and National Opinion Research Center.

Interview

Interviews were conducted when participants are at home, which assumedly produce results that are more ecologically valid, decreasing the amount of stress experienced while being measured. NSHAP Wave 2 data collection included three components: an in-person interview administered via Computer-Assisted Personal Interview (CAPI), biomeasure collection, and a self-administered paper-and-pencil questionnaire (PAPI). The CAPI interview obtained information regarding demographic characteristics, social networks, physical health, history of sexual and intimate partnerships, fertility and menopause, children and grandchildren, mental health, employment and finances, and religion.

The collection of biomeasures was also conducted in the home during the in-person interview with the result directly entered in CAPI. Understandably, values of biological measures are dramatically affected by conditions under which they are collected, transported, and assayed. Because the biomeasures were collected in the respondents' homes by non-medically trained field interviewers, NSHAP could not use clinical laboratory protocols. Therefore, NSHAP adapted and created field methods for biomeasure collection and transportation, prioritizing non-invasive collection techniques and cutting-edge technology that minimized respondent burden.

The NSHAP Wave 2 interview included the collection of up to 15 biomeasures: height, weight, hip circumference, waist circumference, blood pressure, heart rate and pre-ventricular contraction, physical performance measures including timed walk and chair stands, smell, saliva (cortisol), saliva passive drool (dehydroepiandrosterone, estradiol, progesterone, testosterone),

dried blood spots (Epstein–Barr virus antibody titers, C-reactive protein, glycosylated hemoglobin, hemoglobin, cholesterol, high-density lipoprotein), whole blood (cytokines), urine (creatinine, vasopressin, oxytocin), Oragene (genotype), respondent-administered vaginal swabs (bacterial vaginosis, yeast, and vaginal cell cytology), and Actiwatch (sleep patterns and activity).

At the end of the in-person interview, respondents were given a supplemental PAPI, also known as the "Leave-Behind Questionnaire" (LBQ). This questionnaire was incorporated to reduce respondent burden at the time of the interview, and took approximately 30 min for respondents to complete on their own.

Biomeasures

Collection. The collection of biomeasures from respondents is an essential component of NSHAP to objectively estimate the prevalence and severity of diseases and conditions in a community population and the establishment of objective measures of health to use in conjunction with survey-based measures. Therefore, in addition to the report of physician diagnoses, several corresponding biosamples were collected: whole blood collected on filter paper and in a Microtainer®, saliva, and urine, as well as biomeasures for anthropometrics, blood pressure and heart rate, and physical performance measures. My dissertation focuses on chemical indicators of atherosclerosis and heart disease measured in whole blood, both on filter paper and in the Microtainer®. For information on tracking, shipping and cataloguing of the biosamples, see O'Doherty, et al. (2014).

Briefly, in Wave 2 of NSHAP, the field interviewer collected the respondent's blood on pretreated paper that was air dried for 24 hours, stored, and then shipped weekly to the Department of Medicine Biomarker Analysis Laboratory at the University of Washington. In

Wave 2 of NSHAP, participants were instructed to hold hand-warmers to improve circulation in order to increase blood flow and maximize the amount of blood collected. This new protocol enabled the collection of additional biomeasures not feasible with dried blood spot protocols. After dried blood spots were collected, 5 drops (250 microliters (µl)) of whole blood was collected and stored in a Microtainer®; a small, unbreakable plastic tube with a FloTop collector which facilitates fast and efficient blood collection. To prevent the blood from clotting, the microtainers were coated with Dipotassium Ethylenediaminetetraacetic acid (K_2EDTA), an anticoagulant that binds to calcium in the blood needed for clot formation. Uncoagulated whole blood was spun down and the plasma frozen at the University of Chicago Flow Cytometry Core Facility. All samples were void of personal identification material and were given unique laboratory ID's in order to match the biomarker information with the interviews to ensure confidentiality.

Measures Used in This Dissertation

Dependent Variables

Cardiovascular Diseases (CVD) Measures. NSHAP respondents were asked about a variety of health conditions that are known to be highly prevalent in older populations and are predictive of mortality. This study includes several measures of cardiovascular disease. In the morbidity section of the CAPI interview, participants were asked, "Has a doctor told you that you have a heart condition?" If the participant responded yes, then they were asked three additional questions: "Has a doctor told you that you had a heart attack or myocardial infarction (MI)?", "Have you ever had a procedure to treat coronary artery disease (CAD), such as cardiac by-pass surgery or placement of a coronary artery stent?", and "Has a doctor told you that you had congestive heart failure or "CHF"?". For the question on coronary artery disease,

interviewers were instructed to explain, if asked, that this includes balloon angioplasty, but not include diagnostic procedures such as an angiogram.

For the purpose of this study, it is important to note that participants who answered yes to having a heart condition may not have answered yes to any of the three follow-up questions, and are classified here as not surgically treated for CAD or specifically diagnosed with MI or CHF. Further, participants were asked an open-ended question ("Are there any other medical diseases or conditions that are important to your health now, that we have not talked about?), and if they answered yes, were prompted to list off what these conditions were. These conditions were categorized and back-coded for any that were missed on the survey questionnaire; including but not limited to atrial fibrillation and peripheral artery disease.

Inflammation Biomarkers

CRP. C-reactive protein (CRP), which is produced by the liver and serves as a broad marker of inflammation as well as a risk factor for cardiovascular disease, was measured from the dried blood spots. The University of Washington (UW), Department of Medicine Biomarker Analysis Laboratory assayed the dried blood spots. At the laboratory, filter paper and desiccant pack were placed in a Ziploc bag and stored at -70°c until processing. The UW Assay uses an automated ion exchange high-performance liquid chromatography (IE-HPLC) system. A punch from a DBS card containing a patient blood sample is eluted in a buffer solution. The elution solution is transferred to a sample vial, diluted and analyzed. The assay had a high yield: 97.9% valid assay values (O'Doherty et al., 2014).

According to the American Heart Association (AHA) and the Centers for Disease Control (CDC), serum levels of CRP are categorized as "low" if less than 1 mg/L, between 1 and 3 mg/L is "average", and levels between 3 and 10 mg/L are considered "high" risk for cardiovascular

disease, while CRP serum levels greater than 10 mg/L reflect an acute infection (Smith et al., 2004). However, NSHAP directly assayed CRP from dried blood spots, which have been shown to lead to slightly lower values than when measured in serum (McDade, Burhop, & Dohnal, 2003). Although the lab did determine the plasma equivalent using a conversion calculation, we chose to use the directly assayed serum values. Therefore, the CRP cardiovascular risk categories are adjusted to less than 0.76 mg/L is "low", levels from 0.76 to 2.5 mg/L are "average", levels from 2.5 to 8.6 mg/L are "high", and anything over 8.6 mg/L is "acutely high".

Protein Analytes. The University of Chicago Flow Cytometry Core Facility assayed the microtainers of whole blood for multiple proteins including cytokines, chemokines, and fibrinogen. Eighteen-analyte assay panels were run with a Bio-Plex system driven by Luminex xMAP technology. In this assay there are 18 separate bead sets, each internally dyed with a specific fluorescence, and then conjugated with antibodies that bind the analyte of interest. Each bead has antibodies for one particular molecule and a corresponding uniquely colored fluorescence. The plasma sample is then combined with the beads, dilutions are added if necessary, the mixture is stabilized, and the beads adhere to the proteins in the sample. The mixture is then run through the Luminex reader, where beads are detected by flow cytometry and move single-file through a flow cell where red and green lasers function to detect bead color and signal strength, respectively. The analytes of interest for this study include MCP-1, IL-6, IL-1b, and TNF-a.

Currently there are no medically established levels of cytokines or other protein analytes for categorization of risk associated with heart disease. However, higher levels are associated with traditional risk factors and overall cardiovascular disease risk. Therefore, I ran simultaneous analyses using traditional risk factors and these novel markers.

Mental Health

Depressive Symptoms. In order to determine the prevalence of those suffering from Frequent Depressive Symptoms (FDS), NSHAP used an existing 11-item short form of the Center for the Epidemiologic Studies Depression Scale (CES-D), thereby creating the NSHAP Depressive Symptoms Measure (NDSM). NDSM asks participants to describe the frequencies of their depressive symptoms within the past week. NSHAP chose to base its measure of depressive symptoms on a short form of the CES-D, known as the Iowa form from the Established Populations for Epidemiological Studies of the Elderly (EPESE), to minimize respondent burden and the overall interview time of NSHAP's survey (Payne, Hedberg, Kozloski, Dale, & McClintock, 2014).

The NDSM quantifies the frequency of 11 symptoms during the past week using symptom descriptions, and the three response categories for symptom frequency (rarely or none of the time, some of the time, and much or most of the time) were scored as 0, 1, and 2. Scores on each of the 11 items were summed to produce a total score ranging from 0 to 22, with higher scores reflecting more frequent symptoms. Because the CES-D is an integer scale, the cutpoint is 9 or greater for NSHAP's 11 x 3 measure. For a dichotomous variable comparable to original scales and other surveys, NSHAP investigators recommend using ≥ 9 as the cutpoint for Frequent Depressive Symptoms (FDS), which yields a prevalence rate comparable to other epidemiological studies (Payne, et al., 2014).

Independent Variables

Demographic variables included gender, age, race, and education. Gender was coded as binary (female or male), and age was coded as whole numbers. The race variable categorized

participants as White, Black, Hispanic (non-black), or Other. Categories of education included less than high school, high school or equivalent, some college, and bachelor's degree or more.

Participants Selected for Dissertation Analyses. Of the 3,196 age eligible (62-91 years old in Wave 1) participants in NSHAP Wave 2, 3,185 answered the question of whether or not they had ever been doctor diagnosed as having a heart problem (2 refused, 9 did not know). Of these, 3,044 had usable dried blood spot samples with assay values of CRP. Inadequate or lost samples were the largest problems, but of those providing blood samples, 98% had usable CRP data (O'Doherty et al, 2014). Of those who answered the heart condition questions, 2,926 participants provided a sample of whole blood plasma in Microtainers. There were 349 participants excluded because they had CRP values over 8.6 mg/L indicating acute infection, which yielded 2,523 participants (mean age = 72.36 ± 8.04 years, 53.56% female) within the range of normal or chronic inflammation.

Statistical Models

All analyses were run with STATA version 14.0 (StataCorp, 2016). Sample design weights were used to account for the intricate survey design and to generate standard errors and point estimates accurately representative to the composition of the national population of community-residing adults born between 1920 and 1947, which includes an oversampling of African-Americans and Hispanics.

Survey weighted logistic regression models, with gender and age as predictors, were run to determine prevalence of heart disease, surgical treatment of CAD, and specific diagnoses of CHF and MI. Beta coefficients, odds ratios, standard errors, and 95% confidence intervals are reported. Raw CRP and protein analyte values were analyzed, in relation to demographic,

biological, and dependent variables of interest described above, with survey weighted linear regressions and reported beta coefficients, standard errors, and 95% confidence intervals.

References

Jaszczak, A., O'Doherty, K., Colicchia, M., Satorius, J., McPhillips, J., Czaplewski, M., ... & Smith, S. (2014). Continuity and innovation in the data collection protocols of the second wave of the National Social Life, Health, and Aging Project. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *69*(Suppl 2), S4-S14.

McDade, T. W., Burhop, J., Dohnal, J. (2003). High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry, 50*(3), 652-654.

O'Doherty, K., Jaszczak, A., Hoffmann, J. N., You, H. M., Kern, D. W., Pagel, K., McPhillips, J., Schumm, L.P., Dale, W., Huang, E.S., & McClintock, M. K. (2014). Survey field methods for expanded biospecimen and biomeasure collection in NSHAP Wave 2. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *69*(Suppl 2), S27-S37.

Payne, C., Hedberg, E. C., Kozloski, M., Dale, W., & McClintock, M. K. (2014). Using and interpreting mental health measures in the National Social Life, Health, and Aging Project. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *69*(Suppl 2), S99-S116.

Smith, S. C., Anderson, J.L., Cannon, R.O., Fadl, Y.Y., Koenig, W., Libby, P., Lipshultz, S.E., Mensah, G.A., Ridker, P.M., Rosenson, R. (2004). CDC/AHA workshop on markers of inflammation and cardiovascular disease application to clinical and public health practice report from the clinical practice discussion group. *Circulation, 110*, e550-e553.

StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP.

Chapter 3: Sex Differences in Prevalence of Diagnosed Heart Disease in Older U.S. Adults

According to the 2011 update from the American Heart Association, heart disease causes 1 out of every 4 deaths (24.6% of total deaths in 2009) (Kochanek, Xu, Murphy, Miniño, & Kung, 2011). Heart disease is a special concern among older adults. U.S. adults aged 65 and older comprise 80% of people who die from coronary heart disease (Go et al., 2012). Compared to all ages, the death rate attributable to diseases of the heart increases by over 200% at age 65, over 600% at age 75, and over 2,000% for those over 85 years of age (Kochanek et al., 2011). In fact, heart disease is the leading cause of death among men and women ages 65 and over (Go et al., 2012).

Historically, heart disease has been labeled as a "man's disease" and therefore, much of the research has been focused disproportionality on male patients (Shirato & Swan, 2010). However, cardiovascular events are the leading cause of death for women at all ages (Shaw et al., 2006), so perhaps the risk factors established by this research do not generalize to women. Studies suggest that traditional cardiovascular risk factors are able to distinguish between high and low risk in women and men separately, but do not explain the sex difference in prevalence (Kannel, Hjortland, McNamara, & Gordon, 1976). Indeed, there is evidence that rates of traditional risk factors for CHD are higher in women than in men (DeVon, Ryan, Ochs, and Shapiro, 2008; Go et al., 2012), which adds more confusion as CHD mortality rates are consistently found to be higher in men than women (Kannel et al., 1976; Bush, 1990; Kochanek et al., 2011). However, women often develop the disease about 8 years later than men (Bush, 1990), so it is possible that age is confounding these results. In fact, postmenopausal women have higher risk of CHD and rates of cardiovascular disease than premenopausal women (Agrinier et al., 2009; Kannel et al., 1976), and after menopause, women have prevalence rates

of CHD similar to men of the same age (Dabla, Dabla, Dawar, & Arora, 2001). Consequently, there is a need to determine whether guidelines are appropriate to assess heart disease in both women and men throughout older ages.

DeVon, Ryan, Ochs, and Shapiro (2008) found that, despite having no difference in history of coronary heart disease (CHD), women and men hospitalized for acute coronary syndromes (ACS) differ in their presentation of symptomology. Compared to men, women experienced more back pain, dyspnea (shortness of breath), indigestion, palpitations, nausea, fatigue, and weakness, as well as less chest pain. Importantly, these symptoms are considered "atypical" because they are not part of the conventional male model. In addition, they are vague and are often symptoms of many less serious health conditions. The authors argue that this issue may put women and men displaying atypical symptoms at risk for delayed diagnosis, but is a greater risk for women because they are more likely to experience these symptoms (DeVon et al., 2008).

Consequently, over the past 10 to 15 years there has been a large effort to increase awareness of heart disease in women. A major campaign, The Heart Truth, sponsored by the National Heart, Lung, and Blood Institute, an organization of the United States Department of Health and Human Services, was initiated to increase women's awareness of their risk for heart disease, targeting those aged 30-60. This campaign features a Red Dress as a national symbol to signify that heart disease and the accompanying risk factors do not "care what you wear – it's the number 1 killer of women" (Long, Taubenheim, Wayman, Temple, & Ruoff, 2008). From 1997 to 2006, awareness of risk has increased from 30% of women to 57%, which has led to positive lifestyle changes (Long et al., 2008), but it has yet to be seen whether this awareness has transferred to better heart care for women in terms of diagnosis and treatment.

Clearly, the lack of research on heart disease with women as participants has led to gender differences in treatment (Shaw et al., 2006). In patients hospitalized for heart disease, not only do women undergo less diagnostic tests than men, but they also receive fewer therapeutic procedures (Ayanian & Epstein, 1991), and less lifestyle counseling (Gulati, Shaw, & Bairey Merz, 2012). This sex discrepancy is seen in surgical treatments as well. In the year 2010, there were approximately 7.5 million inpatient cardiovascular operations and procedures performed in the U.S.; 4.4 million performed on men, and 3.2 million performed on women (Go et al., 2012).

Sex differences in treatment may have severe consequences, especially for women that are left untreated. Cardiovascular death rates have actually fallen from 1968 to 2000, and about half of this decrease is attributed to medical and surgical treatments such as secondary preventive therapies after MI, revascularization, HF treatments, antihypertensive and lipid-lowering medications (Go et al., 2012). Unfortunately, the decline in cardiovascular deaths lags for women compared to men (Abuful, Gidron, & Henkin, 2005; Gulati et al., 2012).

The range of information collected in the NSHAP population is extensive and includes measures of health and chronic disease, including the most common types of heart disease. While there are many types of heart disease, the most prevalent in the United States is coronary artery disease (CAD, also termed coronary heart disease or CHD), with an estimated 15.4 million American adults affected (Go et al., 2012). CAD is the most common cause of myocardial infarction (MI; 7.6 million), or heart attack, and also increases the risk of congestive heart failure (CHF; 5.1 million) (Go et al., 2012). When blood supply to the heart is reduced, sections of the heart may become damaged or die, leading to an MI. CHF is when the heart is unable to pump the amount of blood and oxygen that the rest of the organs and body requires. The muscles of

the heart become too weak to pump blood into the aorta, and this blood then accumulates in the heart.

Risk of MI increases with age; the average age at first attack for men is 64.7 years and 72.2 years for women (Go et al., 2012). Rates of MI are almost double for men than women (Kudenchuh, Maynard, Martin, Wirkus, & Weaver, 1996; Hanratty et al., 2000; de Torbal et al., 2006; Go et al., 2013). Perhaps because middle-aged men are the "target" population for MI, women and older adults often have worse short-term prognosis, i.e. higher rates of mortality (Hanratty et al., 2000), and there is a higher proportion of unrecognized MI among women (de Torbal et al., 2006). Incidence of CHF is highest in elderly, but results on gender are inconsistent, with some showing higher rates in men (Gottdiener et al., 2000), others reporting higher rates for women (Kudenchuh et al., 1996), and some reporting no difference at all (Go et al., 2013).

The National Social Life, Health, and Aging Project (NSHAP) provides a unique data set in which to address questions of sex discrepancies in issues of heart disease among older people in the United States and whether the differences reported in the literature hold in a representative population. As mentioned, research is usually conducted in clinical samples or laboratory settings and on limited populations. The current study will use data from Wave 2 of NSHAP, which is a national area probability sample of community-residing women and men aged 57 to 85. This design will help address the bias in reporting health disease, as the participants are mainly healthy rather than clinical, of a specific age range, and representative of the national US population, including women and men of various racial backgrounds.

Methods

Sample

The National Social Life, Health, and Aging Project (NSHAP), a study initiated in 2005 by a team of investigators working with the National Opinion Research Center (NORC) at the University of Chicago, is designed to be representative of and yield conclusions about the entire older adult (ages 57-85) population of United States. Broadly, the aim of this survey is to examine the relationship between social support, health and sexuality in aging Americans. This relationship is evaluated in a sociocultural context with a social networks framework. This population-based, longitudinal study uses a national area probability sample of community-residing adults born between 1920 and 1947, which includes an oversampling of African-Americans and Hispanics, to provide sufficient statistical power for nationally representative results.

Wave 1 of NSHAP was conducted from 2005-2006 and resulted in 3,005 interviewed participants. Specific details of the Wave 1 data collection design, implementation, response and cooperation rates are explained in Smith et al., (2009). This study focused on heart disease reports data from Wave 2 of NSHAP, collected from 2010-2011 and included 2,261 of the original participants as well as interviews from 955 of their cohabiting spouses and romantic partners as well as 161 Wave 1 Non-Interviewed Respondents resulting in a total of 3,377 respondents. Detailed description of the collection protocol is described in Jaszczak et al. (2014). All participants provided informed consent and procedures were performed as approved by the institutional review board of the University of Chicago and National Opinion Research Center.

This study will include Wave 2 participants who are age eligible (62-91 years old) with data on heart disease and who did not have a cold or acute infection (CRP > 8.6) on the day of the interview ($N = 2326$). Additionally, when looking at this data, it was clear that age was relating in a non-linear manner for women, so after looking at dot graphs of the data, we decided to split age at 62-76 ($N = 1550$) & 77-91 ($N = 776$) so we could look at everyone versus the oldest old.

Field Data Collection

Cardiovascular Disease (CVD) Measures. NSHAP respondents were asked about a variety of health conditions that are known to be highly prevalent in older populations and are predictive of mortality. This study includes several measures of cardiovascular disease. In the morbidity section of the interview, participants were asked, "Has a doctor told you that you have a heart condition?" If the participant responded yes, then they were asked three additional questions: "Has a doctor told you that you had a heart attack or myocardial infarction (MI)?", "Have you ever had a procedure to treat coronary artery disease (CAD), such as cardiac by-pass surgery or placement of a coronary artery stent?", and "Has a doctor told you that you had congestive heart failure or "CHF"?". For the question on coronary artery disease, interviewers were instructed to explain, if asked, that this includes balloon angioplasty, but not include diagnostic procedures such as an angiogram.

Participants were also categorized as having a heart condition if they indicated one (MI, CAD treatment, CHF, and atrial fibrillation) in response to an open-ended question that asked "Are there any other medical diseases or conditions that are important to your health now, that we have not talked about?". As a result of this back-coding process, 18 additional, independent cases of heart disease (1 MI, 6 CAD treatment, 4 CHF, 2 with atrial fibrillation, 5 other CVD)

were added to the dataset. This process also found 90 cases that had already been reported in the heart condition section of the CAPI.

Heart disease diagnosis was additionally catalogued by certain medications considered by the medical community to be prescribed solely for treatment of heart disease. In the Wave 2 data, there were 55 instances of anti-arrhythmic use, 25 of miscellaneous cardiovascular agents, and 11 vasopressors (1 person was taking both an anti-arrhythmic and vasopressor), resulting in 90 respondents who report medication for treatment of heart disease. Sixty-four (64) of them had answered yes to some sort of heart condition, totaling 26 additional cases added from the medication data. Statin use was examined because they are often prescribed for treatment of CAD. However, due to the high usage among the sample (49%; $N = 2211$), statin use was excluded from analyses. There were many reasons behind this decision. First, it is unclear whether statin use would interfere with the biomarker signals. Next, in this data, statin use was not classified as being prescribed for primary or secondary disease prevention, so some statin users may not yet have begun the disease process.

Traditional Cardiovascular Disease Risk Factors

The American Heart Association (AHA), along with a few other government agencies, releases an annual review of cardiovascular health and metabolic diseases that includes statistics from population studies on disease prevalence, risk and health factors, and disease burden and outcomes. This report outlines key lifestyle behaviors and traditional risk factors related to cardiovascular health. Those included in the Wave 2 NSHAP data collection are as follows.

Diabetes Mellitus. Diabetes mellitus (DM) is a key risk factor for cardiovascular diseases including coronary heart disease (CHD), heart failure, atrial fibrillation, stroke, and peripheral artery disease (PAD). In 2010, the prevalence of DM in adults over the age of 65 was

26.9%, 50% were considered pre-diabetic based on fasting glucose, oral glucose tolerance testing, or HbA1c (Mozaffarian et al., 2016), and 2005-2006 data from NHANES show that 46% of DM cases remain undiagnosed in this age group (Cowie et al., 2009). For the NSHAP Wave 2 data, participants were considered to have DM if they reported a doctor diagnoses or had glycated hemoglobin (HbA1C) levels ≥ 6.5%, as recommended for diagnosing diabetes (American Diabetes Association, 2010; International Expert Committee, 2009).

Hypertension. The AHA has identified untreated blood pressure <120/<80 mm Hg for adults aged over 20 years old as a component of ideal cardiovascular health (Mozaffarian et al., 2016). For health surveillance purposes, the following definition of high blood pressure (HBP) is suggested (Crim et al., 2012): systolic blood pressure (SBP) greater than or equal to 140 mm Hg, diastolic blood pressure (DBP) greater than or equal to 90 mm Hg, taking antihypertensive medicine, or having been told at least twice by a physician or other health professional that one has HBP. Based on NHANES data from 2011-2012, prevalence was 65% among those 60 years of age and older (Nwankwo, Yoon, Burt, & Gu, 2013). The age-adjusted prevalence of hypertension (both diagnosed and undiagnosed) in 2003 to 2006 was 75% for older women and 65% for older men on the basis of data from NHANES (Crescioni, Gorina, Bilheimer, & Gillum, 2010).

Smoking. A health behavior determined important to CVD is smoking. In fact, tobacco smoking was the second leading risk factor for death in the United States in 2010, after dietary risks (Murray et al., 2013). In 2011 to 2013, age-adjusted estimates showed that among people 65 years of age and older, 9.5% of men and 7.3% of women were current smokers. Also in this age group, men were more likely than women to be former smokers (52.1% compared with 32.0%) (Mozaffarian et al., 2016).

Physical Activity. A second health behavior important for CVD and stroke is physical activity or inactivity (Artinian et al., 2010). The US Department of Health and Human Services has set recommended guidelines for adults to get at least 150 minutes of moderate intensity or 75 minutes of vigorous-intensity aerobic activity (or an equivalent combination) per week (Mozaffarian et al., 2016). This is often measured by subjective survey answers and objective measures with wearable monitors. In Wave 2, self-reported physical activity was assessed using four survey questions. All respondents were asked the following question: "On average over the last 12 months, how often have you participated in vigorous physical activity or exercise? By vigorous physical activity, we mean 30 MIN OR MORE of things like sports, exercise classes, heavy housework, or a job that involves physical labor." Answer choices included (a) 5 or more times per week, (b) 3 or 4 times per week, (c) 1–2 times per week, (d) 1–3 times per month, (e) less than 1 time per month, or (f) never. In the current analysis, respondents reporting "3 or 4 activities per week" or "5 or more activities per week" were combined into a single category representing the guideline from the US Department of Health and Human Services. While Wave 2 of NSHAP did include an objective measure of physical activity with accelerometers, these data are not included here because only a subsample ($N = 738$) of the respondents were included (Huisingh-Scheetz et al., 2014).

In 2014, the National Center for Health Statistics reported that physical inactivity was higher among women than men (21.7% versus 28.5%, age adjusted) and increased with age, with those ages 65–74 years at 35.2% and those 75 + at 51.1%.

BMI. Being overweight or obese is a risk factor for CVD. The AHA has determined that for adults, the ideal BMI for cardiovascular health is 25 kg/m^2or less (Mozaffarian et al., 2016).

Based on NHANES data on U.S. adults from 2009-2012, 73% of men and 65% of women were overweight (Mozaffarian et al., 2016).

Blood Cholesterol. Another major risk factor for CVD is high cholesterol. The AHA identifies untreated total cholesterol (TC) less than 200 mg/dL as one of the components of ideal cardiovascular health, with that over 240 mg/dL classified as "high" (Mozaffarian et al., 2016). NHANES data from 2011 to 2012 estimated that 12.9% of US adults aged 20 years and older (11.1% of men and 14.4% of women) had high TC (Carroll, Kit, Lacher, & Yoon, 2012).

Statistical Models

All analyses were run with STATA version 15 (StataCorp, 2017). For analyses on cardiac and cerebrovascular disease prevalence, sample design weights were used to account for the intricate survey design and to generate standard errors and point estimates accurately representative to the composition of the national population.

Following simple chi square analyses determining gender differences, survey weighted logistic regression models (with gender, race/ethnicity, and age as predictors) were run to determine prevalence of CAD, myocardial infarctions (heart attacks), and congestive heart failure. Once the relationship with predictors was determined, the models were expanded to include SES (education). Finally, cardiovascular health behaviors and traditional risk factors were examined separately for women and men. Beta coefficients, odds ratios, standard errors, and 95% confidence intervals are reported.

Results

Cardiovascular Disease Factors

Diabetes Mellitus. Overall, 23.3% of the population were diagnosed with diabetes (N = 2329). Women and men had the same rate of diagnosis at 22.2% and 24.5%, respectively (p =

0.214). Diabetes diagnosis was the same for both age groups; 23.6% at 62-76 and 22.6% at 77-91 ($p = 0.603$).

Hypertension. Overall, 75.7% of the population of older US adults have high blood pressure ($N = 2317$). This is the same for men and women (75.4% and 75.9%) ($p = 0.824$). Those aged 77-91 have a higher percentage (82.3%) than those aged 62-76 (72.9%) ($p < 0.0001$).

Smoking. Only 13.0% of the entire population smoke cigarettes ($N = 2336$). A higher percentage of men (15.3%) smoke than do women (11.0%) ($p = 0.004$). More people smoke when younger (15.0%) than older (8.3%) ($p = 0.0001$).

Physical Activity. Overall, people were pretty active: 44.2% reported rigorous physical activity three or more times a week ($N = 2334$). A higher percentage of men (47.8%) reported rigorous physical activity than women (40.9%) ($p = 0.024$). And those aged 62-76 reported physical activity at a higher percentage than those 77-91 at 46.6% and 38.6%, respectively ($p = 0.012$).

BMI. Using the cutoff for overweight (≥ 25 kg/m^2) shows that in the population, 77.5% ($N = 2336$) of older adults have a BMI that place them in this category. Women are less likely than men to be overweight, at 72.3% and 82.6%, respectively ($p < 0.001$). The younger old were more likely to be over weight than the older group (80.6% vs. 70.1%; $p < 0.001$).

Total Cholesterol. The AHA recommended cutoff of 240 mg/dL shows that 13.9% ($N = 263$) of older adults have high TC. This is similar to the findings from NHANES, as is the result that women are more likely to have high TC than are men (16.6% vs. 11.0%; $p = 0.0018$). There was no difference in the rate of high TC based on age (14.3% in younger old; 12.9% in older old).

Prevalence of Coronary Artery Disease (CAD)

I first looked at the prevalence of older adults with CAD, gathered by the report of having had and survived a surgical CAD treatment. As expected from the literature, fewer women in the US, 62-91 years of age, were diagnosed with CAD than were men (7.5% vs. 18.7%; X^2 (1, N = 2,330) = 93.57, $p < 0.0001$). Overall, women are 65% less likely than men to be diagnosed with CAD (t (50) = -6.37, $p < 0.0001$; Table 3.1; Figure 3.1). Also as expected, the prevalence of CAD increased with age: those 77-91 were 66% more likely than those aged 62-76 to be diagnosed with CAD (t (50) = 2.96, $p = 0.005$; Table 3.1; Figure 3.1). There was no interaction of gender and age.

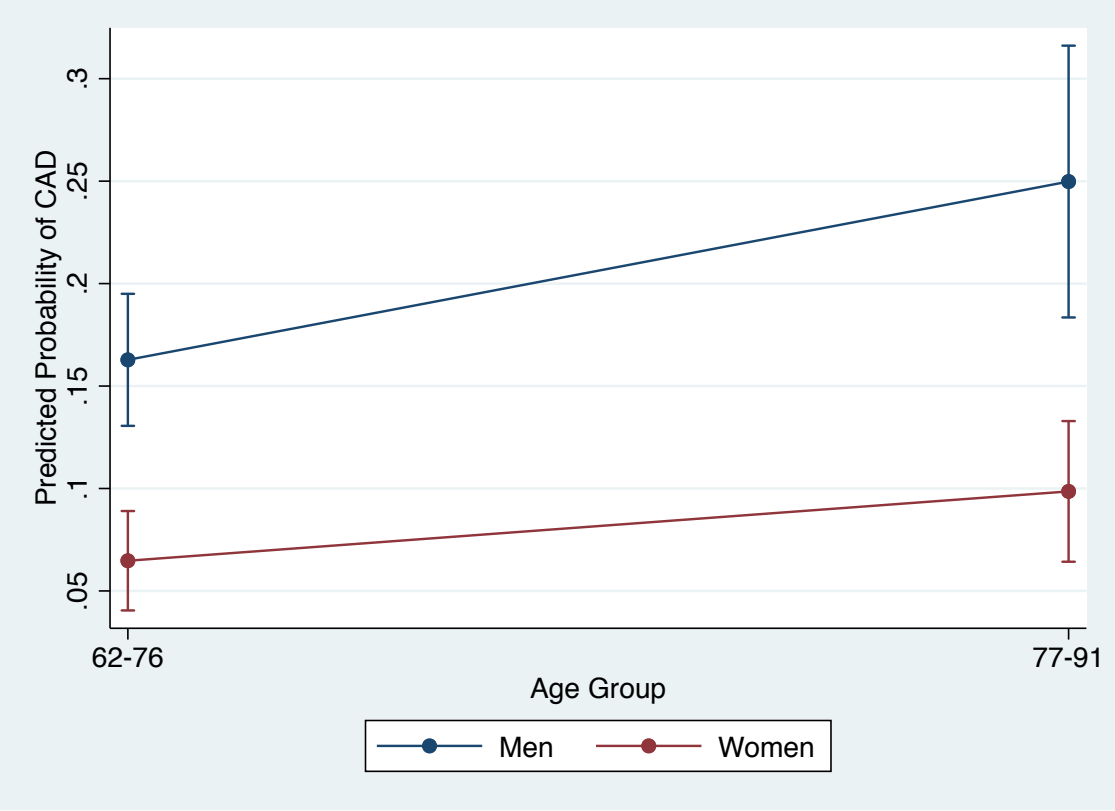

Figure 3.1. US Older Adults Probability of CAD

Table 3.1

Effects of Gender and Age on US Older Adults Prevalence of CAD

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-1.63***	0.20(0.02)	[0.158, 0.244]
Female	-1.06***	0.35(0.06)	[0.247, 0.483]
Age Group (77-91)	0.51**	1.66(0.29)	[1.177, 2.353]

***Note*: N = 2,330; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

There were a few statistically significant findings when looking at the sociodemographics of race/ethnicity and SES/higher education. Black respondents were 46% less likely than whites to have undergone surgical treatment for CAD (t (50) = -2.16, p = 0.035). Participants who had attended at least some college were 39% less likely than those without to have CAD treatment (t (50) = -2.52, p = 0.015). There was an interaction of higher education and age; there was a lower probability of CAD for those with college education aged 62-76, but higher education had no effect for those aged 77-91 (t (50) = 2.85, p = 0.006; Table 3.2; Figure 3.2).

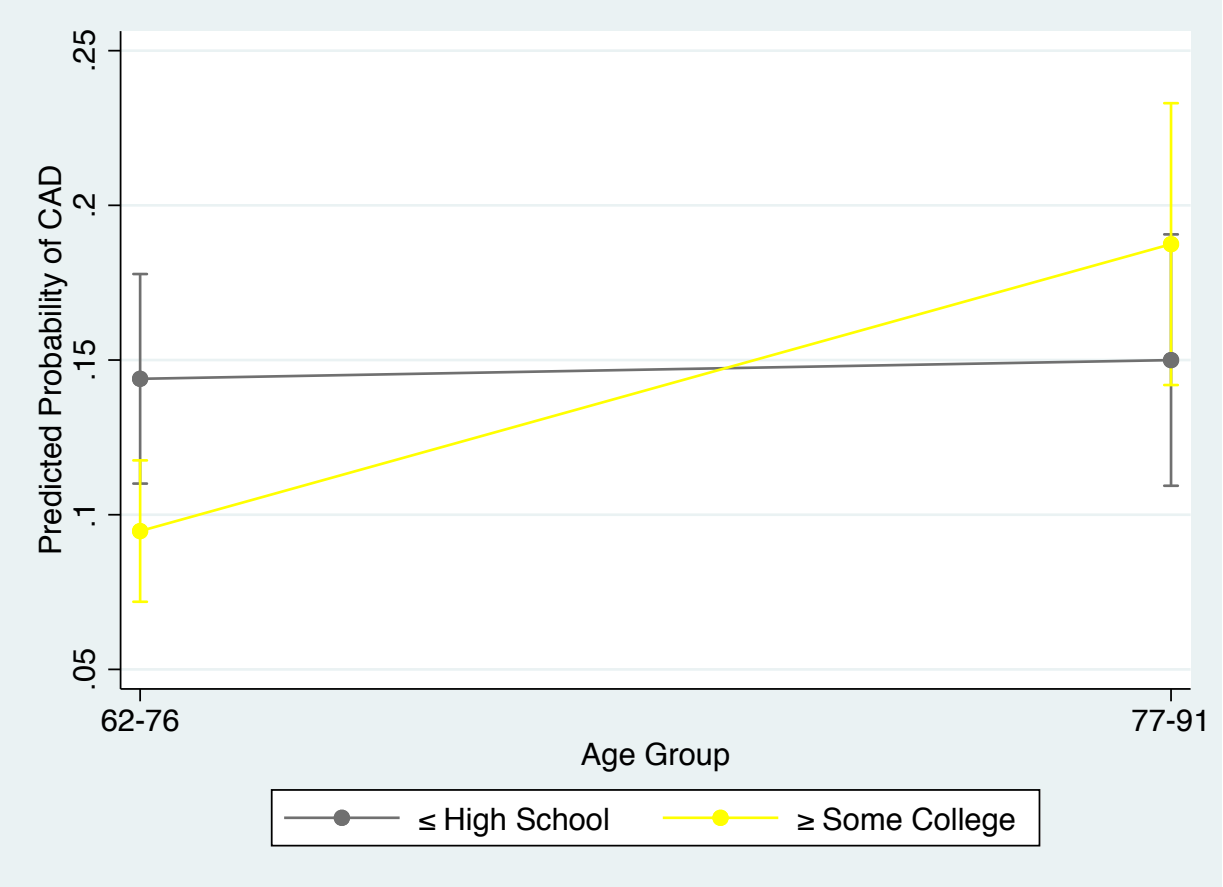

Figure 3.2. Age and Higher Education Effects on Surgical Treatment of CAD

Table 3.2

Effects of Race/Ethnicity and Higher Education on US Older Adults Prevalence of CAD

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-1.29***	0.28(0.04)	[0.203, 0.376]
Sociodemographics			
Female	-1.08***	0.34(0.06)	[0.244, 0.473]
Age Group (77-91)	0.05	1.05(0.24)	[0.660, 1.674]
≥ Some College	-0.49*	0.61(0.12)	[0.415, 0.905]

Table 3.2 continued

Race/Ethnicity			
Black	-0.61*	0.54(0.15)	[0.308, 0.957]
Hispanic, NB	-0.04	0.96(0.27)	[0.544, 1.709]
Other	0.27	1.31(0.65)	[0.483, 3.574]
Interaction			
77-91 x ≥ College	0.77**	2.16(0.58)	[1.255, 3.706]

Note: $N = 2{,}322$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Prevalence of Heart Attack Diagnoses and Survival

As a note, these results report the number of U.S. adults that have been diagnosed and have survived a heart attack, which is important because it is not including those that have had fatal or undiagnosed heart attacks. As expected, fewer women in the US, 62-91 years of age, were diagnosed with a heart attack than were men (4.6% vs. 13.1%; X^2 (1, $N = 2320$) = 76.13, $p < 0.0001$). Overall, women are 80% less likely than men to be diagnosed with a heart attack (t (50) = -6.73, $p < 0.0001$; Table 3.3; Figure 3.3a). Also as expected, the prevalence of a heart attack diagnosis increased with age: those 77-91 were 77% more likely than those aged 62-76 to be diagnosed with a heart attack (t (50) = 2.42, $p = 0.019$; Table 3.3; Figure 3.3a). Older women were less likely to be diagnosed with a heart attack than were men of the same age, and the rate of diagnosis increased with age at a higher rate for women than for men (t (50) = 2.89, $p = 0.006$; Table 3.3; Figure 3.3b).

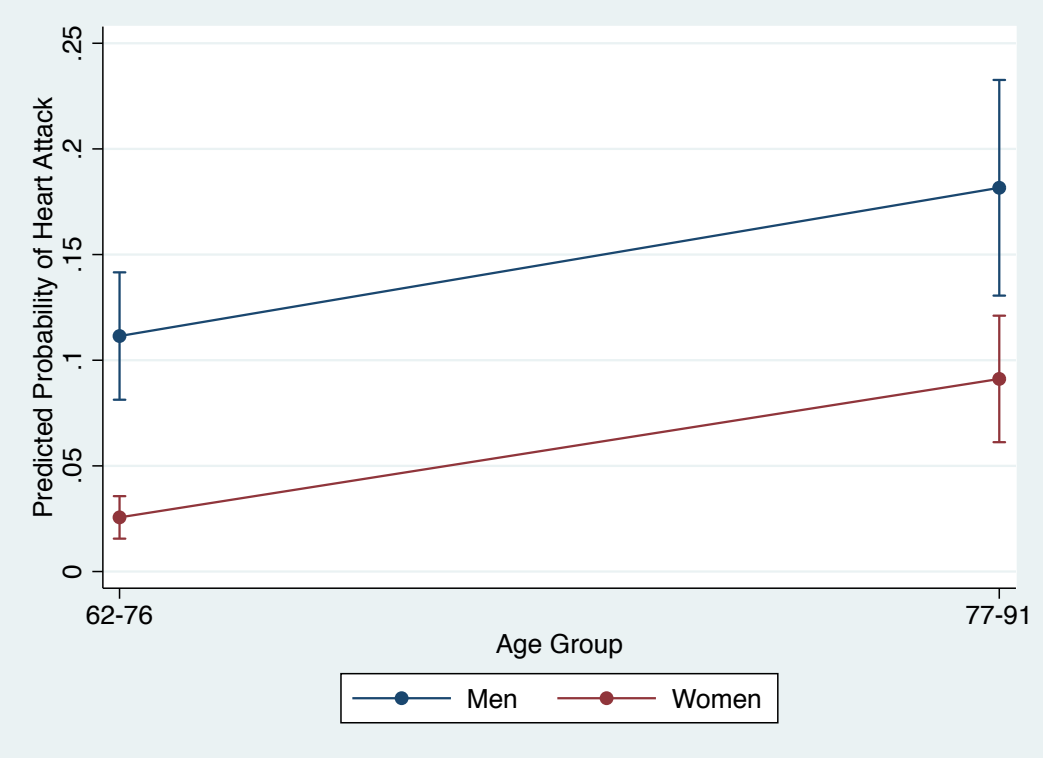

Figure 3.3a. US Older Adults Probability of Heart Attack Diagnosis

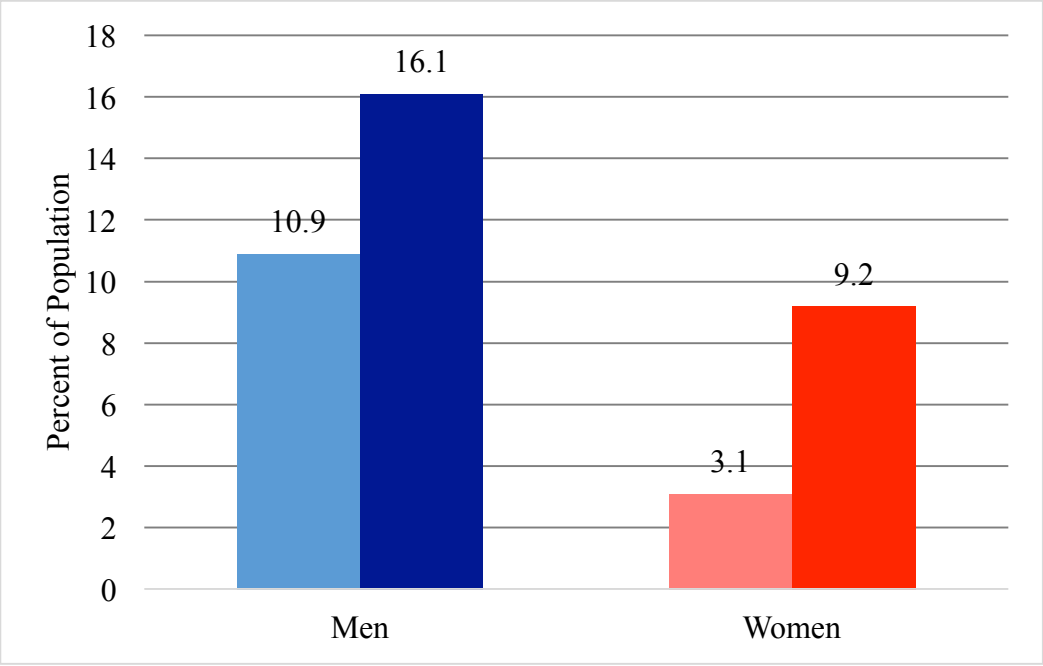

Figure 3.3b. US Older Adults Probability of Heart Attack Diagnosis

Table 3.3

Effects of Gender and Age on US Older Adults Prevalence of Heart Attack

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-2.08***	0.13(0.02)	[0.093, 0.170]
Female	-1.56***	0.21(0.05)	[0.131, 0.334]
Age Group (77-91)	0.57*	1.77(0.42)	[1.101, 2.844]
Interactions			
Female x 77-91	0.77**	2.16(0.57)	[1.264, 3.678]

***Note*: N = 2,320; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

Interestingly, prevalence of heart attack diagnosis was not affected by race/ethnicity (black $p = 0.253$; Hispanic, NB $p = 0.731$; other $p = 0.876$), but was affected by whether one has attended college. Those who had at least some college were 54% less likely than those who had no college to be diagnosed with a heart attack (t (50) = -2.94, $p = 0.005$; Table 3.4; Figure 3.4). As seen for CAD treatment, there was an interaction of higher education and age; while college education seems protective of those aged 62-76, higher education has no effect for those aged 77-91 (t (50) = 2.72, $p = 0.005$; Table 3.4; Figure 3.4).

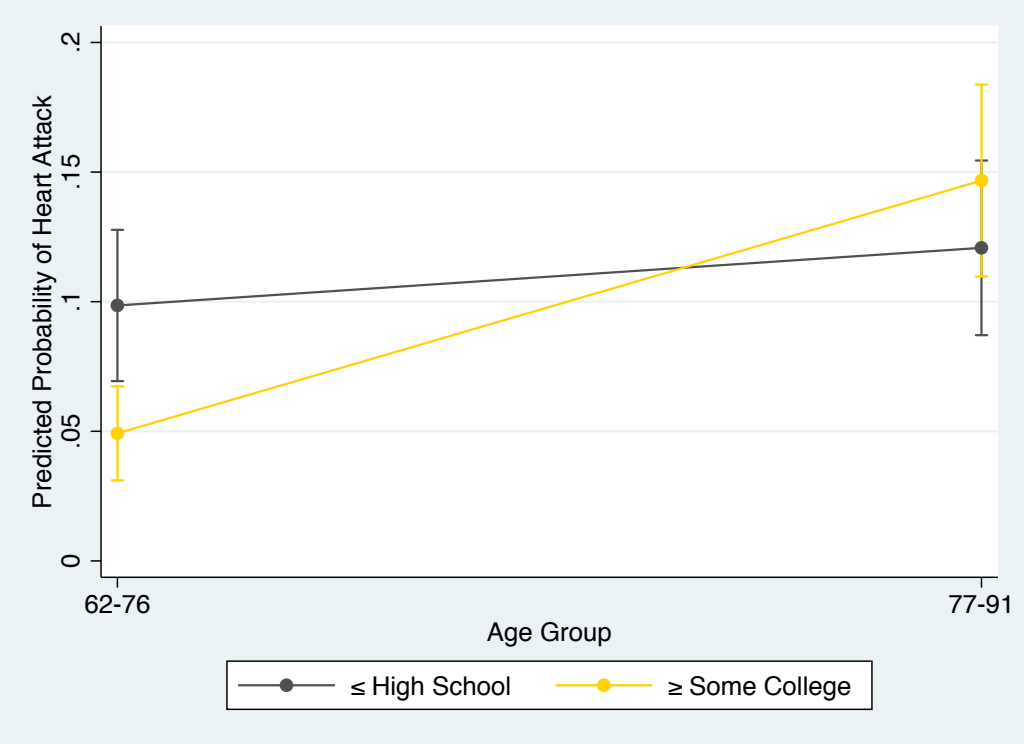

Figure 3.4. Education Effects on Heart Attack Prevalence

Table 3.4

Effects of Higher Education on US Older Adults Prevalence of Heart Attack

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-1.62***	0.19(0.04)	[0.137, 0.287]
Sociodemographics			
Female	-1.62***	0.20(0.04)	[0.126, 0.312]
Age Group (77-91)	-0.16	0.98(0.26)	[0.579, 1.675]
≥ College	-0.77**	0.46(0.12)	[0.272, 0.782]
Interactions			
Female x 77-91	0.84**	2.31(0.59)	[1.382, 3.861]
77-91 x ≥ College	1.00**	2.72(0.93)	[1.375, 5.396]

***Note*: N = 2,320; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

Prevalence of Congestive Heart Failure (CHF)

Finally, I examined the prevalence of CHF in older U.S. adults. Among adults aged 62-91 years of age in the US, women were diagnosed with CHF at the same rate as men (3.8% vs. 5.8%; X^2 (1, N = 2,309) = 7.36, p = 0.0671). The prevalence of CHF diagnosis increased with age: those 77-91 were 94% more likely than those aged 62-76 to be diagnosed with CHF (t (50) = 2.70, p = 0.009; Table 3.5; Figure 3.5). There was no interaction of gender and age.

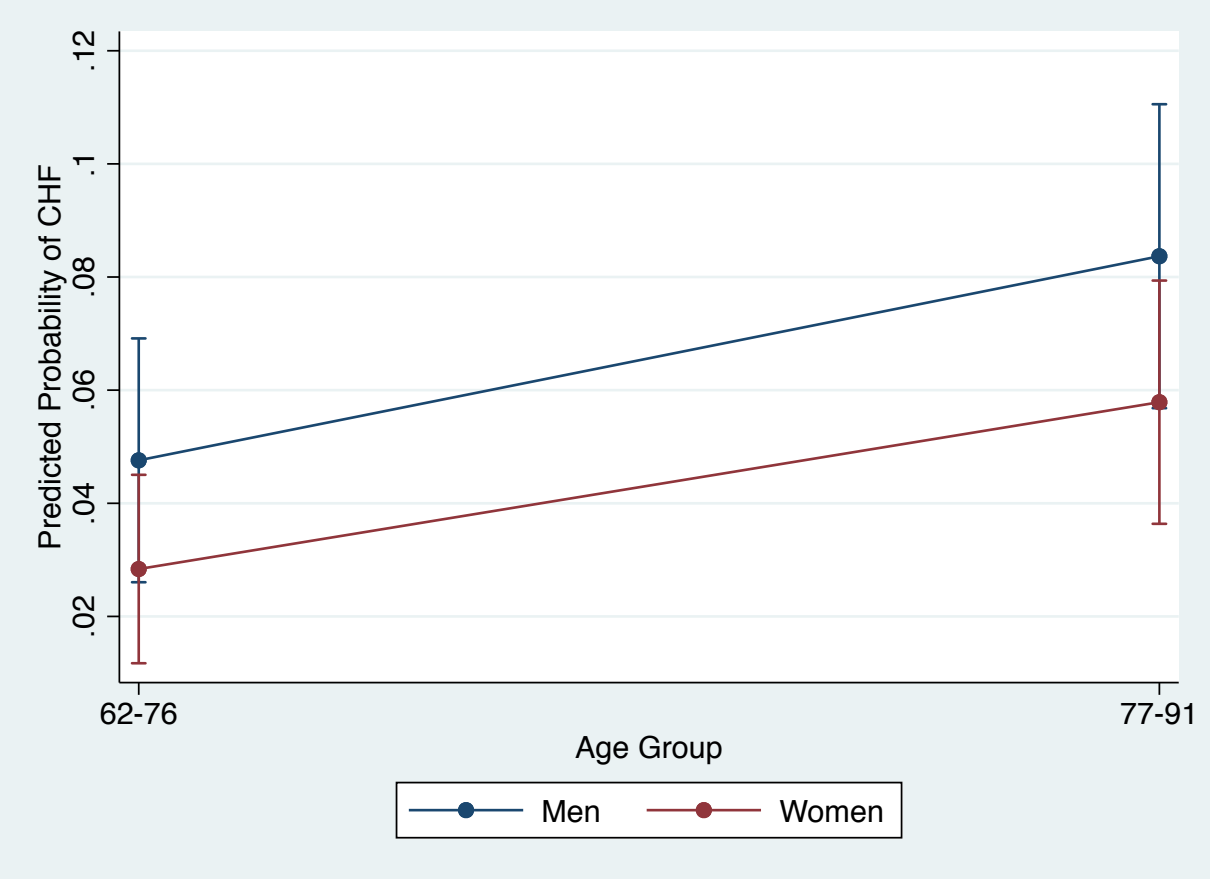

Figure 3.5. US Older Adults Probability of CHF

Table 3.5

Effects of Gender and Age on US Older Adults Prevalence of CHF

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-3.02***	0.05(0.01)	[0.033, 0.072]
Female	-0.48	0.62(0.15)	[0.383, 1.008]
Age Group (77-91)	0.66**	1.94(0.48)	[1.186, 3.183]

***Note*: *N* = 2,309; * = *p*<0.05, **=*p*<0.01, ***=*p*<0.001**

There were a few statistically significant findings when looking at the sociodemographics of race/ethnicity and SES/higher education. Black respondents were 46% less likely than whites to have undergone surgical treatment for CAD (t (50) = -2.16, p = 0.035). Participants who had attended at least some college were 39% less likely than those without to have CAD treatment (t (50) = -2.52, p = 0.015). There was an interaction of higher education and age; while college education seems protective of those aged 62-76, higher education has no effect for those aged 77-91 (t (50) = 2.85, p = 0.006; Table 3.6; Figure 3.6).

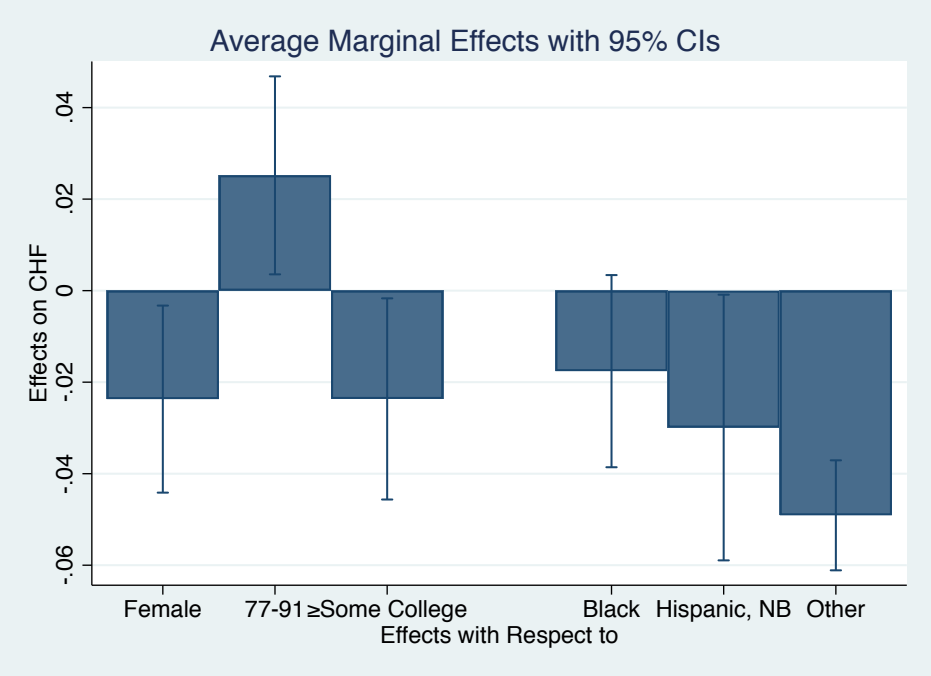

Figure 3.6. Sociodemographic Effects on Congestive Heart Failure

Table 3.6

Effects of Race/Ethnicity and Higher Education on US Older Adults Prevalence of CHF

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-2.57***	0.08(0.02)	[0.044, 0.134]
Sociodemographics			
Female	-0.53*	0.59(0.14)	[0.367, 0.940]
Age Group (77-91)	0.57*	1.76(0.48)	[1.021, 3.049]
≥ Some College	-0.53*	0.59(0.13)	[0.371, 0.928]
Race/Ethnicity			
Black	-0.43	0.65(0.19)	[0.362, 1.158]
Hispanic, NB	-0.89	0.41(0.24)	[0.124, 1.352]
Other	-2.92	0.05(0.06)	[0.007, 0.424]

***Note*: *N* = 2,301; * = *p*<0.05, ** = *p* < 0.01, *** = *p* < 0.001**

After examining the major sociodemographics, I looked at the classic cardiovascular disease factors including diabetes, high blood pressure, smoking, overweight status, physical activity level, and high TC for each of the three heart disease conditions. For many reasons, but mainly to find out how factors affect people differently, I decided to run the regression analyses separately for men and women.

Regarding prevalence of CAD, it appears that while classic cardiovascular health factors, namely diabetes and high blood pressure, have an effect on the treatment of CAD in men, they do not in women (See tables 3.7 & 3.8; Figure 3.7). Additionally, sociodemographics have an effect on the treatment of CAD in women while they do not in men (See tables 3.7 & 3.8; Figure 3.7).

Table 3.7

Effects of Cardiovascular Health Factors on US Older Women Prevalence of CAD

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-3.67**	0.15(0.09)	[0.046, 0.504]
Sociodemographics			
Age Group (77-91)	-0.49	0.61(0.25)	[0.268, 1.392]
≥ College	-0.91*	0.40(0.16)	[0.185, 0.880]
Race/Ethnicity			
Black	-1.01	0.36(0.23)	[0.102, 1.285]
Hispanic, NB	-1.44*	0.24(0.13)	[0.079, 0.703]
Other	1.00	(empty)	
Interactions			
77-91 x ≥ College	1.50**	4.49(2.16)	[1.715, 11.78]

Table 3.7 continued

Black x 77-91	-1.06	0.35(0.44)	[0.027, 4.492]
Hispanic x 77-91	2.16**	8.70(5.50)	[2.445, 30.95]
Cardiovascular Health Factors			
Diabetes	0.49	2.19(0.88)	[0.986, 4.896]
High Blood Pressure	0.12	1.39(0.61)	[0.582, 3.341]
Smoking	0.08	1.08(0.43)	[0.482, 2.419]
Alcohol Use	-0.24	0.50(0.21)	[0.216, 1.138]
Overweight	0.02	0.73(0.25)	[0.370, 1.440]
Physical Activity	-0.19	0.74(0.24)	[0.382, 1.405]
High TC	-0.98	0.67(0.33)	[0.247, 1.816]

***Note*: $N = 950$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

Table 3.8

Effects of Cardiovascular Health Factors on US Older Men Prevalence of CAD

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-1.46***	0.31(0.10)	[0.162, 0.575]
Sociodemographics			
Age Group (77-91)	1.27	0.86(0.28)	[0.447, 1.645]
≥ College	-0.53	0.67(0.15)	[0.432, 1.038]
Race/Ethnicity			
Black	-1.09	0.52(0.20)	[0.241, 1.136]
Hispanic, NB	0.88	0.88(0.23)	[0.512, 1.500]
Other	0.13	1.05(0.65)	[0.308, 3.607]

Table 3.8 continued

Interactions			
77-91 x ≥ College	1.50*	2.17(0.75)	[1.076, 4.359]
Cardiovascular Health Factors			
Diabetes	0.49**	1.74(0.30)	[1.234, 2.464]
High Blood Pressure	0.12*	1.80(0.43)	[1.110, 2.911]
Smoking	0.08	0.66(0.17)	[0.394, 1.107]
Alcohol Use	-0.24	0.76(0.15)	[0.510, 1.124]
Overweight	0.02*	0.66(0.12)	[0.457, 0.965]
Physical Activity	-0.19	0.84(0.16)	[0.569, 1.202]
High TC	-0.98*	0.40(0.22)	[0.133, 0.801]

***Note*: $N = 897$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

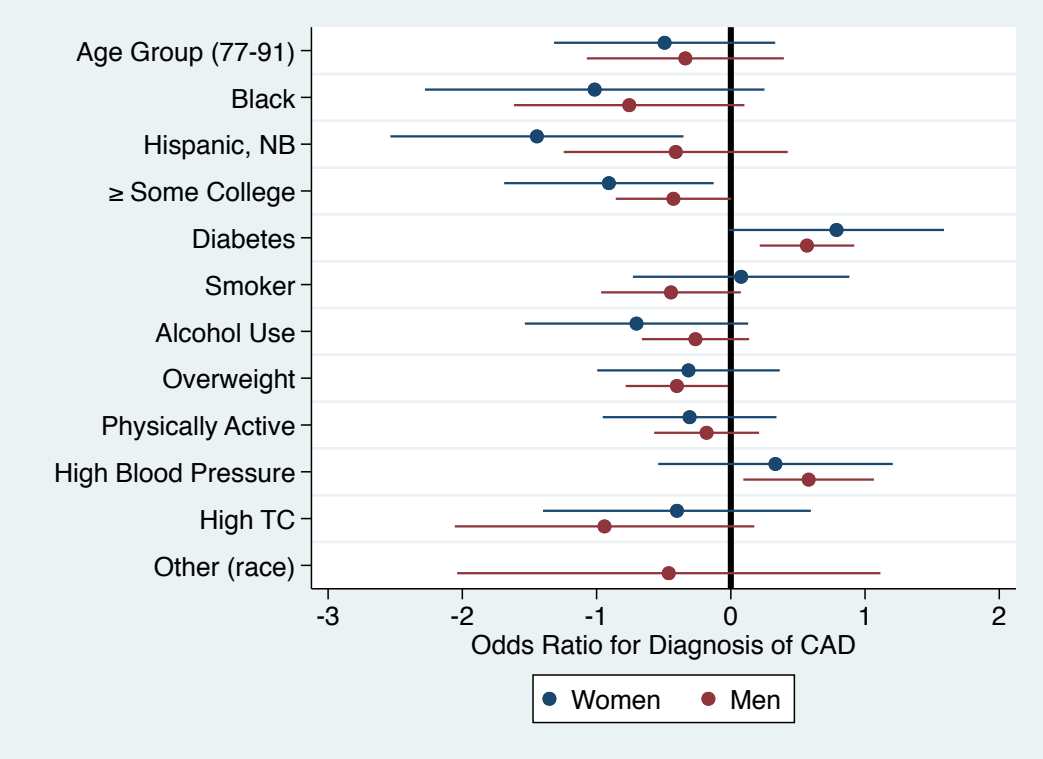

Figure 3.7. CVD Health Factors Differ by Gender for CAD

I turn next to the diagnosis and survival of heart attacks in older U.S. adults. Similar to CAD, the effects of sociodemographics and CVD health factors on heart attack differ for men and women. Age is the biggest factor for women, while no CVD health factors appear to matter. Meanwhile for men, diabetes has the largest effect on heart attack diagnosis and survival. See tables 3.9, 3.10, and Figure 3.8.

Table 3.9

Effects of Cardiovascular Health Factors on US Older Women Prevalence of Heart Attack

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-4.47***	0.01(0.01)	[0.003, 0.044]
Sociodemographics			
Age Group (77-91)	1.50***	4.46(1.45)	[2.327, 8.565]
≥ College	-0.49	0.61(0.26)	[0.262, 1.444]
Race/Ethnicity			
Black	-0.76	0.47(0.21)	[0.188, 1.159]
Hispanic, NB	-1.05	0.35(0.25)	[0.085, 1.446]
Other	-1.81	0.16(0.17)	[0.020, 1.359]
Cardiovascular Health Factors			
Diabetes	0.71	2.03(0.98)	[0.765, 5.359]
High Blood Pressure	0.73	2.08(1.10)	[0.720, 5.989]
Smoking	0.09	1.09(0.64)	[0.336, 3.561]
Alcohol Use	0.01	1.01(0.45)	[0.412, 2.462]
Overweight	0.44	1.56(0.66)	[0.662, 3.650]

Table 3.9 continued

Physical Activity	-0.00	1.00(0.43)	[0.420, 2.380]
High TC	-1.25	0.29(0.28)	[0.039, 2.095]

***Note*: $N = 972$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

Table 3.10

Effects of Cardiovascular Health Factors on US Older Men Prevalence of Heart Attack

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-1.46**	0.30(0.15)	[0.110, 0.807]
Sociodemographics			
Age Group (77-91)	-0.21	0.72(0.27)	[0.346, 1.511]
≥ College	-0.56	0.46(0.15)	[0.239, 0.888]
Race/Ethnicity			
Black	0.55	0.55(0.23)	[0.235, 1.265]
Hispanic, NB	0.82	0.82(0.25)	[0.438, 1.529]
Other	1.83	1.83(1.18)	[0.503, 6.679]
Interactions			
77-91 x ≥ College	1.32*	3.04(1.51)	[1.124, 8.227]
Cardiovascular Health Factors			
Diabetes	0.70**	2.03(0.41)	[1.349, 3.059]
High Blood Pressure	-0.12	1.02(0.30)	[0.566, 1.823]
Overweight	-0.02	0.85(0.40)	[0.326, 2.194]
Physical Activity	-0.25	0.78(0.16)	[0.507, 1.185]

Table 3.10 continued

Smoking	-0.10	1.06(0.34)	[0.562, 2.010]
Alcohol Use	-0.41*	0.71(0.16)	[0.454, 1.120]
High TC	-0.41*	0.41(0.15)	[0.159, 1.076]

***Note*: $N = 894$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$**

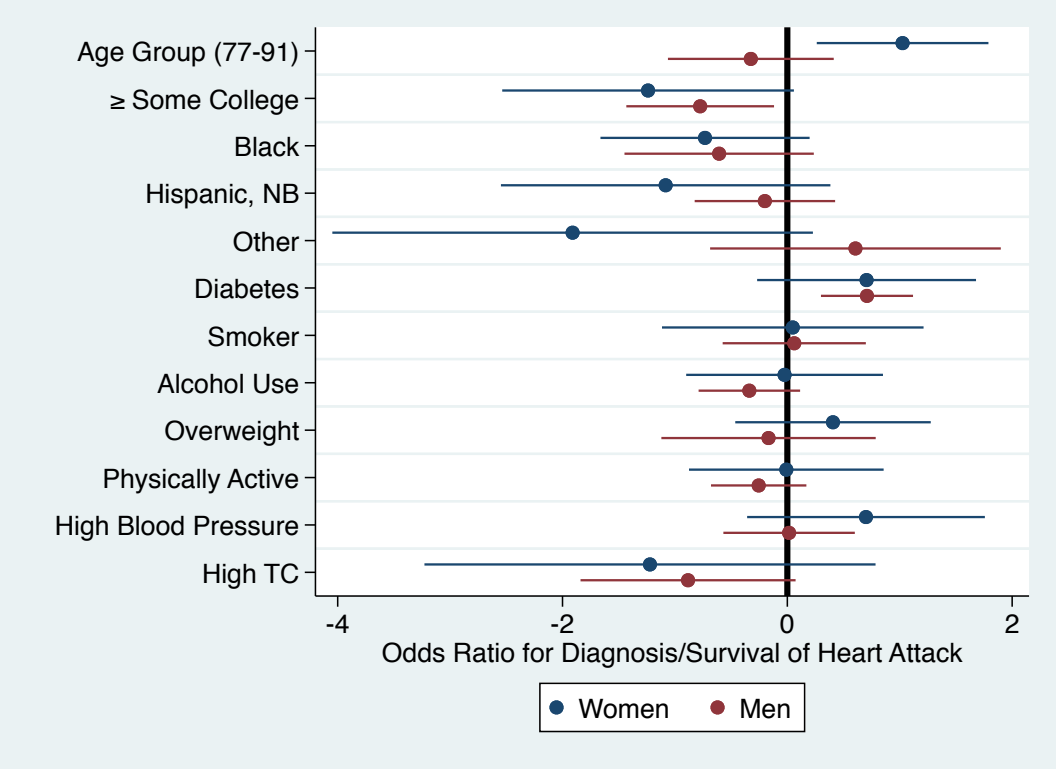

Figure 3.8. CVD Health Factors Differ by Gender for Heart Attack Diagnosis

Lastly, I look at CHF. The story is again quite similar. For women, black participants were less likely to have been diagnosed, and those diagnosed were over 4 times more likely to have high blood pressure at the time of the survey (see table 11). For older men, neither race or higher education was related to a CHF diagnosis, but they were more likely to have CHF if they had diabetes, high blood pressure, or were less likely to be physically active (see table 12). You can also look to Figure 9 to better see the differences for men and women.

Table 3.11

Effects of Cardiovascular Health Factors on US Older Women Prevalence of CHF

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-3.68***	0.03(0.02)	[0.006, 0.099]
Sociodemographics			
Age Group (77-91)	0.31	1.36(0.57)	[0.587, 3.173]
≥ College	-0.68	0.51(0.22)	[0.209, 1.228]
Race/Ethnicity			
Black	-1.25*	0.29(0.16)	[0.093, 0.887
Hispanic, NB	0	(empty)	
Other	-2.02	0.13(0.14)	[0.016, 1.091]
Cardiovascular Health Factors			
Diabetes	0.44	1.55(0.58)	[0.725, 3.293]
High Blood Pressure	1.48**	4.38(2.38)	[1.469, 13.06]
Smoking	-0.98	0.38(0.24)	[0.103, 1.370]
Alcohol Use	-0.15	0.86(0.39)	[0.349, 2.123]
Overweight	-0.29	0.75(0.32)	[0.316, 1.760]
Physical Activity	-0.62	0.54(0.25)	[0.212, 1.363]
High TC	-0.37	0.69(0.39)	[0.221, 2.158]

***Note*: *N* = 883; * = *p*<0.05, **=*p*<0.01, ***=*p*<0.001**

Table 3.12

Effects of Cardiovascular Health Factors on US Older Men Prevalence of CHF

Predictors	**β Coefficient**	**Odds Ratio(SE)**	**95% Confidence Interval**
Constant	-2.84**	0.06(0.05)	[0.012, 0.289]
Sociodemographics			
Age Group (77-91)	0.41	1.51(0.68)	[0.613, 3.715]
≥ College	-0.38	0.68(0.22)	[0.357, 1.299]
Race/Ethnicity			
Black	-0.66	0.52(0.28)	[0.179, 1.504]
Hispanic, NB	-0.90	0.41(0.24)	[0.126, 1.309]
Other	1	(empty)	
Cardiovascular Health Factors			
Diabetes	0.73*	2.07(0.61)	[1.150, 3.723]
High Blood Pressure	1.53*	4.63(3.01)	[1.255, 17.11]
Overweight	-0.71	0.49(0.27)	[0.162, 1.495]
Physical Activity	-1.14*	0.32(0.14)	[0.133, 0.769]
Smoking	-0.38	0.69(0.35)	[0.247, 1.903]
Alcohol Use	-0.31	0.73(0.25)	[0.366, 1.457]
High TC	-0.38	0.68(0.42)	[0.196, 2.375]

***Note*: *N* = 868; * = *p*<0.05, **=*p*<0.01, ***=*p*<0.001**

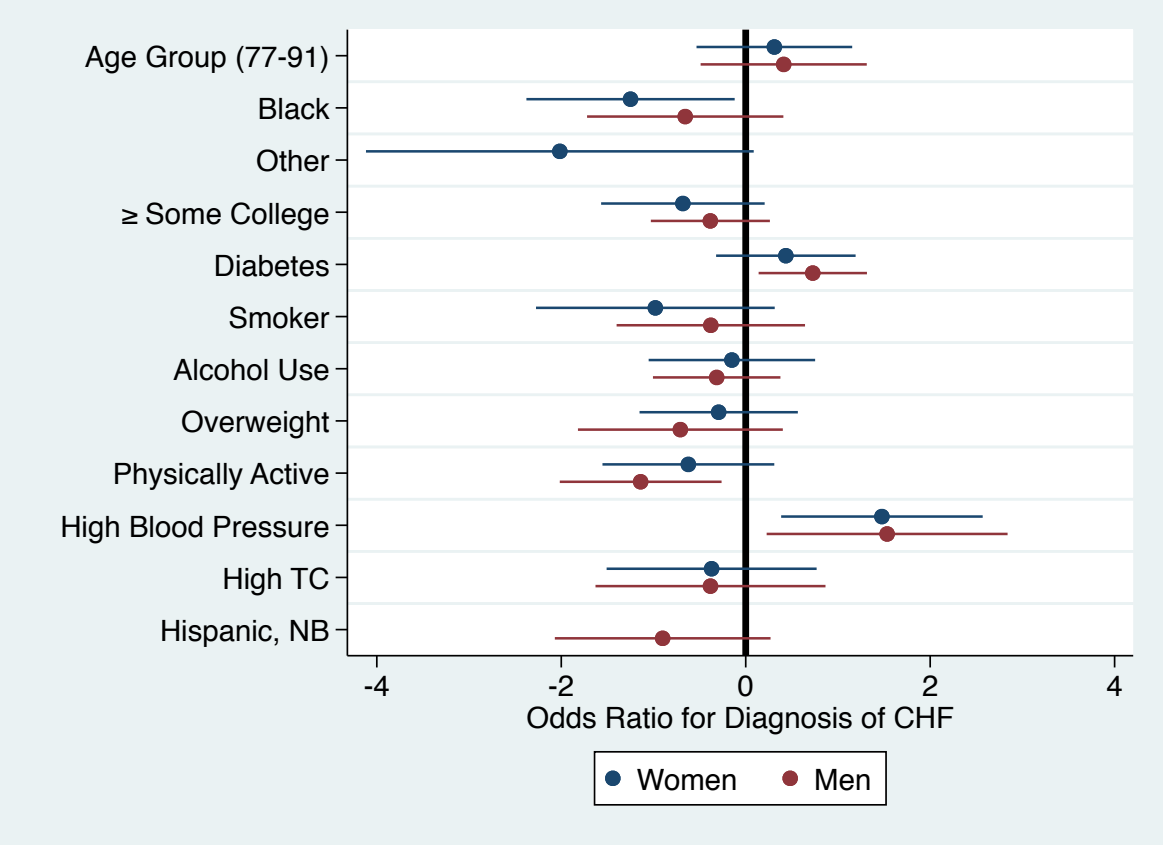

Figure 3.9. CVD Health Factors Differ by Gender for CHF Diagnosis

Discussion

To summarize, we found similarities and discrepancies between previous data and ours in NSHAP. Regarding traditional risk factors for cardiovascular disease; rates of diabetes, physical activity, and total lipids were similar while rates of smoking and being overweight were higher in the NSHAP sample than have been previously reported at the population level (Mozaffarian et al., 2016). Specifically, we found that 11.0% of women smoke, which is over 3% more than was reported in the 2016 update (Mozaffarian et al., 2016). Based on meta-analytic data from 2.4 million people worldwide, women smokers are 25% more at risk for CHD than their smoking male counterparts (Huxley & Woodward, 2011). Further, just the increase alone in rates of being overweight is alarming – we found that, based on calculated BMI, 72.3% of women and 82.6%

of men were overweight. Previous calculations report that 65% of women and 73% of men are overweight (Mozaffarian et al., 2016).

Moving on to disease prevalence, our data support the literature that prevalence of CAD, heart attack, and CHF increases with age. Women are less likely than men to be diagnosed with CAD or heart attack. However, in the case of CHF, we found no differences in prevalence rates between men and women at older ages.

Even further, we were able to provide a more detailed description of traditional risk factors tied to these diseases in older age. While there are nuances, the big picture is that the classic cardiovascular disease factors (diabetes, high blood pressure, smoking, overweight status, physical activity level, & high TC) still matter in older age – for men. For women, the story is different. The only relationship we found was that those women diagnosed with CHF were over 4 times more likely to have high blood pressure. We found many more associations when looking at sociodemographic factors: race and education matter in the cardiovascular health of women.

This is important because it supports the idea that classical guidelines are not appropriate for both men and women at older ages. The fact is that these diseases are killing women and older people, yet the diagnostic criteria and treatment options are geared towards men. This research shows that we need to look into what the risk factors are, specifically for women and older adults.

References

Abuful, A., Gidron, Y., & Henkin, Y. (2005). Physicians' attitudes toward preventive therapy for coronary artery disease: Is there a gender bias? *Clinical Cardiology*, *28*(8), 389-393.

American Diabetes Association. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 33*(Supplement 1), S62-S69.

Agrinier, N., Cournot, M., Dallongeville, J., Arveiler, D., Ducimetiere, P., Ruidavets, J. B., & Ferrieres, J. (2010). Menopause and modifiable coronary heart disease risk factors: A population based study. *Maturitas*, *65*(3), 237-243.

Artinian, N. T., Fletcher, G. F., Mozaffarian, D., Kris-Etherton, P., Van Horn, L., Lichtenstein, A. H., ... & Meininger, J. C. (2010). Interventions to promote physical activity and dietary lifestyle changes for cardiovascular risk factor reduction in adults. A scientific statement from the American Heart Association. *Circulation*, *122*, 406–441.

Carroll, M. D., Kit, B. K., Lacher, D. A., & Yoon, S. S. (2012). Total and high-density lipoprotein cholesterol in adults: National Health and Nutrition Examination Survey, 2009-2010 (pp. 1-8). US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.

Cowie, C. C., Rust, K. F., Ford, E. S., Eberhardt, M. S., Byrd-Holt, D. D., Li, C., ... & Geiss, L. S. (2009). Full accounting of diabetes and pre-diabetes in the US population in 1988–1994 and 2005–2006. *Diabetes Care*, *32*(2), 287-294.

Crescioni, M., Gorina, Y., Bilheimer, L., & Gillum, R. F. (2010). Trends in health status and health care use among older men. Hyattsville, MD: National Center for Health Statistics; 2010. National Health Statistics Report No. 24.

Crim, M. T., Yoon, S. S. S., Ortiz, E., Wall, H. K., Schober, S., Gillespie, C., ... & Hong, Y. (2012). National surveillance definitions for hypertension prevalence and control among adults. *Circulation: Cardiovascular Quality and Outcomes*, 111.

Dabla, P. K., Dabla, V., Dawar, R., & Arora, S. (2011). Approach To Postmenopausal Cardiovascular Risk. *International Journal of Pharmaceutical Sciences Review & Research*, *6*(2), 136-143.

de Torbal, A., Boersma, E., Kors, J. A., van Herpen, G., Deckers, J. W., van der Kuip, D. A., ... & Witteman, J. C. (2006). Incidence of recognized and unrecognized myocardial infarction in men and women aged 55 and older: The Rotterdam Study. *European Heart Journal*, *27*(6), 729-736.

DeVon, H. A., Ryan, C. J., Ochs, A. L., & Shapiro, M. (2008). Symptoms across the continuum of acute coronary syndromes: Differences between women and men. *American Journal of Critical Care*, *17*(1), 14-24.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., ... & Stroke, S. S. (2013). Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*, *127*(1), e6.

Gottdiener, J. S., Arnold, A. M., Aurigemma, G. P., Polak, J. F., Tracy, R. P., Kitzman, D. W., ... & Boineau, R. C. (2000). Predictors of congestive heart failure in the elderly: The Cardiovascular Health Study. *Journal of the American College of Cardiology*, *35*(6), 1628-1637.

Gulati, M., Shaw, L. J., Merz, B., & Noel, C. (2012). Myocardial ischemia in women: Lessons from the NHLBI WISE study. *Clinical Cardiology*, *35*(3), 141-148.

Hanratty, B., Lawlor, D. A., Robinson, M. B., Sapsford, R. J., Greenwood, D., & Hall, A. (2000). Sex differences in risk factors, treatment and mortality after acute myocardial infarction: An observational study. *Journal of Epidemiology and Community Health*, *54*(12), 912-916.

Huisingh-Scheetz, M., Kocherginsky, M., Schumm, P.L., Engelman, M., McClintock, M.K., Dale, W., Magett, E., Rush, P., & Waite, L. (2014). Geriatric syndromes and functional status in NSHAP: Rationale, measurement, and preliminary findings. *Journals of Gerontology, Series B: Psychological Sciences and Social Sciences, 69*(8), S177–S190.

Huxley, R. R., & Woodward, M. (2011). Cigarette smoking as a risk factor for coronary heart disease in women compared with men: A systematic review and meta-analysis of prospective cohort studies. *The Lancet*, *378*(9799), 1297-1305.

Kannel, W. B., Hjortland, M. C., McNamara, P. M., & Gordon, T. (1976). Menopause and risk of cardiovascular disease: The Framingham study. *Annals of Internal Medicine*, *85*(4), 447-452.

Kochanek, K.D., Xu, J., Murphy, S.L., Miniño, A.M., & Kung, H.C., (2011). Deaths: Final data for 2009. *National Vital Statistics Reports, 60*(3), 1-117.

Kudenchuk, P. J., Maynard, C., Martin, J. S., Wirkus, M., Weaver, W. D., & MITI Project Investigators. (1996). Comparison of presentation, treatment, and outcome of acute myocardial infarction in men versus women (the Myocardial Infarction Triage and Intervention Registry). *The American Journal of Cardiology*, *78*(1), 9-14.

International Expert Committee. (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*, *32*, 1327–1334.

Long, T., Taubenheim, A. M., Wayman, J., Temple, S., & Ruoff, B. A. (2008). The Heart Truth: Using the power of branding and social marketing to increase awareness of heart disease in women. *Social Marketing Quarterly*, *14*(3), 3-29.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., ... & Howard, V. J. (2016). Heart disease and stroke statistics—2016 update. *Circulation*, *133*(4), e38-e360.

Murray, C. J., Abraham, J., Ali, M. K., Alvarado, M., Atkinson, C., Baddour, L. M., ... & Bolliger, I. (2013). The state of US health, 1990-2010: Burden of diseases, injuries, and risk factors. *JAMA*, *310*(6), 591-606.

Nwankwo, T., Yoon, S. S., Burt, V., & Gu, Q. (2013). Hypertension among adults in the United States: National Health and Nutrition Examination Survey, 2011-2012. *NCHS Data Brief*, (133), 1-8.

Shaw, L. J., Merz, C. N. B., Pepine, C. J., Reis, S. E., Bittner, V., Kelsey, S. F., ... & Sopko, G. (2006). Insights from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study Part I: Gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. *Journal of the American College of Cardiology*, *47*(3s1), S4-S20.

Shirato, S., & Swan, B. A. (2009). Women and cardiovascular disease: An evidentiary review. *Medsurg Nursing: Official Journal of the Academy of Medical-Surgical Nurses*, *19*(5), 282-286.

Chapter 4: Sex Differences in Markers of Inflammation and Heart Disease

The biological process of inflammation plays a role in both myocardial infarction (MI) and congestive heart failure (CHF). Atherosclerosis, a systemic disease process in which fatty deposits (trapped low-density lipoprotein (LDL) molecules), inflammation, cells, and scar tissue build up within the arteries, is the underlying cause of the majority of clinical cardiovascular events (Stary et al., 1992). Over the past couple of decades there has been a vast effort to include inflammatory molecules as biomarkers of cardiovascular disease. Researchers have consistently found links between measures of inflammation and prevalence of heart disease. However, this research has thus far been unable to provide consistent evidence in order to establish guidelines for risk prediction or disease treatment (Koening & Khuseyinova, 2006). Inconsistencies in results may be due to variations among participants in regards to their gender, age, and specific disease state. Yet, as described, much research on cardiovascular risk assessment and categorization is based on limited populations not generalizable to women or, more broadly, older adults.

In this regard, NSHAP again delivers an exceptional opportunity to examine controversies existing in the literature concerning age and sex, as the data are representative of a non-clinical population and are restricted to those aged 57 to 85. Along with the aforementioned measures of heart health the project collected biomeasures of inflammation, which are non-specifically (C-reactive protein) and specifically (monocyte chemoattractant protein–1) related to cardiovascular inflammatory processes (see Special Issue papers for specific details of Wave 2 data collection). In addition, all NSHAP interviews were conducted when participants are at home, decreasing the amount of stress experienced while being measured, which will assumedly produce results that are more ecologically valid, especially for biomarkers.

C-Reactive Protein (CRP). Elevated levels are associated with increased risk of CHD in women and men (Pai et al., 2004). For example, in the National Health and Nutrition Examination Survey (NHANES) data, which is a multiethnic sample of healthy adults aged 30-74 representative of the U.S. population, CRP was related to the 10-year estimated risk of CHD in both men and women (Wong, Pio, Valencia, & Thakal, 2001). While it is not clear if CRP is related to all types of CHD, elevated CRP levels have been associated with an increased risk of heart failure in many population wide studies (Gottdiener et al., 2000; Vasan et al., 2003; Cesari et al., 2003; Kardys et al., 2006; Bekwelem et al., 2007), and CRP levels have also been correlated with severity of CHF (Huang et al., 2004). In fact, among all known cardiovascular diseases, CRP has been found to be most associated with CHF (Rosen et al., 2007).

However, there are noted differences in CRP level based on age and sex. While one of the first studies linking CRP to increased risk of CHD was conducted solely on men aged 35-57 (Kuller, Tracy, Shaten, & Meilahn, 1996), many studies have begun to look at broader age rages in both men and women. In the NHANES III study, CRP levels increased with age (10-year intervals) for men, but did not for women (Wong et al., 2001). Also in the NHANES III data, women were more likely than men to have elevated levels of CRP, and while CRP was associated with 10-year estimated risk of CHD, the probabilities for women were anywhere from 10-15% less than that of men (Wong et al., 2001). The Cardiovascular Health Study (CHS), a sample of primarily white men and women aged 55 and over, also showed that CRP levels were higher in women, but, in contrast, CRP levels provided prediction for CHD death more accurately in men than in women (Cushman et al., 2005). In the Health, Aging, and Body Composition (ABC) Study, representative of U.S. women and men aged 70-79 without existing CHD, high CRP levels were associated with incidence of CHF, but not of MI, angina pectoris, or

stroke (Cesari et al., 2003), but this study did not look at differences based on sex or age. Data from the Cardiovascular Health Study, a population based study on Americans over the age of 65, show that CRP is related to CHF more strongly in women than it is in men (Gottdiener et al., 2000). Further, in Americans aged 45-64, women comprise a larger proportion of people with CRP > 3mg/L than men, and CRP levels >3 mg/L drove the association with increased risk of HF (Bekwelem et al., 2007). However, in the Framingham Heart Study, which is representative of Americans over the age of 65, men had higher CRP levels than women, and elevated CRP levels were related to incidence of CHF only when established risk factors were not accounted for (Vasan et al., 2003). Additionally, in the Rotterdam Study, on healthy men and women 55+ living in the Netherlands, elevated CRP levels were associated with heart failure in men, but only in women with prevalent CHD when not accounting for traditional risk factors (Kardys et al., 2006).

Overall, the use of CRP as an indicator of heart disease is well established in the literature. However, in addition to inconsistencies in statistical methods, the data from these studies express that CRP levels are dependent on age, sex and specific diagnosis of participants. There is a strong need to determine whether elevated levels of CRP have the same relationship to heart disease for women and men of increasing age, and for exactly which types of heart disease this association holds true. It is possible that CRP has different physiological influences for men and women, presumably because of distinctive biological pathways, such as those involving sex hormones. Further, because CRP is a broad marker of inflammation, it may be indicating disease other than that of the cardiovascular system.

Monochemoattractant Protein (MCP)-1. Monocyte chemoattractant protein (MCP)-1 is directly involved in atherogenesis, as it is the most important chemokine in regulating the

migration of macrophages (Koening & Khuseyinova, 2006). MCP-1 is specifically involved in heart disease, as it causes chronic inflammation in vasculature, induces proliferation and migration of smooth muscle and endothelial cells, oxidative stress, and neovascularization in plaques, and thrombosis (Egashira, 2003). A relationship between MCP-1 and plaque destabilization is supported as well (Koening & Khuseyinova, 2006).

In fact, large case-cohort studies have found that elevated levels of MCP-1 predicted future CHD events. The Dallas Heart Study, a population based study of adults aged 30 to 65, found that MCP-1 levels increase with age, did not differ between men and women, were only mildly correlated with CRP, but were correlated with levels of subclinical atherosclerosis (Deo et al., 2004). In the MONICA/KORA study, a population based Augsburg cohort study of men and women ages 35 – 74 years old, baseline MCP-1 levels increased with age, were higher in men, and were higher in participants of both sexes who, at the 11-year follow up, had experienced sudden cardiac death, fatal MI, or nonfatal MI compared to those that had not (Herder et al., 2006). In the A to Z (Aggrastat to Zocor) trial, a large population study of patients (21-80 years old) stabilized after acute coronary syndrome (ACS), MCP-1 levels were higher in women and participants with prior MI or CHF, increased with age, and levels > 238 pg/ml were associated with mortality, MI, CHF, readmission for ACS, and stroke even after controlling for other risk factors and CRP levels (de Lemos et al., 2007). This study showed that MCP-1 levels more accurately predicted major adverse coronary events than did CRP levels, that the two biomarkers were not correlated, and suggested that MCP-1, reflecting atherosclerotic burden and plaque vulnerability, is a marker of chronic pathophysiological processes rather than an acute marker like CRP (de Lemos et al., 2007).

Overall, there are key discrepancies in the existing literature. Women have a higher prevalence of traditional CHD risk factors, but men have higher rates of incidence than women, until after menopause when incidence rates are similar. Further, women and physicians are unaware of the high risk of heart disease for women, who usually present symptoms atypical of the commonly accepted male model. Together, this leads to less aggressive treatment in women. In addition, rates of MI are higher in men, but there is no consistency in the data on rates of CHF. In regards to novel biomarkers of heart disease, inconsistencies are also apparent. There is contradictory evidence to whether CRP levels differ based on sex and age, whether levels are related to only certain types of heart disease, and if CRP is actually related independently to heart disease or functions best as a broad marker of inflammation. Unfortunately, studies on a more specific marker of heart disease, MCP-1, are not as abundant as those on CRP, so there are still questions regarding its role in heart disease for elderly men and women.

Therefore, the goals of this study were two-fold. We examined whether sex differences in the prevalence rates of heart disease were sustained in older ages. Perhaps women are wrongfully under-diagnosed and left untreated, resulting in rates that are similar between men and postmenopausal women. Further, I present representative data on rates of CAD treatment and diagnoses of MI and CHF in the elderly. In addition, I explored whether MCP-1 is more sensitive to differences in gender and age than CRP, a standard but non-specific biomarker.

Methods

Whole Blood Biosamples. In addition to the report of physician diagnoses, and to provide a richer characterization of their health, several corresponding biosamples were collected: whole blood collected on filter paper and in a Microtainer®, saliva, and urine, as well as biomeasures for anthropometrics, blood pressure and heart rate, and physical performance

measures. This study focuses on chemical indicators of atherosclerosis and heart disease measured in whole blood, both on filter paper and in the Microtainer. For information on tracking, shipping and cataloguing of the biosamples, see O'Doherty, et al. (2014).

Briefly, in Wave 2 of NSHAP, the field interviewer collected the respondent's blood on pretreated paper that was air dried for 24 hours, stored, and then shipped weekly to the Department of Medicine Biomarker Analysis Laboratory at the University of Washington. In Wave 2 of NSHAP, participants were instructed to hold hand-warmers to improve circulation in order to increase blood flow and maximize the amount of blood collected. This new protocol enabled the collection of additional biomeasures not feasible with dried blood spot protocols. After died blood spots were collected, 5 drops (250 microliters (µl)) of whole blood was collected and stored in a Microtainer®; a small, unbreakable plastic tube with a FloTop collector which facilitates fast and efficient blood collection. To prevent the blood from clotting, the microtainers were coated with Dipotassium Ethylenediaminetetraacetic acid (K_2EDTA), an anticoagulant that binds to calcium in the blood needed for clot formation. Uncoagulated whole blood was spun down and the plasma frozen at the University of Chicago Flow Cytometry Core Facility. All samples were void of personal identification material and were given unique laboratory ID's in order to match the biomarker information with the interviews to ensure confidentiality.

Laboratory Assays

CRP. C-reactive protein (CRP), which is produced by the liver and serves as a broad marker of inflammation as well as a risk factor for cardiovascular disease, was measured from the dried blood spots. The University of Washington (UW), Department of Medicine Biomarker Analysis Laboratory assayed the dried blood spots.

According to the American Heart Association (AHA) and the Centers for Disease Control (CDC), serum levels of CRP are categorized as "low" if less than 1 mg/L, between 1 and 3 mg/L is "average", and levels between 3 and 10 mg/L are considered "high" risk for cardiovascular disease, while CRP serum levels greater than 10 mg/L reflect an acute infection (Smith et al., 2004). However, NSHAP directly assayed CRP from dried blood spots, which have been shown to lead to slightly lower values than when measured in serum (McDade, Burhop, & Dohnal, 2003). Although the lab did determine the plasma equivalent using a conversion calculation, we chose to use the directly assayed serum values. Therefore, the CRP cardiovascular risk categories are adjusted to less than 0.76 mg/L is "low", levels from 0.76 to 2.5 mg/L are "average", levels from 2.5 to 8.6 mg/L are "high", and anything over 8.6 mg/L is "acutely high" (McDade et al., 2003).

MCP-1. Monocyte chemoattractant protein (MCP) -1, which is produced by macrophages, monocytes, smooth muscle cells, and endothelial cells within atherosclerotic plaques, promotes atherosclerosis when at increased levels (de Lemos, et al., 2007). Atherosclerosis can be described as an inflammatory response to injury, and every stage of atherogenesis involves cytokines such as MCP-1.

The University of Chicago Flow Cytometry Core Facility assayed the microtainers of whole blood. Eighteen-analyte assay panels were run with a Bio-Plex system driven by Luminex xMAP technology. In this assay there are 18 separate bead sets, each internally dyed with a specific fluorescence, and then conjugated with antibodies that bind the analyte of interest. Each bead has antibodies for one particular molecule and a corresponding uniquely colored fluorescence. The plasma sample is then combined with the beads, dilutions are added if necessary, the mixture is stabilized, and the beads adhere to the proteins in the sample. The

mixture is then run through the Luminex reader, where beads are detected by flow cytometry and move single-file through a flow cell where red and green lasers function to detect bead color and signal strength, respectively. The analyte of interest for this study, MCP-1, had the highest number of values within the range of the standard curve, and therefore, good density of data.

Currently there are no medically established levels of MCP-1 for categorization of risk associated with heart disease. However, higher levels of MCP-1 are associated with traditional risk factors, an increased risk of myocardial infarction (Charo & Taubman, 2004), and higher risk of CVD (Herder et al., 2006).

Eligible Wave 2 Participants

Of the 3,377 participants in Wave 2, 3,366 answered the question of whether or not they had ever been doctor diagnosed as having a heart problem (2 refused, 9 did not know). Of these, 3,044 had usable dried blood spot samples with assay values of CRP. Inadequate or lost samples were the largest problems, but of those providing blood samples, 98% had usable CRP data (O'Doherty et al., 2014). There were 365 participants excluded because they had CRP values over 8.6 mg/L indicating acute infection, which yielded 2,679 participants (mean age = 72.36 ± 8.04 years, 53.56% female) within the range of normal or chronic inflammation.

Of those who answered the heart condition questions, 2,926 participants provided a sample of whole blood plasma in Microtainers. While the majority of samples yielded assay results, currently 344 samples have been reported, verified and made available for analysis. All of these 344 participants answered the heart condition questions. Three did not have usable CRP data and 43 had CRP values over 8.6 mg/L, but were still included in analyses on MCP-1 because CRP is less specific to atherosclerosis. One participant had an MCP-1 value over 8000 pg/mL, >3 SD above the mean, and was excluded as a statistical outlier from the analysis,

yielding 343 participants (mean age = 72.24 ± 7.78 years, 56.85% female) with interpretable MCP-1 values.

MCP-1 levels had a strong positively skewed distribution and were normalized by transformation to square roots from raw scores. Due to the small sample size available, it was not necessary to run survey statistics on the MCP-1 data, which were analyzed with linear regression models that included gender, age, heart condition, MI and CHF diagnoses, and CAD surgical treatment as predictors. Therefore, MCP-1 results reported here (beta coefficients, standard errors, 95% confidence intervals) are not representative or generalizable to the US national population of older adults.

Results

Biomarkers of Heart Disease

CRP. Inflammation is a key component of cardiovascular disease; high sensitivity C-reactive protein (hs-CRP) is a non-specific indicator of inflammation and is used by the medical community as a guideline for increased heart disease risk and treatment. Therefore, those diagnosed with CAD (as indicated by surgical treatment), MI, or CHF might be expected to have higher circulating levels of hs-CRP than those without these most prevalent types of heart conditions. Counter to this hypothesis, there was not a significant difference in CRP levels between those US older men or women with a heart condition, who had or did not have a specific diagnosis of CAD, MI or CHF (type of diagnosis $p = 0.534$; gender $p = 0.210$; age $p = 0.611$; Table 4.1).

These three diagnoses are typically accompanied by various treatments to reduce inflammation and risk for future cardiovascular events (Ayanian & Epstein, 1991; Go et al., 2012). Therefore, we hypothesized that participants with these specific diagnoses would have

lower inflammation at older ages due to the treatments that they may have received, whereas those without diagnoses would not. Moreover, because women were more likely to have an unspecified heart condition and tended to have higher levels of CRP indicating broad inflammation, we hypothesized that any participants, regardless of gender, who did not have surgical treatment for CAD or an MI or CHF diagnosis, would have higher levels of CRP, consistent with untreated disease.

Indeed, CRP levels were lower at older ages for the men and women who had been surgically treated for CAD or diagnosed with MI or CHF (age $p = 0.043$; gender $p = 0.162$; Table 4.1; Figure 4.1). In contrast, CRP levels did not decline significantly with age among men and women without surgical treatment or specific diagnosis of their cardiovascular disease (age $p = 0.606$; gender $p = 0.684$; Table 4.1; Figure 4.1). This shows that participants diagnosed with heart conditions but left without surgical treatments or a specific diagnosis have CRP levels that do not decrease as they age. Thus, it could be reasoned that these participants have CRP levels that do not necessitate medical intervention or treatment.

Table 4.1

Effects of Diagnosis Type and Age on Older US Adults High Sensitivity C-Reactive Protein Levels

Predictor	*β*	Linearized Std. Err.	*t*	*p*	95% Conf. Interval
All Heart Conditions (*N* = 705)					
CAD, MI, CHF(no)	0.0807	0.0656	1.23	0.225	[-0.0511, 0.2126]
Gender (men)	-0.0069	0.0034	-2.04	0.046	[-0.0137, -0.0001]
Age	0.0818	0.0500	1.64	0.108	[-0.0186, 0.1823]
Constant	0.9777	0.2381	4.11	0.000	[0.4994, 1.4560]
Treated CAD, Diagnosis of MI or CHF (*N* = 420)					
Gender(men)	0.1459	0.1027	1.42	0.162	[-0.0603, 0.3522]
Age	-0.0123	0.0060	-2.07	0.043	[-0.0241, -0.0004]
Constant	1.4670	0.4497	3.26	0.002	[0.5637, 2.3702]
Heart Condition without Surgical Treatment or Diagnosis of MI or CHF (*N* = 285)					
Gender(men)	0.0707	0.1726	0.41	0.684	[-0.2761, 0.4174]
Age	0.0058	0.0111	0.52	0.606	[-0.1657, 0.0281]
Constant	0.0861	0.8223	0.10	0.917	[-1.5657, 1.7378]

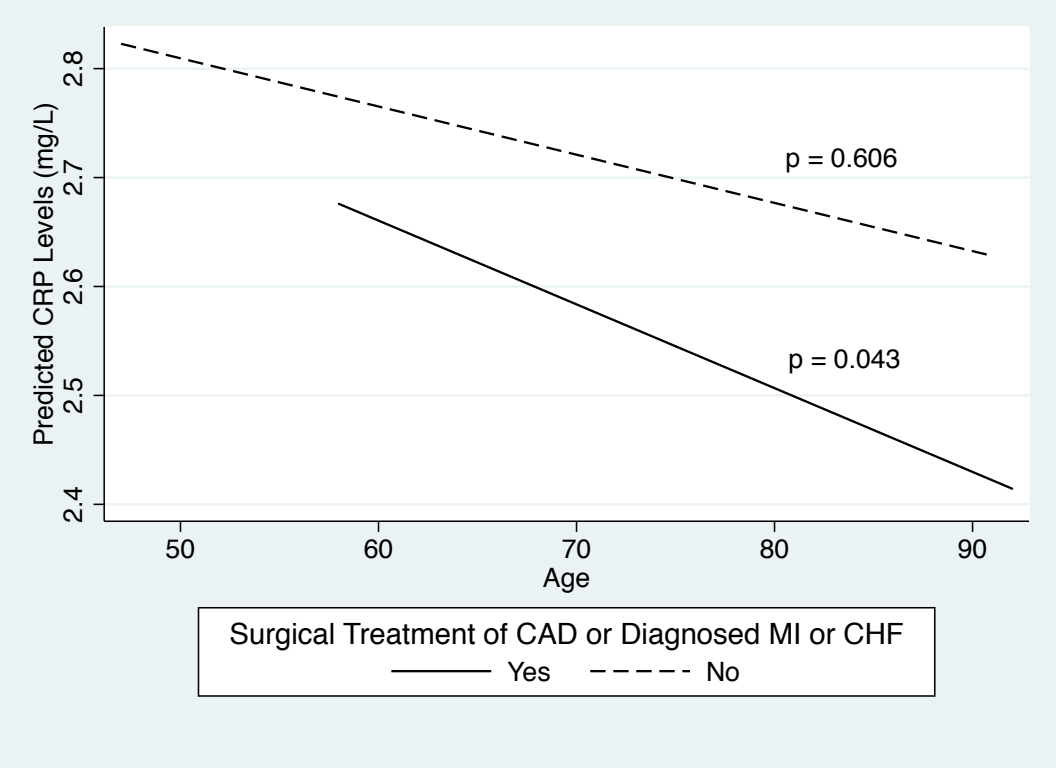

Figure 4.1. C-Reactive Protein Levels Association with Age and Treatment of CAD or Specific Diagnosis of MI or CHF among those with a Heart Condition

MCP-1. CRP is part of the acute phase broad inflammatory response to injury, but is not specific to heart disease. In contrast, MCP-1 is related more directly to inflammation of the cardiovascular system, as it is produced as an inflammatory response to injury within atherosclerotic plaques. Therefore, we hypothesized that MCP-1 would be a more sensitive physiological indicator of cardiovascular system inflammation, and reveal an inflammatory process in participants with a heart condition, but who had not been surgically treated for CAD or specifically diagnosed with MI or CHF.

Indeed, MCP-1 levels were significantly higher at older ages among participants who had not had surgical treatment of CAD or a specific diagnosis of MI of CHF, and were lower among those who had had these specific treatment and diagnoses (interaction between age and diagnostic status b = -0.59, t (97) = -2.12, p = 0.038; Table 4.2; Figure 4.2). Because MCP-1 is a

more distinctive biomarker of underlying atherosclerotic burden and corresponding cardiovascular disease, the MCP-1 increase in older ages suggests that some of these people are undertreated or under-diagnosed.

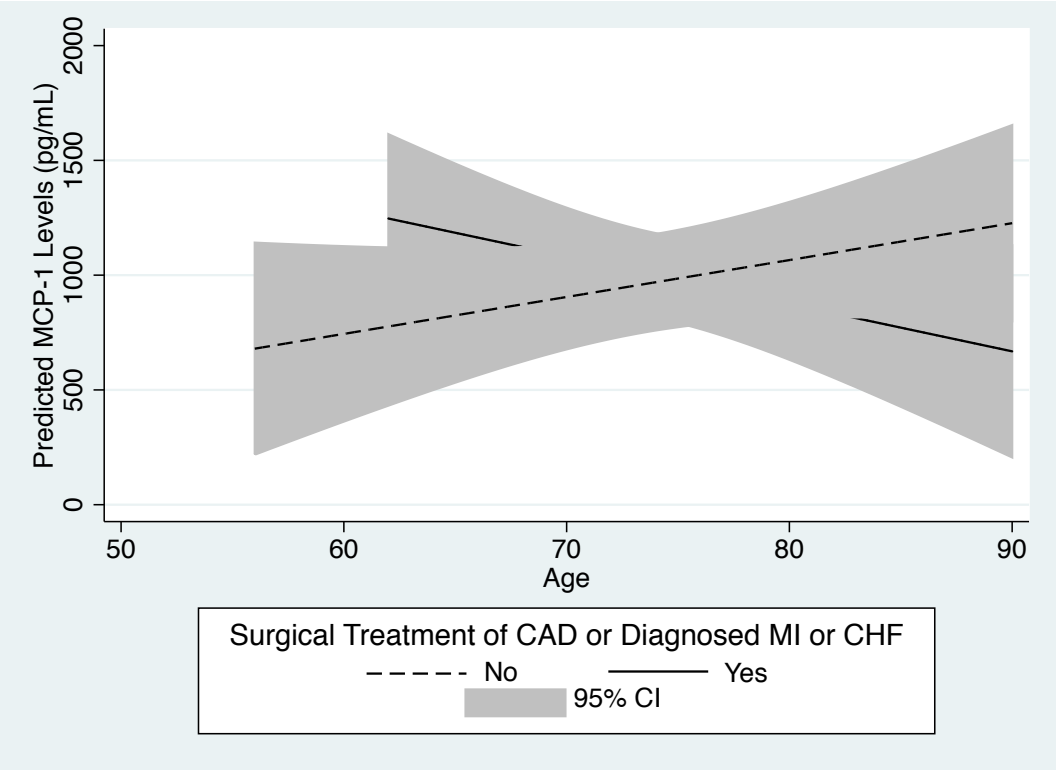

Figure 4.2. MCP-1 Levels Association with Age and Treatment of CAD or Specific Diagnosis of MI or CHF among those with a Heart Condition

Table 4.2

Effects of Diagnosis Type and Age on Older US Adults Monocyte Chemoattractant Protein - 1 Levels

Predictor	*β*	Linearized Std. Err.	*t*	*p*	95% Conf. Interval
All Heart Conditions (*N* = 98)					
CAD, MI, CHF(no)	43.355	20.592	2.11	0.038	[2.4697, 84.2395]
Age	0.2267	0.1852	1.22	0.224	[-0.1409, 0.5944]
Interaction	-0.5863	0.2769	-2.12	0.037	[-1.1361, -0.0365]
Constant	12.7140	13.7789	0.92	0.359	[-14.6441, 40.0722]
Treated CAD, Diagnosis of MI or CHF (*N* = 57)					
Gender(men)	-1.1246	3,1860	-0.35	0.725	[-7.5122, 5.2630]
Age	-0.3404	0.2248	-1.51	0.136	[-0.7911, 0.1103]
Constant	55.1032	16.4414	3.35	0.001	[22.1401, 88.0663]
Heart Condition without Surgical Treatment of Diagnosis of MI or CHF (*N* = 41)					
Gender(men)	-2.9443	3.2683	-0.90	0.373	[-9.5606, 3.6720]
Age	0.2076	0.1727	-1.20	0.237	[-0.1420, 0.5571]
Constant	15.9977	13.2631	1.21	0.235	[-10.8522, 42.8475]

In the previous chapter, I reported that women were less likely to be treated for CAD or diagnosed with MI or CHF, raising the hypothesis that women might have lower inflammatory markers that serve as risk factors for cardiovascular disease and indicators for treatment, such as CRP or MCP-1. However, there were no gender differences in MCP-1 levels, either among

those who had had surgical treatment of CAD or a specific diagnosis of MI or CHF ($p = 0.725$) or among those without these treatments and diagnoses ($p = 0.373$; Table 4.2). Likewise, there were no gender differences in CRP levels (Table 4.1).

Discussion

We hypothesized that differences noted in diagnosis and treatment of heart disease could be explained by the use of biomarkers of inflammation, as they may be less biased concerning the significance of individual atherosclerotic lesions. However, we found that, among U.S. older adults with a heart condition, levels of CRP, a non-specific marker of inflammation, were not different between those that had or did not have a specific diagnosis of CAD, MI or CHF. Nevertheless, we did find an age-related effect of treatment or diagnosed MI or CHF on CRP levels. Levels of CRP were lower at older ages for people surgically treated for CAD or diagnosed with MI or CHF, but CRP levels were stable across all ages for those that had not received surgical treatment or specific diagnosis of their cardiovascular disease. When considering the previous result that people who had not received CAD treatment have higher incidence of CHF, these results on CRP levels are congruent with a recent study showing that, among patients with CAD, high CRP levels are related to future incidence of CHF (Eisen, Benderly, Behar, Goldbourt, & Haim, 2014).

Further, we sought to determine whether CRP had the same relationship to heart disease for women and men. Previous studies have strived to relate CRP to the risk and incidence of heart disease, but there have been no consistent results to date. There is disagreement whether men or women have higher levels of CRP, and whether it holds more predictive value for women or men. For example, while women are more likely than men to have elevated CRP, this increase is not related to a higher risk of cardiac events or death in women (Doran, Zhu, &

Muennig, 2013). However, other results show that CRP may actually hold more predictive importance for women when looking at ischaemia rather than coronary artery disease (Shaw et al., 2006). Inconsistencies such as this stress the need for guidelines or cutpoints that are sensitive to gender and age differences, as proteins such as CRP vary based on sex and hormonal status. Sex-specific ranges of CRP and associated predictive value for heart disease are a target area of research that we plan to examine with the NSHAP data.

Our results showed that there were no differences in CRP levels between men and women, but rather CRP levels only decrease with age if someone had been diagnosed with MI, CHF, or CAD. This is supportive of studies showing that, while CRP is related to heart disease, it may be a bystander of sorts, in that it is not specific to vascular inflammation, but rather a broad marker of metabolic changes and general inflammation (Lyngbaek et al., 2013). In fact, in the Cardiovascular Health Study, a population study of US adults over 65 years of age, baseline CRP levels were related, at the 9-year follow-up, to overall declines in physical and cognitive functions, but were not related to cardiovascular events in (Jenny et al., 2012). Further, CRP levels were related most strongly to all-cause mortalities occurring within a few years of measurement, which suggests it may be involved in a final pathway common to various diseases resulting in death (Gussekloo, Schaap, Frölich, Blauw, & Westendorp, 2000).

To date, CRP is the most widely used biomarker in predicting occurrence of heart disease, but it has not been shown, consistently, to serve as an independent predictor above traditional risk factors (Koening & Khuseyinova, 2006). Overall, our results confirm some of the more recent research that lends evidence in support of the view that CRP, while related indirectly to cardiovascular health, is not useful as a direct, independent risk factor/predictor for heart disease. While studies have shown that CRP is present at the site of atherosclerotic

plaques, research has not been able to confirm a direct causal relationship to the progression of atherogenesis (Koening & Khuseyinova, 2006).

Therefore, we examined whether MCP-1, a chemokine more specifically related to vascular inflammation, would relate more strongly to heart disease. As we found with CRP, MCP-1 levels increased with age among those with a heart condition that had not received surgical treatment or a diagnosis of MI or CHF. This result is consistent with the literature, as increases of MCP-1 with age have been reported in other population-based studies (Deo et al., 2004). However, MCP-1 levels decreased with age among those that had received treatment for CAD or a diagnosis of MI or CHF, a relationship that was not seen with CRP. Further, there were no differences in MCP-1 levels between women and men.

Studies have suggested that MCP-1 mediates the atherogenic effects of some of the risk factors of heart disease; including hypertension, hypercholesterolemia, and diabetes (Deo et al., 2004). In fact, it is found that macrophages, which are present in all artery intima but higher in advanced lesion types, contain genes that code for proteins, such as MCP-1, associated with formation of advanced lesions (Stary et al., 1995). Thus, MCP-1 appears to be related to cardiovascular inflammation, and may be helpful in determining which patients are at risk for future adverse cardiac events and should therefore undergo preventative treatment or therapy. Among older adults, higher MCP-1 levels could result from being more likely to have a heart condition left untreated or undiagnosed. Our data support this interpretation, because lack of treatment and diagnosis was associated with higher cardiovascular inflammation (MCP-1) at older ages.

The results of this study show that, among the elderly US population, women are less often diagnosed with a heart condition, less often given a specific diagnosis of MI or CHF, and

less often receive treatment for CAD than are men. Levels of CRP levels, a non-specific inflammatory diagnostic indicator, were similar for women and men, and so could not explain this discrepancy in diagnosis. While CRP levels were stable across age for those without CAD treatment, levels did decrease with age for those that had received CAD treatment, suggesting that treatment did reduce inflammation, but also that CRP is likely involved in many other age-related diseases. In contrast, MCP-1 not only decreased among those treated, but also increased among those left untreated, as would be expected in an inflammatory marker specific to cardiac disease. Importantly, MCP-1 levels were similar for women and men within diagnostic status, indicating that that the sex differences in CHD prevalence have more to do with biases in diagnosis and treatment of MI, CHF and CAD than sex differences in physiology.

Further evidence supporting this hypothesis could incorporate a more detailed diagnoses roster to determine what cardiac conditions women are diagnosed with if not MI, CHF and CAD. Indeed, future analyses from Wave 3 of NSHAP will include incidence of atrial fibrillation. In addition, our plan to analyze further data on traditional risk factors, hormones, medication use, and mortality rates should aid in explaining the sex and age differences in heart disease and the use of related biomarkers.

References

Ayanian, J. Z., & Epstein, A. M. (1991). Differences in the use of procedures between women and men hospitalized for coronary heart disease. *New England Journal of Medicine*, *325*(4), 221-225.

Bekwelem, W., Lutsey, P. L., Loehr, L. R., Agarwal, S. K., Astor, B. C., Guild, C., ... & Folsom, A. R. (2011). White blood cell count, C-reactive protein, and incident heart failure in the Atherosclerosis Risk in Communities (ARIC) Study. *Annals of Epidemiology*, *21*(10), 739-748.

Cesari, M., Penninx, B. W., Newman, A. B., Kritchevsky, S. B., Nicklas, B. J., Sutton-Tyrrell, K., ... & Pahor, M. (2003). Inflammatory markers and onset of cardiovascular events results from the Health ABC Study. *Circulation*, *108*(19), 2317-2322.

Charo, I. F., & Taubman, M. B. (2004). Chemokines in the pathogenesis of vascular disease. *Circulation Research, 95*(9), 858-866.

Cushman, M., Arnold, A. M., Psaty, B. M., Manolio, T. A., Kuller, L. H., Burke, G. L., ... & Tracy, R. P. (2005). C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: The Cardiovascular Health Study. *Circulation*, *112*(1), 25-31.

de Lemos, J. A., Morrow, D. A., Sabatine, M. S., Murphy, S. A., Gibson, C. M., Antman, E. M., ... & Braunwald, E. (2003). Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation*, *107*(5), 690-695.

Deo, R., Khera, A., McGuire, D. K., Murphy, S. A., Neto, J. D. P. M., Morrow, D. A., & de Lemos, J. A. (2004). Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *Journal of the American College of Cardiology*, *44*(9), 1812-1818.

Doran, B., Zhu, W., & Muennig, P. (2013). Gender differences in cardiovascular mortality by C-reactive protein level in the United States: Evidence from the National Health and Nutrition Examination Survey III. *American Heart Journal, 166*(1), 45-51.

Egashira, K. (2003). Molecular mechanisms mediating inflammation in vascular disease special reference to monocyte chemoattractant protein-1. *Hypertension*, *41*(3), 834-841.

Eisen, A., Benderly, M., Behar, S., Goldbourt, U., & Haim, M. (2014). Inflammation and future risk of symptomatic heart failure in patients with stable coronary artery disease. *American Heart Journal, 167*(5), 707-714.

Gottdiener, J. S., Arnold, A. M., Aurigemma, G. P., Polak, J. F., Tracy, R. P., Kitzman, D. W., ... & Boineau, R. C. (2000). Predictors of congestive heart failure in the elderly: The

Cardiovascular Health Study. *Journal of the American College of Cardiology*, *35*(6), 1628-1637.

Gussekloo, J., Schaap, M. C., Frölich, M., Blauw, G. J., & Westendorp, R. G. (2000). C-reactive protein is a strong but nonspecific risk factor of fatal stroke in elderly persons. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*(4), 1047-1051.

Herder, C., Baumert, J., Thorand, B., Martin, S., Löwel, H., Kolb, H., & Koenig, W. (2006). Chemokines and incident coronary heart disease results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(9), 2147-2152.

Huang, W. P., Yin, W. H., Jen, H. L., Chiang, M. C., Feng, A. N., & Young, M. S. (2004). C-reactive protein levels in chronic congestive heart failure. *Acta Cardiologica Sinica*, *20*(1), 7-14.

Jenny, N. S., French, B., Arnold, A. M., Strotmeyer, E. S., Cushman, M., Chaves, P. H., ... & Newman, A. B. (2012). Long-term assessment of inflammation and healthy aging in late life: The Cardiovascular Health Study All Stars. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 67*(9), 970-976.

Kardys, I., Knetsch, A. M., Bleumink, G. S., Deckers, J. W., Hofman, A., Stricker, B. H. C., & Witteman, J. (2006). C-reactive protein and risk of heart failure: The Rotterdam Study. *American Heart Journal*, *152*(3), 514-520.

Koenig, W., & Khuseyinova, N. (2007). Biomarkers of atherosclerotic plaque instability and rupture. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*(1), 15-26.

Kuller, L. H., Tracy, R. P., Shaten, J., & Meilahn, E. N. (1996). Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *American Journal of Epidemiology*, *144*(6), 537-547.

Lyngbæk, S., Marott, J. L., Sehestedt, T., Hansen, T. W., Olsen, M. H., Andersen, O., ... & Jeppesen, J. (2013). Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *International Journal of Cardiology*, *167*(6), 2904-2911.

McDade, T. W., Burhop, J., Dohnal, J. (2003). High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry, 50*(3), 652-654.

O'Doherty, K., Jaszczak, A., Hoffmann, J. N., You, H. M., Kern, D. W., Pagel, K., McPhillips, J., Schumm, L.P., Dale, W., Huang, E.S., & McClintock, M. K. (2014). Survey field methods for expanded biospecimen and biomeasure collection in NSHAP Wave 2. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *69*(Suppl 2), S27-S37.

Pai, J. K., Pischon, T., Ma, J., Manson, J. E., Hankinson, S. E., Joshipura, K., ... & Rimm, E. B. (2004). Inflammatory markers and the risk of coronary heart disease in men and women. *New England Journal of Medicine*, *351*(25), 2599-2610.

Rosen, B. D., Cushman, M., Nasir, K., Bluemke, D. A., Edvardsen, T., Fernandes, V., ... & Lima, J. A. (2007). Relationship between C-reactive protein levels and regional left ventricular function in asymptomatic individuals: The Multi-Ethnic Study of Atherosclerosis. *Journal of the American College of Cardiology*, *49*(5), 594-600.

Shaw, L. J., Merz, C. N. B., Pepine, C. J., Reis, S. E., Bittner, V., Kelsey, S. F., ... & Sopko, G. (2006). Insights from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study Part I: Gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. *Journal of the American College of Cardiology*, *47*(3s1), S4-S20.

Smith, S. C., Anderson, J.L., Cannon, R.O., Fadl, Y.Y., Koenig, W., Libby, P., Lipshultz, S.E., Mensah, G.A., Ridker, P.M., & Rosenson, R. (2004). CDC/AHA Workshop on markers of inflammation and cardiovascular disease application to clinical and public health practice report from the clinical practice discussion group. *Circulation, 110*, e550-e553.

Stary, H. C., Blankenhorn, D. H., Chandler, A. B., Glagov, S., Insull Jr, W., Richardson, M., ... & Wagner, W. D. (1992). A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, *85*(1), 391.

Vasan, R. S., Sullivan, L. M., Roubenoff, R., Dinarello, C. A., Harris, T., Benjamin, E. J., ... & D'Agostino, R. B. (2003). Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction the Framingham Heart Study. *Circulation*, *107*(11), 1486-1491.

Wong, N. D., Pio, J., Valencia, R., & Thakal, G. (2001). Distribution of C-reactive protein and its relation to risk factors and coronary heart disease risk estimation in the National Health and Nutrition Examination Survey (NHANES) III. *Preventive Cardiology*, *4*(3), 109-114.

Chapter 5: Validating Blood Sample Field Methods for Measuring Protein Analytes

There is a need to validate a field method for analyzing blood levels of analytes used as markers of disease, or biomarkers, as the conditions are often extremely different than laboratory conditions that are used in most clinical research. In clinical settings, blood samples are usually obtained through venipuncture, which requires trained phlebotomists and the blood samples would need to be processed and stored immediately under exceptionally controlled conditions. This procedure is invasive and not feasible for large population based studies. In Wave 2 of NSHAP, non-medically trained field interviewers obtained whole blood by finger-prick with a disposable lancet and collected the samples into a small container, or Microtainer®, which was kept cool and then shipped to the laboratory for storage and processing. Such field methods have been validated for other analytes, namely CRP (Cordon, Elborn, Hiller, & Shale, 1991; McDade, Burhop, & Dohnal, 2004), but there remains a need to validate similar methods for other protein analytes such as cytokines and chemokines.

Cytokines and chemokines are protein molecules in the immune system, and they are vast in number and in function. In general, they serve as markers of inflammatory processes, and are often specifically related to chronic conditions, such as cardiovascular disease and depression. Previous research has primarily measured levels of individual cytokines or of the "proinflammatory triad" of TNF-α, IL-1, and IL-6. The cytokine triad has been shown to be a reliable predictor of disease. However, just as the immune system itself is complex, communication via cytokines is equally complex with many interactions and cascade effects among the different proteins. Therefore, it was decided, for Wave 2 of NSHAP, to take an innovative measurement of 22 analytes: IFN-γ, IL-10, IL-1b, IL-6, MCP-1, TGF-α, TNF-α, GM-

CSF, IL-12, IL-13, IL-1ra, IL-2, sIL-2ra, IL-3, IL-4, IL-5, TNF-β, VEGF, Fibrinogen, Adiponectin, NGAL, and Apolipoprotein-B.

Whole blood collected from respondents was assayed with a Bio-Plex system, driven by Luminex xMAP technology, to measure analyte levels. In these assays, a set of six known concentrations, or standards, are measured. These obtained values are graphed against the expected concentrations, and are used to calculate what is known as a standard curve. Field samples, of unknown concentrations, are then assayed and the resulting measurements and corresponding standard curve are used to calculate concentrations for these samples. Assay results from a subset of samples revealed that many were so low that they were not detectable, or out of the reliable range of the standard curve (3.2-10,000 pg/mL). Therefore, we decided to conduct two validation studies.

This chapter will describe in detail these two studies and the implications of their results. In Study 1, we asked whether our method of dilution was producing our low assay levels and if we could get more values without dilution. In Study 2, we sought to determine whether these low values were due to the NSHAP field methods, or, whether they reflected true values in the population of older US adults. Both of these validation studies led us to deem our data as accurate and useful. However, there was still the issue of how to deal statistically with such novel data. Therefore, for Study 3, we describe the distribution of these data, the methods we employed to analyze them, and a relationship between our inflammatory profiles and health.

Study 1: Effect of Sample Dilution on Out of Range Assay Values

Preliminary analyses from a subset of Wave 2 NSHAP data resulted in an unreasonably low number of data points within the standard range, with many of the cytokines producing non-detectable amounts. With such assays, it is standard practice to add diluent solution to the

sample because contaminant analyte concentrations are often above the range of the assays. However, as is supposed in our case, this standard dilution can reduce targeted analyte concentrations to below the assay range. In order to determine whether the sensitivity of the bead technology assay could be increased, an experiment was run. In this study, samples were each run twice: at the standard 1:2 dilution and without being diluted ("neat"). The results showed that sensitivity of the assay could indeed be increased by running samples neat rather than dilute, with over 10% more of the samples being in the detectable and measurable range of the standard curve (neat = 60% on the standard curve, dilute = 48% on the standard curve).

Methods

Subjects

For this study, we used a subset of 78 samples from NSHAP Wave 2 participants (37.2% male, 61.5% female, 1.3% unknown gender; 76.9% white, 12.8% black, 9% non-Black/Hispanic). Ages ranged from 50-89 years old, with a mean age of 72.7 years and standard deviation of 8.4 years. The respondents were varied in their educational background: 19.2% less than high school, 23.1% high school degree or equivalent, 34.6% some college, and 21.8% bachelors or higher. In this sample, 59% were married or living with a partner and 41% were separated, divorced, or widowed.

Field Sample Collection, Preparation, and Assay Procedure

Field Sample Collection and Storage. In order to collect blood drops from a finger prick in the field, we had to use collection tubes coated with an anticoagulant so the blood could run down into the tube without clotting. We also needed to increase the volume of blood collected in order to run the multiplex assays proposed for Wave 2. Participants were instructed to hold hand-warmers to improve circulation in order to increase blood flow and maximize the

amount of blood collected. This new protocol enabled the collection of additional biomeasures not feasible with dried blood spot protocols. Five drops (250 microliters (μl)) of uncoagulated whole blood was collected and stored in a Microtainer®; a small, unbreakable plastic tube with a FloTop collector which facilitates fast and efficient blood collection. Unclotted whole blood in the Microtainer® was spun down and the plasma frozen at the University of Chicago Flow Cytometry Core Facility. All samples were void of personal identification material to ensure confidentiality.

Assay. In a regular ELISA, antibodies are created that are specific to the non-functioning portion of a protein, or cytokine. A potential florescence is attached to the antibody, and luminesces when the target protein binds to the antibody. The standard curve is built with protein samples of known quantities. The sample, of unknown amount, is run through the assay and the fluorescence is measured. The observed fluorescence is compared to the standard curve, and the value for each analyte is estimated. With this method, one would need to run this assay 18 times to measure the molecules considered in NSHAP Wave 2. Instead, blood samples were run through a bead-based multiplex assay, enabling the *simultaneous* measurement of multiple analytes.

Assays were run with a Bio-Plex system, driven by Luminex xMAP technology, at the University of Chicago Flow Cytometry Core Facility. In this assay, there are 18 separate beads, each of which is internally dyed with a specific fluorescence, and is then conjugated with antibodies that bind the analyte of interest. Each bead has antibodies for 1 particular molecule and a corresponding uniquely colored fluorescence. The blood sample is combined with the beads, dilutions are added if necessary, the mixture is stabilized, and the beads adhere to the proteins in the blood sample. The mixture is then run through the Luminex reader, where beads

are detected by flow cytometry and move single file through a flow cell where red and green lasers function to detect bead color and assay signal strength.

The Luminex assay technology returns a range of values: missing, out of range (OOR), extrapolated, and actual observed or calculated with the standard curve. Extrapolated values are calculated with the measured fluorescence and standard curve equation as the actual values are, but are beyond the confidence interval and may result in less certain estimations. For this project, extrapolated values were deemed acceptable and were treated as actual observed values in all analyses.

In this substudy, there were 78 samples tested on 4 plates. Each sample was run neat and at a 1:2 dilution, and the corresponding samples were run on different plates, e.g. subject #123456 was run neat on plate 1 and diluted on plate 2. The plates were set up as follows:

Plate 1: Samples 1-19 were run at a 1:2 dilution
Samples 20-39 were run neat

Plate 2: Samples 1-19 were run neat
Samples 20-39 were run at a 1:2 dilution

Plate 3: Samples 40-58 were run at a 1:2 dilution
Samples 59-78 were run neat

Plate 4: Samples 40-58 were run neat
Samples 59-78 were run at a 1:2 dilution

Results

Overall, the neat samples produced more actual observed values (60%) than when the samples were run at a dilution (48%). Specifically, sensitivity was increased for 16 out of 18

analytes, of notable importance are analytes IL-1b, IL-6, TNF-a, and MCP-1, which led to the decision to run all further cytokine assays neat. See Table 5.1 and Figure 5.1.

Table 5.1

Dilution Effect on Types of Data Values for Each Analyte

	OOR <		Extrapolated		Actual	
Analyte	Dilute	Neat	Dilute	Neat	Dilute	Neat
GM-CSF	42	21	13	12	23	45
IFN-g	20	6	36	35	22	37
IL-10	60	53	10	6	8	19
IL-12	53	50	11	14	14	14
IL-13	65	60	4	4	9	14
IL-1b	18	2	13	7	47	69
IL-1ra	19	13	4	0	55	65
IL-2	68	56	2	4	8	18
IL-3	42	47	22	28	14	3
IL-4	61	54	1	2	16	22
IL-5	47	35	30	39	1	4
IL-6	60	46	3	7	15	25
MCP-1	12	0	1	0	65	78
TGF-a	35	9	31	33	12	36
TNF-a	9	0	11	1	58	77
TNF-b	53	57	0	5	11	16
VEGF	11	14	0	0	67	64
sIL-1Ra	58	41	0	1	20	36

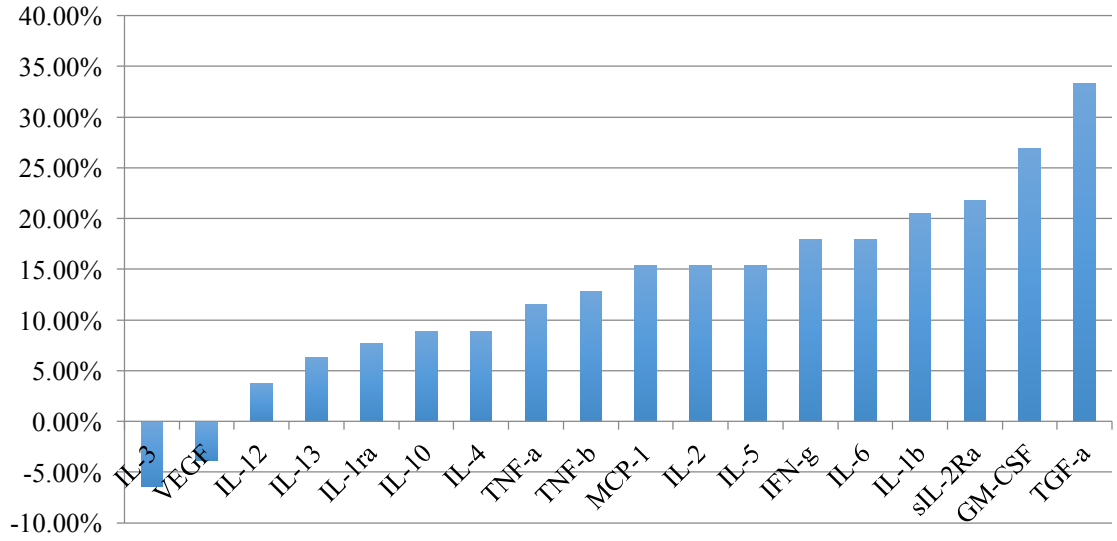

Figure 5.1. Percentage change in the number of samples with actual observed values on the standard curve when running the assay neat compared to at a dilution. A positive value on the graph signifies a higher number of actual values for undiluted neat samples than for samples diluted 1:2.

Discussion

Running samples neat, without the standard dilution, increased the percentage of actual observed values. Nonetheless, the assay returned out of range low values for many analytes, so it is necessary to determine the best way to deal with them. There are a few ways to handle management of the OOR values: 1. Set OOR values to zero or missing, 2. Replace values with lower bound of standard curve, or a value just a magnitude less than the lower limit, 3. Convert observed fluorescence values to actual observed values with the standard curve equation, or 4. Fit regressions that censor the OOR values.

However, before making the decision on how to manage OOR values, the circulating levels of the measured cytokines must be specified. Based on reported levels in the literature, the low levels may be reasonable for certain analytes. See Table 5.2.

Table 5.2

Cytokine Levels Reported in Literature

Analyte	Sample	*N*	Technology	Level	Reference
IFN-γ	Plasma	20	Multiplex (beads)	90 pg/ml	Khan, 2012
IL-10	Plasma	30	ELISA	0.66 pg/ml	Martin, et al., 2006
IL-1β	Plasma	20	Multiplex (beads)	Undetected	Khan, 2012
IL-6	Plasma	30	ELISA	0.92 pg/ml	Martin, et al., 2006
MCP-1	Plasma	19	Multiplex (beads)	17 pg/ml	Khan, 2012
TGF-α	Serum	30	ELISA	9.2 U/l	Passoja, et al., 2010
TNF-α	Plasma	20	Multiplex (beads)	Undetected	Khan, 2012
GM-CSF	Plasma	17	Multiplex (beads)	9 pg/ml	Khan, 2012
IL-12	Plasma	7	Multiplex (beads)	11 pg/ml	Khan, 2012
IL-13	Plasma	30	ELISA	0.99 pg/ml	Martin, et al., 2006
IL-1ra	Plasma	20	Multiplex (beads)	42 pg/ml	Khan, 2012
IL-2	Plasma	30	ELISA	2.3 pg/ml	Martin, et al., 2006
sIL-2ra	Serum	1679	ELISA	1.7-2.4 pg/ml	Maier, et al., 2009
IL-3	Plasma	20	Multiplex (beads)	68 pg/ml	Khan, 2012
IL-4	Plasma	20	Multiplex (beads)	2 pg/ml	Khan, 2012
IL-5	Plasma	20	Multiplex (beads)	8 pg/ml	Khan, 2012
TNF-β	Plasma	20	Multiplex (beads)	Undetected	Khan, 2012
VEGF	Plasma	9	Multiplex (beads)	8 pg/ml	Khan, 2012

After examining cytokine levels referenced in the literature, it is clear that while some cytokines may have low circulating levels, there is no reason to believe that the Wave 2 NSHAP data should have produced such a high number of OOR values for so many of the analytes. In light of these results, it was decided that all OOR values would be best handled by running censored regressions.

Study 2: Effect of Field Blood Collection Methods on Assay Values

As expected in a sample of older adults, we have found that a lower than expected number of samples produce a value which fall on the standard curve, even after running samples without dilution to maximize sensitivity. This issue has not been reported explicitly in the literature, presumably because the low values are imputed conservatively as the value of the lower limit. This second study was designed to determine whether having low values was an artifact of the NSHAP field methods, or, whether they reflect true values in the population of older US adults living in their homes.

NSHAP field methods differ from typical clinical methods in two observable ways: collection method and timing of sample preparation. First, we have noted that most, but not all, studies work with serum while NSHAP works with plasma. Serum forces clotting of blood, while plasma keeps it unclotted. The process of clotting may force more cytokines out of the entrapped cells, leading to higher concentrations of cytokines when using serum samples. Using plasma may result in lower values. Alternatively, certain cytokines may be complementary to coagulation and thus would be in higher concentration for serum than plasma samples. This methods study will directly examine the effect of allowing blood to clot on cytokine levels by comparing values in plasma and serum. Second, the time between blood draw and assay during Wave 2 of NSHAP may be considered a long period of refrigeration. In clinical practice, the

goal is to have blood drawn, centrifuged, and frozen within 4 hours. In NSHAP Wave 2, this interval is anywhere from 24-72 hours. Cytokine levels may or may not be stable over time during shipping, despite maintaining refrigerated temperatures. This study will also examine whether this time delay has an effect on the protein analyte levels in our assay.

To examine the effects of blood collection, shipment, and assay techniques, this study uses participants recruited at South Shore Senior Clinic. To review, this study will compare cytokine values obtained from serum (clotted, clinical protocol) and plasma (unclotted, field protocol) and determine the difference in values based on whether the time between collection and freeze is 4 (clinical protocol) or 48 hours (field protocol).

Methods

In NSHAP Wave 2, non-medically trained field interviewers obtained blood samples by lancing the finger and collecting it in an anticoagulant-coated Microtainer® tube, thus accomplishing a home-based blood collection in the field. Samples were immediately stored on frozen refrigerant bricks in a thermally insulated container, and shipped overnight to the assay laboratory, whereupon they were spun down and the plasma was frozen.

For this study comparing field and clinical methods, we obtained blood samples using typical venipuncture procedures and four separate vacutainers – one for each condition (plasma 48 hours, plasma 4 hours, serum 48 hours, and serum 4 hours). The blood from each subject under all four collection and handling conditions was assayed in the same way. Two tubes were coated with anticoagulant (K_2EDTA) for plasma, and the other two were allowed to clot, yielding serum. Then, one tube each of plasma and serum was spun down within four hours, and the serum/plasma collected and frozen for storage at -80^{o}F. The second tubes of plasma and serum were placed on the same frozen refrigerant packs used in the field, and kept at this cold

temperature for 48 hours, again, as happened in the field, before being spun down and stored at -80^{o}F.

Subjects

Subjects were recruited from the University of Chicago Senior Outpatient Center at South Shore. Each subject recruited from the clinic was there to receive venipuncture for unrelated reasons. A phlebotomist employed by the clinic completed all blood draws. Respondents were asked to donate an additional 12 ml of blood for this project (collected in 4 vacutainers), to be drawn with the same venipuncture. No identifying information was collected – only age and sex. Participants read and signed an informed consent form before the venipuncture procedure, which took approximately one minute. Respondents were compensated $5 towards transportation to the clinic.

Subject Characteristics. Sixty subjects were English speaking males and females born between 1920 and 1947, matching the NSHAP Wave 2 sample. Subjects were recruited from the Senior Outpatient Center at South Shore. The physicians and clinic staff agreed to facilitate the study. The physicians mentioned the study if they determined that the subject was having a venipuncture for clinical tests as part of their normal clinic visit and that there were no health risks associated with providing the additional blood tubes. No additional venipuncture was required. The research assistant, who coordinated with phlebotomy and handled the samples, approached and obtained informed consent from willing patients.

Results

Results suggest that, for the majority of analytes, in comparison to typical clinical methods, NSHAP field methods did not reduce the likelihood of getting values on the standard

curve. In fact, NSHAP field methods yielded more standard curve values: plasma samples with a 48 hour delay returned more actual observed values than serum samples with a 4 hour delay.

Table 5.3

Percent Actual Observed Values for Serum No Delay and Plasma Delay

Analyte	Clinical Method	NSHAP Field Method	Difference
IL-4	25%	95%	70%
TGF-α	22%	67%	45%
IL-1α	52%	90%	38%
IFN-γ	52%	87%	35%
Fibrinogen	67%	100%	33%
IL-1β	50%	80%	30%
TNF-β	72%	97%	25%
GM-CSF	62%	87%	25%
IL-6	70%	90%	20%
IL-13	68%	87%	19%
IL-12	73%	92%	19%
IL-2	57%	75%	18%
IL-10	85%	98%	13%
TNF-α	87%	98%	11%
IL-1rα	82%	92%	10%
VEGF	92%	97%	5%
IL-3	37%	38%	1%
NGAL	100%	100%	0%

Table 5.3 continued

MCP-1	100%	100%	0%
Apo-B	100%	100%	0%
Adiponectin	98%	98%	0%
IL-5	87%	87%	0%

Discussion

As with the first methodological study, this validation study indicates the success of our field methods, assesses their relationship with traditional clinical protocols, and addresses methodological concerns. The information from these results has allowed the NSHAP Quality Control Group to develop suggestions for researchers interested in using these data.

Study #3: Patterns and Cluster Analysis of Cytokine Levels in U.S. Older Adults

Cytokines regulate inflammation in many chronic conditions. However, they are typically measured in clinical samples, with methods infeasible for population studies. Therefore, little is known about the distribution of individual cytokines or cytokine profiles in a general population of older adults.

For this study, our goals were to: 1) describe the distributions of cytokines among the general population of older U.S. adults, 2) develop multivariate methods for analyzing multiple analytes within individuals, and 3) study the association between inflammatory profiles and health conditions.

Methods

Subjects

Data are from the National Social Life, Health, and Aging Project (NSHAP, 2010-2011, *N* = 2,745; 62-91 years old; 55% women; 72% white, 14% AA, 11% Hispanic). We assayed 18 cytokines, run with a Bio-Plex system driven by Luminex xMAP technology.

Statistical Analyses

First, quantile regressions were used for each cytokine to allow for relationships with health conditions to vary across different levels of the cytokines. This confirmed the importance of analyzing elevated cytokine levels. For example, one can see that the relationship between MCP-1 and depressive symptoms only holds above the 60th percentile of MCP-1 levels.

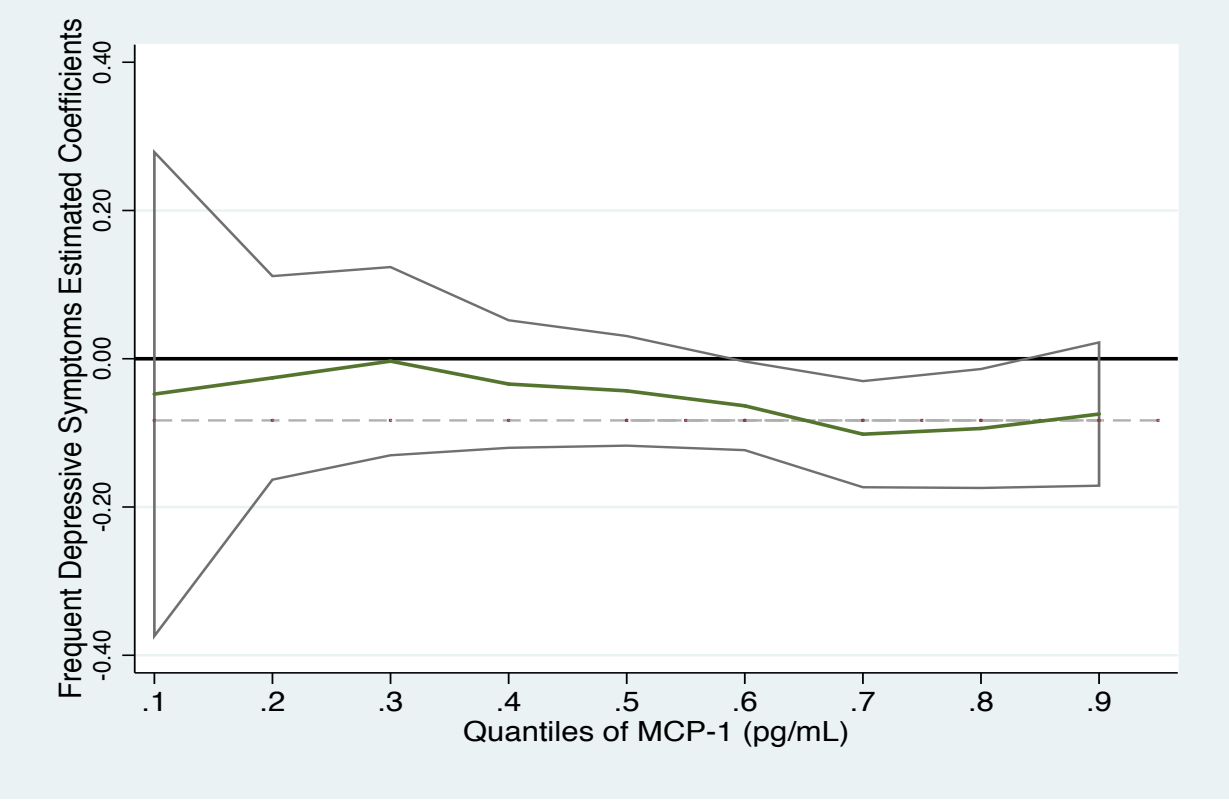

Figure 5.2. Estimated Regression Coefficients and 95% CI of Frequent Depressive Symptoms for Quantiles of MCP-1.

Next, we ran a K-means cluster analysis (Jaccard similarity coefficient) to determine the biggest sources of variation in the set of 18 cytokines that were examined simultaneously in the Bio-Plex assay. We used the 80th percentile in this analysis, because this was determined to be the most appropriate among all cytokines based on the quantile regressions described above. The cluster analysis emphasized the co-occurrence of elevated cytokines to identify groups (clusters) of people sharing similar cytokine profiles (see Table 1 for frequencies of individual within the clusters).

Cluster	Frequency	Percent
1	454	16.5%
2	195	7.1%
3	305	11.1%
4	285	10.4%
5	188	6.9%
6	239	8.7%
7	213	7.8%
8	642	23.4%
9	224	8.2%
Total	2,745	100.0%

Figure 5.3. Results of Cluster Analysis Using 80th Percentile of Each Cytokine

For the sake of this paper, I focus here on four exemplary clusters with different profiles of elevated cytokines and health risks. The first is cluster 1 in the table above, in which none of the individuals ($N = 454$) had elevated values for any of the 18 cytokines. In comparison (see figure 4), in cluster 8 ($N = 642$) over 60% of them had elevated values of 6 cytokines (IL-10, IL-

13, IL-2, IL-4, IL-6, and TNF-β), were more likely to have a cold during the interview (*RR* = 2.23, *p* = 0.002), and were less likely to be female (*RR* = 0.64, *p* = 0.001) or an ethnic minority (AA: *RR* = 0.57, *p* = 0.007; Hispanic: *RR* = 0.40, *p* < 0.0001).

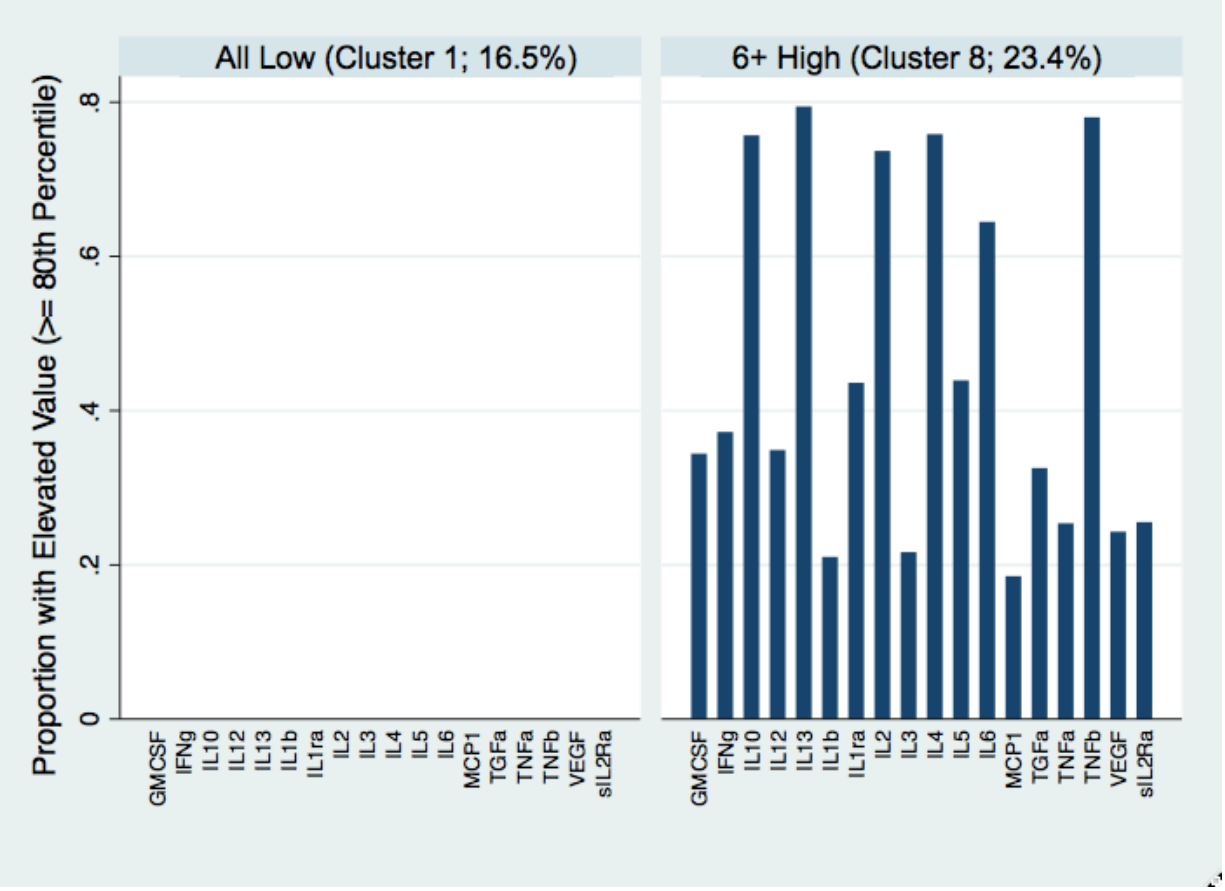

Figure 5.4. Comparison of All Low and 6+ High Clusters

Also important for this study are clusters 4 (*N* = 285) and 6 (*N* = 239) (see figures 5.5 and 5.6). Everyone (100% of people) in cluster 4 had elevated MCP-1 and they were less likely to have Frequent Depressive Symptoms (FDS) (*RR* = 0.58, *p* = 0.015). Levels of IL-1β and TNF-α were elevated in over 60% of those in cluster 6, who were likely to have diabetes (*RR* = 1.77, *p* = 0.003).

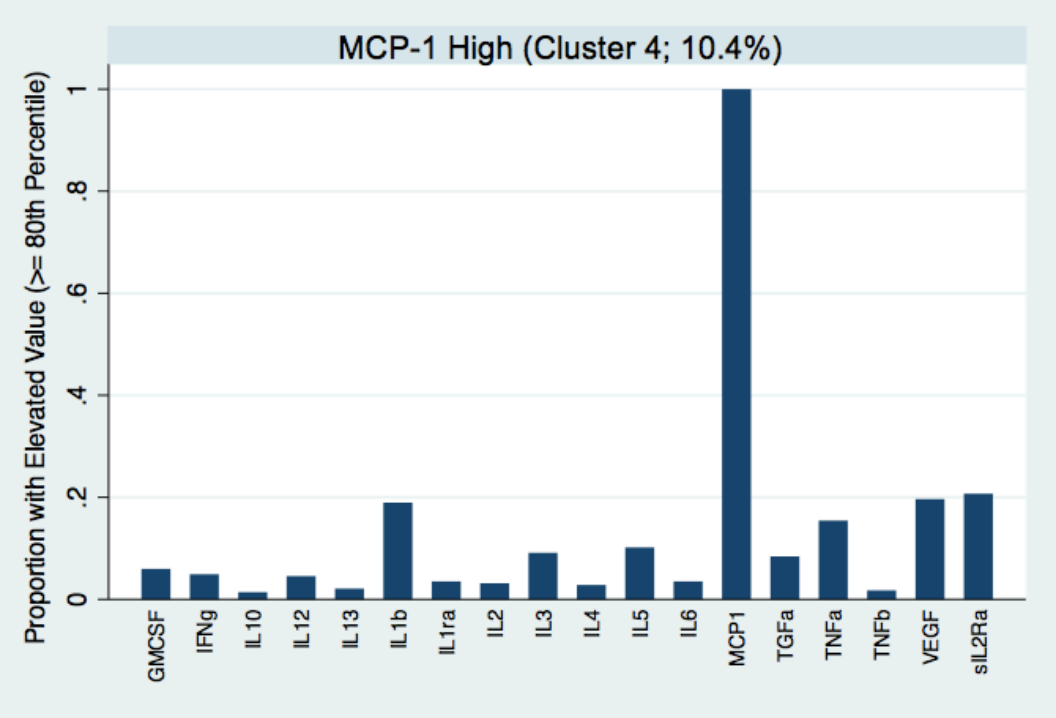

Figure 5.5. Cytokine levels in MCP-1 High Cluster

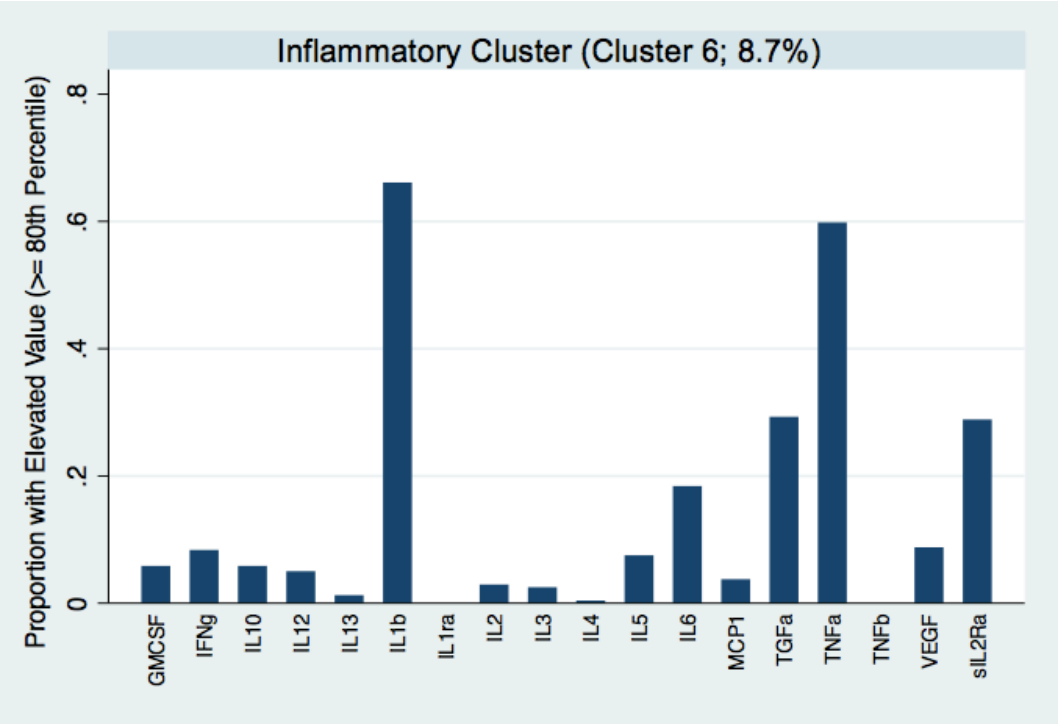

Figure 5.6. Cytokine Levels in the Inflammatory Cluster

Discussion

Cytokine profiles may work best as omnibus indicators of multi-morbidity health classes, while analyses of individual cytokines may be more relevant to specific health conditions. In our novel data, distinct cytokine profiles are identifiable among the general population of U.S. older adults, which are associated with the presence of a cold or acute infection, depression, and diabetes. A limitation is that these distinct profiles, however, do not identify specific mechanisms as there are multiple pathways involved. Nevertheless, cluster analysis, when used to define cytokine profiles, builds upon and improves the traditional approach of conducting a series of separate analyses of individual cytokines.

References

Cordon, S. M., Elborn, J. S., Hiller, E. J., & Shale, D. J. (1991). C-reactive protein measured in dried blood spots from patients with cystic fibrosis. *Journal of Immunological Methods*, *143*(1), 69-72.

McDade, T. W., Burhop, J., Dohnal, J. (2004). High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry, 50*(3), 652-654.

Chapter 6: Summary, Conclusions, and Future Research

Aging, in and of itself, is a complex process that incorporates and affects every aspect of one's body and mind. To understand the complexities of how biological, psychological, and social changes interact has been the interest of modern researchers in transdisciplinary areas. This dissertation, as a prime example, examined both inflammatory mechanisms and psychosocial factors of heart disease in older adults.

Overall, several expected differences in prevalence rates of cardiovascular disease were found in the survey data used. Prevalence of CAD and heart attack was higher in men than women and increased with age for everyone. CHF also increased with age, but we found no gender differences in prevalence. Although there are contradictions in the literature, this is consistent with the most current rates found (Go et al., 2013). These results served as an internal validation for the NSHAP cardiovascular measures.

The findings from examination of cardiovascular risk factors showed that many factors, including biological, psychological, and social, were found to explain important sex differences that can help us understand heart disease. With the NSHAP data, we were able to provide a new big picture: classic cardiovascular disease factors (diabetes, high blood pressure, smoking, overweight status, physical activity level, & high TC) still matter in older age, but maybe only for men. The story is different for older women. In general, the classic or traditional risk factors do not matter, at least using the current guidelines. However, we did find that certain sociodemographics are related to heart disease. A Hispanic ethnicity and a college education both serve as protective factors for the heart health of older women. This is a step in the right direction to develop inclusive diagnostic guidelines for women and older adults.

In Chapter 4, I examined biomarkers of inflammation to determine that gender differences in cardiovascular disease prevalence may have more to do with biased diagnoses and treatment of MI, CHF and CAD than with actual differences in physiology. I show that, among the elderly US population, women are less often given a specific diagnosis of MI or CHF, and less often receive treatment for CAD than are men. This discrepancy cannot be explained by inflammatory processes, as both the levels of CRP, a non-specific inflammatory diagnostic indicator, and MCP-1, a newly proposed specific cardiovascular disease indicator, were similar for women and men. Additionally, CRP and MCP-1 levels decreased for all older adults that had received CAD treatment. Based on the results, I further suggest that MCP-1 may function as an inflammatory marker specific to cardiac disease, because MCP-1 levels increased in age for those who were diagnosed with a heart condition but left untreated. This is in contrast to CRP, which showed levels stable across age for those without treatment, suggesting inflammation was reduced only partially and that CRP is likely involved in many other diseases seen in older adults.

Finally, I completed a report on validation studies for blood collection, assays, and statistical techniques used for the NSHAP biomeasures used in this dissertation. I provide evidence for the success of our field methods and address methodological concerns, which allowed the NSHAP Quality Control Group to develop suggestions for researchers using these data. After preliminary examination of cytokine levels in our data and those previously referenced in the literature, there was no reason to believe that the Wave 2 NSHAP data should have a high number of OOR values for so many of the analytes. The remainder of samples were then run neat, without the standard dilution, which increased the percentage of observed values.

Still, the final data returned many OOR values, so it was decided that these values would be best handled by running censored regressions.

Lastly, we were able to show that cluster analysis can successfully be used to define cytokine profiles, which builds upon and improves the traditional approach of conducting a series of separate analyses of individual cytokines. I found 8 distinct cytokine profiles identifiable among the general population of U.S. older adults. Additionally, these profiles were robustly associated with the presence of a cold or acute infection, depression, and diabetes.

Future Research

In the future, I would like to further explore gender differences in cardiovascular disease using the longitudinal NSHAP data, which currently has 3 waves of data. Perhaps accounting for the complex mechanistic network of inflammation, mental health, and physical disease over time will help understand discrepancies found in diagnosis and treatment of cardiovascular disease among older women and men. My plan to analyze further data on traditional risk factors, hormones, medication use, and mortality rates should aid in explaining the sex and age differences in heart disease and the use of related biomarkers. This line of research is only the start, as it has generated ideas to include other health conditions, psychological, and social variables.

Already, we have completed a study showing that the IL-1Ra^{high}-IL-4^{low}-IL-13^{low} cytokine profile, adapted from the IL-1Ra^{high}-IL-4^{low} profile correlated with physical frailty (Pérez-Suárez, et al., 2016), is related to olfactory dysfunction (Darnell et al., 2019). It is hopeful that this will be clinically useful as a biomarker to identify patients at high-risk for health problems.

In this dissertation, I found cytokine profiles to be related to other inflammatory diseases. Therefore, future analyses will examine conditions of diabetes, arthritis, and also incidence of atrial fibrillation, which was added in Wave 3 of NSHAP, in relation to cytokine profiles and individual cytokines. Further, I currently have 2 undergraduate students who will be writing theses next year on the relationship between cognitive processes and inflammatory mechanisms, using the NSHAP data of course. Future research of my own and my students will be the highlight of my career. Overall, the work I completed in this dissertation was extremely informative and like most research, has led me to develop even more questions that I will continue to investigate.

References

Darnell, E.P., Wroblewski, K.E., Pagel, K.L., Kern, D.W., McClintock, M.K., & Pinto, J.M. (2019). IL-1Ra^{high}-IL-4^{low}-IL-13^{low}, A novel plasma cytokine signature of olfactory dysfunction in older US adults. Manuscript submitted for publication.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., ... & Stroke, S. S. (2013). Heart disease and stroke statistics--2013 update: A report from the American Heart Association. *Circulation*, *127*(1), e6.

Pérez-Suárez, T. G., Gutiérrez-Robledo, L. M., Ávila-Funes, J. A., Acosta, J. L., Escamilla-Tilch, M., Padilla-Gutiérrez, J. R., ... & Torres-Carrillo, N. M. (2016). VNTR polymorphisms of the IL-4 and IL-1RN genes and their relationship with frailty syndrome in Mexican community-dwelling elderly. *Aging Clinical and Experimental Research*, *28*(5), 823-832.