THE ROLE OF INFLAMMATION IN THE ASSOCIATION BETWEEN
AUTONOMIC NERVOUS SYSTEM DYSREGULATION AND COGNITIVE
DYSFUNCTION IN CARDIOVASCULAR DISEASE

A dissertation to be defended in partial fulfillment of the requirements for
the degree of Doctor of Philosophy in Clinical Psychology

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<td>α-blocker</td>
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</tr>
<tr>
<td>ACE inhibitors</td>
<td>angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td>ASA</td>
<td>acetylsalicylic acid (i.e., aspirin)</td>
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<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
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<td>AVR</td>
<td>aortic valve replacement</td>
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<td>β</td>
<td>standardized regression weight</td>
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<td>β-blocker</td>
<td>beta-blocker</td>
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<tr>
<td>B</td>
<td>unstandardized regression weight</td>
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<td>BDI</td>
<td>Beck Depression Inventory</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<tr>
<td>BRS</td>
<td>baroreceptor sensitivity</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>cc</td>
<td>cubic centimeter(s)</td>
</tr>
<tr>
<td>CHF</td>
<td>congestive heart failure</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>d</td>
<td>Cohen’s measure of effect size for comparing two sample means</td>
</tr>
<tr>
<td>Δ</td>
<td>Greek letter delta, referring to increment of change</td>
</tr>
<tr>
<td>COWA</td>
<td>Controlled Oral Word Association</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>DV</td>
<td>dependent variable</td>
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<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<td>EF</td>
<td>ejection fraction</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>f²</td>
<td>effect size index for a multiple correlation</td>
</tr>
<tr>
<td>g</td>
<td>gravity</td>
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<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
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<tr>
<td>HRV</td>
<td>heart rate variability</td>
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<td>HR</td>
<td>heart rate</td>
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<td>HR recovery</td>
<td>heart rate recovery</td>
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<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>IGNITE Rehab</td>
<td>Integrating Growth, Neurocognitive, and Inflammation research To Enhance Rehabilitation</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>IV</td>
<td>independent variable</td>
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LNS  Letter Number Sequencing
LPS  lipopolysaccharide
µL  microliter
µg/mL  microgram per milliliter
M  mean
METs  metabolic equivalents
MI  myocardial infarction
MoCA  Montreal Cognitive Assessment
MVR  mitral valve repair/replacement
mmHg  millimeters of mercury
MMSE  Mini Mental Status Exam
N  total sample size
n  subsample size
NSAID  non-steroidal anti-inflammatory drug
O₂/kg/min  millimeters of oxygen per kilogram (of body weight) per minute
p  statistical abbreviation referring to probability
PAD  peripheral arterial disease
PNS  parasympathetic nervous system
r  estimate of the Pearson product-moment correlation coefficient
ROCF  Rey-Osterrieth Complex Figure
SBP  systolic blood pressure
SD  standard deviation
SNS  sympathetic nervous system
t  sample value of the t-test statistic
TMT  Trail Making Test
WAIS  Wechsler Adult Intelligence Scale
Χ²  sample value of the chi-square statistic
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I am very grateful for the many individuals who have been influential in the pursuit of this dissertation and my overall educational goals. First, I would like to express my sincere gratitude for my co-advisors and dissertation committee co-chairs, Drs. Joel Hughes and John Gunstad, for their guidance and mentorship during not only the formulation of this dissertation, but throughout my graduate training. Second, I would like to acknowledge Colleen Cole Mattson, Tracy Hammonds, James Rosneck, and Dr. Donna Waechter for their assistance in executing this dissertation project. Third, I wish to extend my heartfelt appreciation to my family and friends for their support and encouragement during the dissertation process and every other phase of my education. Lastly, I dedicate this dissertation to my loving husband, Dr. Jonathan Keary. I am truly blessed by his enduring love and understanding.

“Whatever you do, work at it with all your heart, as working for the Lord” (Colossians 3:23).
INTRODUCTION

Overview

Cardiovascular disease (CVD) has been known to adversely impact cognitive function. Recent research has shown that dysregulation of the autonomic nervous system is also associated with cognitive impairment in this population. The underlying pathophysiological mechanisms of this association are poorly understood. However, one candidate mechanism implicates the inflammatory cytokines regulated by the vagus nerve, which may have a detrimental impact on cognition. The goal of the present study was to examine the associations among cardiovascular ANS regulation, inflammation, and cognitive functioning (particularly attention, executive, and psychomotor speed functions) in patients with CVD, with the expectation that inflammation was a partial mediator between ANS dysregulation and cognitive dysfunction.

The first section of the following introduction describes the burden of cardiovascular disease, with particular focus on the cognitive sequelae of the disease. The second provides the reader with a short tutorial of the structure and function of the two major divisions of the autonomic nervous system (ANS), and briefly describes the role of the ANS in the context of cardiovascular health and disease. The third section reviews research linking ANS dysregulation to cognitive impairment in CVD patients. The fourth section provides a short tutorial of inflammation and discusses its role in CVD and cognitive functioning.

The Burden of Cardiovascular Disease
Cardiovascular disease is a comprehensive term that refers to several conditions of the heart, blood vessel system (e.g., arteries, capillaries, and veins), and circulation, including such pathology as atherosclerosis, arteriosclerosis, angina pectoris, cardiomyopathy, hypertension, myocardial infarction, congestive heart failure, stroke, cardiac arrhythmias, coronary artery disease, and peripheral artery disease. CVD conditions are often co-morbid with one another, and approximately 81 million American adults (i.e., roughly one in four) have one or more types of CVD (American Heart Association, 2010). Hypertension, which affects more than 73 million Americans, is the most prevalent CVD condition (Lloyd-Jones et al., 2009).

Although considerable progress has been made in its prevention and treatment, CVD remains the single leading cause of death in the United States (Kung, Hoyert, Zu, & Murphy, 2008) and worldwide (World Health Organization, 2008). In addition to the substantial physical health consequences, CVD is associated with a tremendous financial burden (Goetzel et al., 2004; Matson Koffman et al., 2005). The estimated direct and indirect costs of CVD within the United States for 2009 was a staggering $475.3 billion (Lloyd-Jones, et al., 2009); this cost estimate is approximately three times the monetary expenditure related to CVD health care 15 years ago (National Heart, 1996). Unfortunately, the burden of CVD not only consists of considerable physical and financial costs, but also frequently includes impairment in cognitive functioning.

**Cardiovascular Disease and Cognitive Impairment**

CVD has been known to adversely impact cognitive functioning in varying degrees, from subtle to severe. Interestingly, the presence of even sub-clinical CVD has
been shown to be correlated with poorer cognitive performance in both middle-aged and elderly men (Muller, Grobbee, Aleman, Bots, & van der Schouw, 2007). CVD has been related to the development of subtle cognitive deficits well before the onset of both stroke and dementia (R. A. Cohen et al., 1999; Grubb, O'Carroll, Cobbe, Sirel, & Fox, 1996; Kalra, Jackson, & Swift, 1993; Mazzucchi et al., 1986; Moser et al., 1999; O'Reilly, Grubb, & O'Carroll, 2003; Rafnsson, Dreary, Smith, Whiteman, & Fowkes, 2007; Trojano et al., 2003). The deleterious effect of CVD on cognitive functioning has been estimated to be equivalent to approximately five years of additional aging (Elwood, Pickering, Bayer, & Gallacher, 2002). Even slight decrements in cognitive functioning may be considered practically significant, as cognitive deficits have been linked to poorer quality of life in this population (R. A. Cohen, et al., 1999). Cognitive deficits are of clinical concern because patients with CVD who are cognitively impaired show reduced adherence to treatment (Farmer et al., 1990; Vinyoles, De La Figuera, & Gonzalez-Segura, 2008) and reduced benefit from cardiac rehabilitation (Kakos et al., 2010). More moderate cognitive deficits are also of clinical concern because Mild Cognitive Impairment, a clinical condition between the cognitive decline of normal aging and the more severe cognitive deficits (e.g., dementia), precedes at least 50% of dementia onsets (Palmer, Fratiglioni, & Winblad, 2003).

In addition to its relationship with more subtle cognitive deficits, CVD is frequently associated with more severe brain pathology and cognitive dysfunction, including stroke, vascular dementia, and Alzheimer’s disease (de la Torre, 2006; Hofman et al., 1997; Kivipelto et al., 2001; Newman et al., 2005; van Oijen, de Jong, Witteman,
Hofman, & Breteler, 2007; Witt, Ballman, Brown, Jacobsen, & Roger, 2006). One study estimated that women with a history of myocardial infarction are five times more likely to develop dementia than those without a history of myocardial infarction (Aronson et al., 1990). Moreover, research has suggested that there is a strong dose-response relationship between the number of cardiovascular pathologies and degree of cognitive impairment (van Exel, Gussekloo, Houx, et al., 2002).

**The Autonomic Nervous System in the Context of Cardiovascular Health and Disease**

The ANS consists of nerves that regulate the body’s internal environment, particularly smooth muscle, glands, and cardiac muscle. The ANS manages several vital functions, including heart rate (HR), blood pressure, respiration rate, digestion, and internal body temperature. The ANS consists of two anatomically separate divisions, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). At the most basic level, the SNS governs functions related to arousal and energy expenditure, while the PNS controls the body’s vegetative, restorative, and growth functions. Accordingly, the PNS generally exerts a calming or stabilizing effect, while the SNS exerts an arousing or stimulating effect. The two divisions of the ANS can vary reciprocally, independently, or coactively with one another (Berntson, Cacioppo, & Quigley, 1991, 1993). Depending upon the relative input of the SNS and PNS at any given moment, the cumulative effect of ANS functioning can be either excitatory or inhibitory in nature.
The ANS plays a vital role in modulating the physiological properties and activity of the heart and vasculature. Both divisions of the SNS contain afferent (i.e., sensory) and efferent (i.e., motor) nerve fibers that subserve communication and interaction between the heart and the nervous system. Originating in the cervical and thoracic sympathetic ganglia, SNS nerves innervate the ventricles of the heart. Efferent SNS nerve signals increase heart rate and cardiac contractility and to dilate the coronary arteries. Afferent SNS nerves send pain signals from the heart back to the central nervous system. Originating in the dorsal motor nucleus of the medulla oblongata, PNS nerves traverse the vagus nerve and ultimately innervate several organs in the chest and abdomen, including the heart. Efferent PNS nerves serve to slow heart rate and constrict the coronary arteries. Afferent PNS nerves mediate cardiac reflexes (Malhotra, Edelman, & Lilly, 2003; Waldstein, Snow, Muldoon, & Katzel, 2001).

ANS dysfunction in patients with several types of CVD was initially discussed nearly four decades ago (Eckberg, Drabinsky, & Braunwald, 1971). Subsequent research employing a variety of cardiovascular indices of ANS function has demonstrated that patients with CVD tend to exhibit signs of attenuated PNS activity and elevated SNS tone (e.g., Frenneaux, 2004), as indicated by significantly lower heart rate variability (HRV), diminished baroreceptor sensitivity (BRS), and reduced (or slower) heart rate recovery (HR recovery) values than those of healthy (i.e., non-cardiac) controls. More specifically, middle-aged men and women with CVD demonstrate substantially lower HRV values on multiple HRV indices than healthy controls (Bigger et al., 1995; Kuch, Parvanov, Hense, Axmann, & Bolte, 2004; Saul et al., 1988). Impairment of the
baroreflex (i.e., reduced baroreceptor sensitivity) has also been shown to play a critical role in the development and progression of CVD and event occurrence (LaRovere, Pinna, & Raczk, 2008). Diminished baroreflex control of heart rate has been implicated in several manifestations of CVD, including hypertension, myocardial infarction, and coronary artery disease (Bristow, Honour, Pickering, Sleight, & Smyth, 1969; Eckberg & Sleight, 1992; Head, 1994; Ylitalo et al., 1997). In addition, previous research has shown that HR recovery is significantly reduced (or slower) in patients with CVD (Imai et al., 1994; Lipinski, Vetrovec, & Froelicher, 2004).

**Cardiovascular ANS dysregulation and Cognitive Dysfunction**

Recent research has provided evidence that cardiovascular ANS dysregulation is related to impaired cognitive functioning in CVD patients. For example, both blood pressure variability (BP variability) and HRV have been linked to neurocognitive outcome in persons with CVD (Britton et al., 2008; Gunstad et al., 2009; Keary et al., 2007; Kim et al., 2006). In addition, results from another recent investigation of CVD patients have indicated that faster HR recovery two minutes after exercise termination was correlated with better performance in multiple cognitive domains, including global cognitive functioning (Modified Mini Mental Status Exam), speeded executive function (Trail Making Test B), and language (Boston Naming Test short form). Of note, the relationships between ANS-mediated cardiovascular functioning and cognitive tasks appear to be independent of cardiovascular fitness (as measured by exercise capacity), age, years of education, and β-blocker usage (Keary et al., in press).
The underlying pathophysiological mechanisms of the association between cardiovascular ANS dysregulation and impaired cognitive functioning are not yet well-understood. However, one candidate mechanism implicates the inflammatory cytokines of the vagus nerve. More specifically, diminished BP variability, reduced HRV, and slower HR recovery may be a reflection of a more fundamental dysregulation of the cholinergic anti-inflammatory pathway, which is discussed in greater detail below.

**Inflammation**

**An introduction to inflammation.** Inflammation is an immune system response which occurs in response to mechanical injury, chemical toxins, invasion by microorganisms (e.g., bacteria and viruses), and hyper-sensitivity reactions (Rankin, 2004). Inflammation can be classified as either acute or chronic. Acute inflammation is characterized as having a rapid onset and short duration (i.e., lasting anywhere from a few minutes to a few days), while chronic inflammation is typified by a slower onset and longer duration, lasting anywhere from a few weeks to several years (Cotran, Kumar, & Collins, 1999).

Cytokines are protein molecules produced by immune system cells (e.g., macrophages, neutrophils, lymphocytes) that mediate the process of inflammation by either promoting or inhibiting the inflammatory response (Cotran, et al., 1999; Rankin, 2004). Cytokines are often classified as either pro-inflammatory or anti-inflammatory. Examples of pro-inflammatory cytokines include interleukin (IL)-1, IL-6, IL-18 and tumor necrosis factor-alpha (TNF-α). C-reactive protein (CRP), an acute phase protein produced by the liver in response to IL-6, is another pro-inflammatory marker that has
been widely investigated. Examples of anti-inflammatory cytokines include IL-4, IL-10, IL-11, and IL-13 (Kronfol & Remick, 2000; Opal & DePalo, 2000). During the acute phase response to injury or infection, immune system cells release pro-inflammatory cytokines, which, in turn, trigger a local inflammatory response (Bauman & Gauldie, 1994; Koj, 1996). Immune system cells can also release anti-inflammatory cytokines to restrain the inflammatory response and to confine it to the immediate site of infection; this reduces the likelihood that a local inflammatory response will spread into the bloodstream and impair remote areas of the body (Tracey, 2005).

The magnitude of the inflammatory response is a critical factor because an insufficient response can result in immunodeficiency, while an excessive response can result in morbidity and mortality (Tracey, 2002). When the immune system is functioning properly, cytokines help to successfully eradicate invading pathogens and restore immunological homeostasis without causing excessive tissue damage. However, when the magnitude of the invading pathogen is overwhelming or when counter-regulatory mechanisms (e.g., anti-inflammatory cytokine production) that typically suppress the release of pro-inflammatory cytokines is insufficient, chronic or systemic inflammation can cause more injury to the organism than the inciting infection or injury (Czura, Friedman, & Tracey, 2003; Pavlov & Tracey, 2005; Pavlov, Wang, Czura, Friedman, & Tracey, 2003; Tracey, 2002, 2005). Chronic inflammation has been known to contribute to the initiation and progression of several diseases, including rheumatoid arthritis, Crohn’s disease, diabetes, Alzheimer’s disease, tuberculosis, and systemic lupus
erythematous (Cotran, et al., 1999). As discussed below, inflammation is also thought to play a critical role in the pathogenesis and progression of many CVD conditions.

**The cholinergic anti-inflammatory pathway.** A relatively recent discovery has been the identification of a bi-directional neural communication route between the central nervous and immune systems that reflexively monitors and regulates the inflammatory response (Tracey, 2002). Local inflammatory cells activate afferent (i.e., sensory) signals of the vagus nerve so that information regarding the magnitude of the inflammatory response can be communicated to the central nervous system via the hypothalamic-pituitary-adrenal axis. In response, the efferent (i.e., motor) arm of the vagus nerve can release the neurotransmitter acetylcholine, which binds to nicotinic α7 receptors. The binding of acetylcholine on the nicotinic receptors suppresses pro-inflammatory cytokine synthesis and stimulates anti-inflammatory cytokine production (Wang et al., 2003). Tracey and colleagues (Pavlov & Tracey, 2005; Pavlov, et al., 2003; Tracey, 2002, 2007) have termed this efferent neural neuroimmunomodulatory mechanism the *cholinergic anti-inflammatory pathway* because acetylcholine is the principle parasympathetic/vagal postganglionic neurotransmitter involved. Thus, in addition to its role in regulating vital ANS functions like HR and BP, the PNS (and more specifically, the vagus nerve) also appears to play an important role in moderating the inflammatory response (Pavlov & Tracey, 2005; Tracey, 2002). According to this model, reduced activity of the cholinergic anti-inflammatory pathway may result in an unchecked inflammatory response, resulting in unnecessary tissue damage and contributing to disease pathogenesis and progression (Tracey, 2002, 2005).
**Inflammation and CVD.** According to recent conceptualization, inflammation contributes to the pathogenesis and progression of many manifestations of CVD (Berliner et al., 1995; Hansson, 2005; Koenig, 2001; Libby, Ridker, & Maseri, 2002; Mulvihill & Foley, 2002; Robbins & Topol, 2002; Ross, 1993). For example, atherosclerosis, a condition in which arteries become thickened and hardened, was previously regarded as simply a disease of lipid accumulation. However, research conducted during the past two decades has provided important insights regarding the role of inflammation in atherosclerosis. As a result, atherosclerosis is increasingly considered to be a chronic inflammatory disorder initiated by injuries to the vascular endothelium and endocardium, the layers of cells that line blood vessels and the heart (Libby, et al., 2002; Ross, 1993, 1999).

A rather extensive body of research has indicated that increased levels of certain inflammatory markers predict future cardiovascular morbidity and mortality in apparently healthy (i.e., non-cardiac) individuals. CRP has been the most extensively investigated inflammatory marker. At least 32 prospective cohort studies conducted worldwide have shown that a single measure of CRP is a predictor of future risk of first cardiovascular events and cardiovascular death (Cesari et al., 2003; Cushman et al., 2005; Hung et al., 2008; and Kritchevsky, Cesari, & Pahor, 2005; Luc et al., 2003; Pai et al., 2004; for a reviews of 27 studies, see Ridker, 2003). A series of three meta-analyses conducted by Danesh and colleagues (Danesh, Collins, Appleby, & Peto, 1998; Danesh et al., 2004; Danesh et al., 2000) have compared persons in the lower tertile of CRP with those in the upper tertile. Results of the most recent of these meta-analyses (i.e., Danesh, et al., 2004)
reported that the odds ratio for coronary heart disease was 1.5 (95% confidence interval = 1.3 to 1.7) in a comparison of participants in the top third of the group with respect to base-line CRP values with those in the bottom third.

IL-6 also appears to play an important role in predicting cardiovascular risk in the apparently healthy (Bermudez, Rifai, Buring, Manson, & Ridker, 2002; Cesari, et al., 2003; Luc, et al., 2003; Pai, et al., 2004; Ridker, Rifai, Stampfer, & Hennekens, 2000; Vasan et al., 2003; Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000). It should be noted that measures of IL-6 are less stable within individuals across time as compared to CRP levels, making IL-6 somewhat less reliable (Cava, Gonzalez, Pascual, Navajo, & Gonzalez-Buitrago, 2000). However, a recent systematic review (N= 17 studies) of IL-6 levels and subsequent risk of CVD attempted to adjust for the within-subjects variability of IL-6 levels by quantifying the extent to which single measurements reflect their long-term average (i.e., “usual”) levels (Danesh et al., 2008). Results indicated that increased long-term average IL-6 levels are correlated with greater risk of clinical coronary outcomes, including myocardial infarction and coronary death. Furthermore, population-based cohort studies of initially healthy individuals have also shown that levels of IL-8 (Boekholdt et al., 2004), IL-10 (van Exel, Gussekloo, de Craen, et al., 2002), IL-18 (Blankenberg et al., 2003), P-selectin (Ridker, Buring, & Rifai, 2001), E-selectin (Hwang et al., 1997), intercellular soluble adhesion molecule (ICAM)-1 (Ridker, Hennekens, Roitman-Johnson, Stampfer, & Allen, 1998), TNF-α (Cesari, et al., 2003), and fibrinogen (Kannel, Wolf, Castelli, & D'Agostino, 1987; Luc, et al., 2003) are associated with elevated risk of cardiovascular morbidity and mortality.
In addition to research documenting an association between inflammatory markers and subsequent risk of CVD in the apparently healthy, a growing body of research has examined inflammatory markers as predictors of recurrent CVD morbidity and cardiovascular mortality. Numerous investigations have demonstrated that CRP predicts new cardiovascular events in patients with angina (stable and unstable), as well as in those with history of myocardial infarction (Bazzino, Ferreiros, Pizarro, & Corrado, 2001; Biasucci, 2001; Biasucci et al., 1999; Bickel et al., 2002; Haerkate, Thompson, Pyke, Gallimore, & Pepys, 1997; Lindahl, Toss, Siegbahn, Venge, & Wallentin, 2000; Liuzzo et al., 1994; Milazzo et al., 1999; Niccoli et al., 2008; Ridker et al., 1998; Zebrack et al., 2002; Zebrack, Muhlestein, Horne, & Anderson, 2002). Other inflammatory markers, including TNF-α, IL-6, fibrinogen, and IL-10 have also been shown to predict risk of recurrent cardiovascular events and mortality in those who have some form of CVD (Bickel et al., 2002; Deswal et al., 2001; Heeschen et al., 2003; Lindmark, Diderholm, Wallentin, & Siegbahn, 2001; Ridker et al., 2000; Volpato et al., 2001).

**Inflammation and cognitive functioning.** Ample research supports the assertion that inflammation, as indexed by elevated levels of inflammatory biomarkers, is related to cognitive dysfunction. Cross-sectional population-based studies have found that elevated levels of CRP, IL-6, and IL-18 are related to cognitive dysfunction (Baune et al., 2008; Gimeno, Marmot, & Singh-Manoux, 2008; Marsland et al., 2006; Ravaglia et al., 2005; Teunissen et al., 2003; Wright et al., 2006). In addition, inflammation has been implicated in the pathogenesis of dementia (Peila & Launer, 2006; Weninger & Yankner, 2001; Wilson, Finch, & Cohen, 2002), and IL-6 has been shown to be elevated in patients
with Alzheimer’s disease and vascular dementia (Angelopoulos et al., 2008; Singh & Guthikonda, 1997). Moreover, several prospective investigations have observed that CRP, IL-6, and ICAM-1 levels are associated with increased risk of cognitive decline (Alley, Crimmins, Karlamangla, Hu, & Seeman, 2008; Komulainen et al., 2007; Rafnsson, et al., 2007; Schram et al., 2007; Weaver et al., 2002; Yaffe et al., 2004; Yaffe et al., 2003) and dementia (Engelhart et al., 2004; Jordanova, Stewart, Davies, Sherwood, & Prince, 2007; Ravaglia et al., 2007; Schmidt et al., 2002).

Four known studies have examined the relationship between inflammation and cognitive functioning in CVD patients (Gunstad et al., 2006; Hoth et al., 2008; Mangiafico, Samataro, Mangiafico, & Fiore, 2006; van Exel et al., 2003). Mangiafico et al. (2006) compared the cognitive performances of patients with peripheral arterial disease (PAD) with healthy age-, sex- and education-matched controls, and examined whether CRP was independently associated with cognitive performance. Results indicated that patients with PAD performed significantly worse than controls on measures of working memory (Digit Span Backward), psychomotor speed (Trail Making Test part A), visuoconstruction (Rey-Osterrieth Complex Figure [ROCF] Copy) and visual memory (ROCF Delayed recall). Of greater note, results of multiple linear regression analyses showed that CRP was a significant, independent predictor of cognitive performance on all four measures in patients with PAD.

Gunstad et al. (2006) examined the relationship between CRP and cognitive functioning in a sample of patients with diverse CVD conditions. Results indicated that CRP was significantly and independently correlated with performance in multiple
cognitive domains, including global cognitive performance (Dementia Rating Scale total score), attention/psychomotor function (Trail Making Test A), executive function (Similarities), verbal and non-verbal/visual memory (California Verbal Learning Test Short Form Free Recall, Brief Visuospatial Memory Test- Revised Trials 1-3 and Delayed Recall), and visuospatial abilities (Block Design, Hooper Visual Organization Test). Notably, the investigations by both Gunstad et al. (2006) and Mangiafiaco et al. (2006) yielded quite a similar pattern of results in terms of cognitive domains impacted, despite differences in the type of CVD patient sample studied (i.e., specifically PAD patients versus a diverse CVD patient sample) and the specific cognitive measures utilized.

Taking a somewhat different methodological approach to the study of inflammation and cognitive function in patients with CVD, van Exel et al. (2003) tested the hypothesis that a “pro-inflammatory response” is related to reduced cognitive performance in CVD patients. The ratio of TNF-α to IL-10 was calculated for each participant; larger ratios were considered indicative of a greater pro-inflammatory response. Results indicated that there was a significant negative association between the magnitude of the pro-inflammatory response and performance on several measures of cognitive functioning, including tests of global cognitive functioning (Mini Mental Status Exam), attention (Stroop Test), cognitive speed (Coding Test), and verbal memory (Word Learning Test). Individuals in the uppermost quartile of the TNF-α/IL-10 ratio were 2.8 times as likely to have dementia, as compared to those in the lowest quartile. Such
results provided preliminary evidence that a pro-inflammatory cytokine response is associated with cognitive impairment and dementia in CVD patients.

In a prospective study, Hoth et al. (2008) examined the relationship between CRP and change in cognitive functioning during a 1-year follow-up period. Higher CRP levels at baseline were related to a greater decline in cognitive performance in the attention-executive-psychomotor speed domain during the subsequent year; this association remained significant after adjusting for age and cognitive performance at study entry. The investigators noted that the declines observed during the short 1-year follow-up period, though subtle, may be a precursor to more severe cognitive impairment; prospective investigation with a much longer follow-up period are necessary to test this proposition.

**Inflammation and pathological brain changes.** Inflammatory processes are related to pathological changes within the brain. For example, higher levels of CRP and other inflammatory markers have been linked to greater cerebral atrophy (Jefferson et al., 2007) and the presence of cerebral small vessel disease (van Dijk et al., 2005; Wada et al., 2008), which are both known to adversely impact cognitive functioning (Prins et al., 2005; Rusinek et al., 2003; Whitwell, 2008). In addition, cytokines from the peripheral immune system can penetrate the blood-brain barrier via vagal nerve stimulation or active transport mechanisms (Banks, 2005; Banks, Farr, & Morley, 2002; Banks, Kastin, & Broadwell, 1995; Pan & Kastin, 1999). Once inside the brain, cytokines can have a detrimental impact on cognition because they can mediate the cellular mechanisms (e.g., acetylcholine and dopamine pathways) involved in cognition (Wilson, et al., 2002). Of
note, recent evidence suggests that any systemic challenge that promotes a systemic inflammatory response may contribute to the progression of chronic neurodegenerative diseases like dementia (for a review, see Perry, 2004).

**Inflammation section summary and integration.** Recent theory by Tracey and colleagues (Pavlov & Tracey, 2005; Pavlov, et al., 2003; Tracey, 2002, 2007) asserts that chronic inflammation may be perpetuated by a failure of the ANS (i.e., reduced vagal control) to properly regulate the inflammatory response. A rather substantial body of evidence indicates that chronic inflammation contributes to both the pathogenesis and progression of CVD. Inflammatory processes promote pathological changes within the brain (e.g., cerebral atrophy and cerebral small vessel disease) known to adversely impact cognition, and cytokines within the brain can interfere with the cellular mechanisms involved in cognition. Given these observations, it is not surprising that inflammation, as indexed by elevated levels of pro-inflammatory biomarkers, is related to poorer cognitive function and dementia in patients with CVD. Three cross-sectional studies have shown that inflammation is also related to cognitive dysfunction in CVD samples (Gunstad, et al., 2006; Mangiafico, et al., 2006; van Exel, et al., 2003), and one prospective study of CVD patients has shown that inflammation is correlated with larger declines in cognitive function over time (Hoth, et al., 2008).

Considered collectively, these observations may help to explain why ANS dysregulation is associated with cognitive impairment in CVD patients. More specifically, they provide preliminary support for the theory that inflammation, which may be perpetuated by a failure of the ANS to properly regulate the inflammatory
response, is a mechanistic contributor to the cognitive impairment observed in patients with CVD.

**The Present Study**

The present study simultaneously examined the associations among cardiovascular ANS regulation, inflammation, and cognitive functioning in patients with CVD. The expectation was that inflammation was a partial mediator between ANS dysregulation and cognitive dysfunction. See Figure 1.

*Figure 1.* Hypothesized relationships among ANS dysregulation, inflammation, and cognitive function in individuals with CVD.
**Cardiovascular ANS regulation.** Cardiovascular ANS regulation was operationally defined in terms HR recovery. Heart rate rapidly decreases during the first one to two minutes after the termination of exercise, and gradually decreases thereafter. HR recovery is the term used to describe this reduction in heart rate immediately after the sympathetically active state of exercise. HR recovery is considered to be a cardiovascular ANS index that is sensitive to parasympathetic activity. Because the return to resting HR following exercise termination is predominately accomplished via vagus nerve activity (often termed *vagal reactivation*), slower HR recovery is thought to be a marker of reduced PNS activity (Huang, Leu, Chen, & Lin, 2005; Imai, et al., 1994; Pierpont, Stolpman, & Gornick, 2000; Pierpont & Voth, 2004). Conversely, faster HR recovery is linked with cardiovascular health and a higher level of physical fitness (Imai, et al., 1994; Lauer & Froelicher, 2002).

In the present study, HR recovery was operationally defined as the difference in beats per minute (bpm) between peak HR and HR two minutes after exercise termination (Cole, Foody, Blackstone, & Lauer, 2000; Hughes et al., 2006; Lipinski, et al., 2004). Although HR recovery has also been computed from HR one minute after exercise termination, HR recovery at 2 minutes predicted mortality better than other indices in a large study of male patients completing treadmill tests and coronary angiography (Shetler et al., 2001), and in another study, only HR recovery at 2 minutes predicted the presence of coronary artery disease (Lipinski, et al., 2004).

**Inflammatory markers.** P-selectin and IL-10 were the inflammatory markers assessed in the present study. P-selectin was considered to be a pro-inflammatory marker
and IL-10 was regarded as an anti-inflammatory marker. Each inflammatory marker is briefly described below.

**P-selectin.** P-selectin is a cell adhesion molecule involved in the initial recruitment of leukocytes to the site of injury during inflammation. P-selectin helps to mediate platelet-leukocyte interaction and is expressed after exposure to inflammatory cytokines (Ludwig, Schon, & Boehncke, 2007; Woollard & Chin-Dusting, 2006). In a prospective epidemiological evaluation of apparently healthy women, elevated baseline levels of soluble P-selectin were associated with increasing risks of future myocardial infarction, stroke, coronary revascularization, and cardiovascular death; of note, this association was independent of age, smoking status, and several lipid and non-lipid cardiovascular risk factors (Ridker, et al., 2001). Previous work has documented that P-selectin levels are significantly higher in patients with stable coronary artery disease as compared to matched health controls (Atalar et al., 2000). In addition, P-selectin levels have been associated with the extent of coronary stenosis in CAD (Fang et al., 2004). Also of note, higher levels of P-selectin have been associated with more severe white matter lesions (de Leeuw et al., 2002).

**IL-10.** IL-10 is an anti-inflammatory cytokine known to suppress the immune response via inhibition of the production of a several pro-inflammatory cytokines, including IL-2, TNF-α, and IFN-γ (Moore, de Waal Malefyt, Coffman, & O'Garra, 2001; Pestka et al., 2004). Because of its anti-inflammatory properties, IL-10 is thought to be atheroprotective and to have anti-atherogenic potential (Galkina & Ley, 2009; Kleeman, Zadelaar, & and Kooistra, 2008). Elevated IL-10 levels have been associated with a
more favorable prognosis in patients with acute coronary syndromes and elevated CRP levels (Heeschen, et al., 2003). In addition, previous work has documented that older adults with low IL-10 production levels have an increased risk of stroke (van Exel, Gussekloo, de Craen, et al., 2002).

**Cognitive functioning.** The cognitive tests selected for the present study represent indices of attention, executive, and psychomotor speed functions. Although CVD can be linked to impairment in several cognitive domains, measures of attention, executive function and psychomotor speed were intentionally selected for several reasons. First, impairments of attention, executive, and psychomotor functions are the hallmarks of both vascular cognitive impairment and vascular dementia (Rockwood, 2002; Vinkers et al., 2005). Second, compared to healthy (i.e., non-cardiac) controls, deficits in attention and executive control have been observed in a similar sample of cardiac rehabilitation participants (Moser, et al., 1999). Third, previous work from our lab has documented an association between performance on a task of speeded executive function (i.e., TMT-B) and 2-minute HR recovery in a sample of cardiac rehabilitation patients. Fourth, three cross-sectional investigations which have examined the relationship between inflammation and cognitive functioning among CVD patients have shown that inflammation is related to performance on tasks of attention, executive function, and psychomotor speed (Gunstad, et al., 2006; Mangiafico, et al., 2006; van Exel, et al., 2003). Finally, recent research has shown that higher baseline inflammation (i.e., CRP level) is associated with decline in the attention-executive-psychomotor performance domain over the course of a 1-year follow-up period (Hoth, et al., 2008).
**Hypotheses**

**Aim 1.** The first aim of the present study was to examine the relationships among cardiovascular ANS regulation, inflammation, and cognitive functioning in CVD patients.

*Hypothesis 1:* It was hypothesized that cardiovascular ANS regulation would be significantly related to cognitive functioning. More specifically, it was hypothesized that faster HR recovery would be associated with better cognitive test performance.

*Hypothesis 2:* It was hypothesized that cardiovascular ANS regulation would be significantly related to inflammation. More specifically, it was hypothesized that heart rate recovery values would be: 1) directly related to an anti-inflammatory cytokine level (i.e., P-selectin) and, 2) inversely related to a pro-inflammatory cytokine level (i.e., IL-10).

*Hypothesis 3:* It was hypothesized that inflammation would be significantly related to cognitive functioning. More specifically, it was hypothesized that cognitive test performance would be: 1) directly related to an anti-inflammatory cytokine level (i.e., P-selectin) and, 2) inversely related to a pro-inflammatory cytokine level (i.e., IL-10).

**Aim 2:** The second aim of the present study was to examine inflammation as a *partial* mediator of cardiovascular ANS dysregulation and cognitive dysfunction.
Hypothesis 4: It was hypothesized that the strength of the relationship between HR recovery values and cognitive test performance would be significantly reduced when controlling for inflammatory cytokine levels.
METHOD

The Institutional Review Boards (IRBs) of Summa Health System and Kent State University approved the following study protocol. The present study is a part of a larger study entitled IGNITE Rehab (Integrating Growth, Neurocognitive, and Inflammation research To Enhance Rehabilitation), which was aimed at assessing the effects of psychosocial factors, physical functioning, and cognitive abilities on inflammation in cardiac rehabilitation patients.

Before consenting to participate, participants were provided with full verbal and written descriptions of the study's procedures and were given the opportunity to have questions or concerns addressed. A copy of the IRB-approved informed consent form is located in Appendix A. Participants enrolled in the study granted the researchers permission to gather information from their medical records. Information obtained from the clinical database included treadmill exercise stress test data, cognitive screening data [i.e., Mini Mental Status Exam (MMSE) score], sociodemographic data (e.g., age, sex, marital status), and medical history data (e.g., current medications, reason for referral to cardiac rehabilitation). A copy of the Health Insurance Portability and Accountability Act (HIPAA) consent form is located in Appendix B.

Participant Screening and Recruitment

The Administrative Coordinator of the Center for Cardiopulmonary Research at Summa Health System conducted the initial screening of patients entering the phase II cardiac rehabilitation program in order to determine which patients were eligible for
recruitment into the present study (see next section below for a description of eligibility and exclusion criteria). Two Clinical Psychology doctoral students recruited patients who met the eligibility criteria following their intake evaluation. The cardiac rehabilitation intake evaluations, which were conducted by either a registered nurse or exercise physiologist, included an exercise stress test, brief cognitive screening assessment (i.e., administration of the MMSE), and completion of self-report measures. Eligible participants who agreed to participate were scheduled to complete the research protocol within their first two weeks of cardiac rehabilitation; this two week limit was designed to limit/control for the potential confound of regular exercise on patients’ inflammatory marker profile (Abramson & Vaccarin, 2002).

**Participant Eligibility and Exclusion Criteria**

Male and female patients between 40 and 85 years of age who were enrolled in Summa Health System’s phase II cardiac rehabilitation program at Akron City Hospital between July 2009 and August 2010 were screened according to the following eligibility/exclusion criteria. Patients were deemed ineligible to participate if they had a history of: 1) dementia, as defined by a score lower than 24 on the MMSE (Folstein, Folstein, & McHugh, 1975), 2) a major neurological disorder (e.g., Alzheimer’s disease, stroke), 3) a major psychiatric disorder (e.g., schizophrenia, substance abuse), 4) intellectual disability (formerly known as mental retardation), or 5) a chronic inflammatory condition besides CVD (e.g., fibromyalgia, chronic kidney disease, rheumatoid arthritis, hepatitis). Patients who did not speak English as their first language were not recruited.
Patients with diagnoses hierarchical to coronary artery disease or coronary heart disease were not recruited for participation. For example, patients with heart failure [i.e., those with an ejection fraction (EF) ≤ 40%], which is often considered the so-called ‘endpoint’ of many types of cardiovascular disease, were excluded. In addition, patients who had recently undergone a cardiac procedure that required sternotomy [i.e., coronary artery bypass graft surgery (CABG), mitral valve repair/replacement (MVR), aortic valve replacement (AVR) within the past three months] were not recruited because they can evidence a significant systemic inflammatory response secondary to sternotomy and subsequent sternal healing (Ishida et al., 2006; Larmann & Theilmeier, 2004).

Cardiac rehabilitation patients who did not complete a treadmill exercise stress test were also not recruited for the present study because they did not have HR recovery data available for statistical analysis. Physicians referring to cardiac rehabilitation occasionally waive an entry stress test for patients who had orthopedic or other limitations. Cardiac rehabilitation patients who had any type of stress test other than a treadmill exercise stress test (e.g., bicycle exercise stress test, dobutamine or adenosine stress test, nuclear stress test) were also not recruited.

Upon completion of the study, participants’ data were excluded from statistical analysis if: 1) they reported symptoms of current infection, or 2) if they display inflammatory marker levels more than three standard deviations away from the sample mean (which may have indicated the presence of an acute infection).

Procedure
Within completion of the first two weeks of cardiac rehabilitation, participants completed a short battery of self-report questionnaires, a brief battery of cognitive functioning tests, and a blood draw. The blood samples were always drawn prior to the cardiac rehabilitation exercise sessions in an effort to minimize the acute effect of exercise on the inflammatory marker levels (Pedersen & Saltin, 2006; Ploeger, Takken, de Greef, & Timmons, 2009). Participants received monetary compensation for their participation in the form of a $40 (United States dollars) check.

**Measures**

**Beck Depression Inventory.** The Beck Depression Inventory (BDI; Beck & Steer, 1987) was administered as part of standard intake procedure for Summa’s phase II cardiac rehabilitation program. The BDI assesses depressive symptomatology during the previous two weeks. Each of the twenty-one items on the BDI consists of four statements representing increasing degrees of severity with scores ranging from 0 to 3. The total score on the BDI can range from zero (no depression) to a maximum score of 63 (severe state of depression). Patients with a BDI score of 10 or greater are considered to show at least mild to moderate symptoms of depression. Using a cutoff score of 10, the BDI has a sensitivity of 82% and specificity of 79% for diagnosing major depression in patients diagnosed with myocardial infarction (Strik, Honig, Lousberg, & Denollet, 2001).

**Cognitive screening measure.** The MMSE (Folstein, et al., 1975) was administered as part of standard intake procedures in cardiac rehabilitation. As noted above, the MMSE was used for the purposes of excluding cardiac rehabilitation patients who may have had dementia, as defined by a score lower than 24 (out of a maximum
possible 30 points). The MMSE is a widely used, well-validated screening instrument for evaluation of cognitive impairment. It is comprised of several short tasks that briefly measure orientation to time and place, immediate recall, short-term verbal memory, calculation, language, and visuoconstruction ability. Each area tested has a designated point value, with the maximum possible score of 30 points.

**Cognitive functioning measures.** Two Clinical Psychology doctoral students administered cognitive functioning testing on a one-on-one basis within completion of the first two weeks of cardiac rehabilitation. Administration of the cognitive test battery required approximately 30 minutes to complete. The cognitive measures represented a subset of cognitive functioning instruments administered as part of the larger IGNITE Rehab study. As noted above, the cognitive tests selected for the present study represent indices of attention, executive, and psychomotor speed functions. See Table 1 for the specific abilities assessed by each measure.

Table 1

*Tests of attention, executive, and psychomotor speed functions*

<table>
<thead>
<tr>
<th>Cognitive test</th>
<th>Specific Ability Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trail Making Test A (TMT-A)</td>
<td>psychomotor speed</td>
</tr>
<tr>
<td>Trail Making Test B (TMT-B)</td>
<td>cognitive set shifting (i.e., ability to switch between competing cognitive sets)</td>
</tr>
<tr>
<td>Controlled Oral Word Association (COWA)</td>
<td>phonemic verbal fluency, reflecting generativity (i.e., ease with which words can be produced given certain parameters)</td>
</tr>
<tr>
<td>Ruff 2 &amp; 7 Selective Attention Test (Ruff 2 &amp; 7)</td>
<td>focused, sustained attention</td>
</tr>
<tr>
<td>Letter Number Sequencing (LNS)</td>
<td>complex auditory attention and working memory</td>
</tr>
</tbody>
</table>
The cognitive functioning measures administered in the current study included:

1. **Trail Making Test A and B (TMT-A and TMT-B)**: TMT (Reitan, 1958) consists of two tasks, parts A and B. TMT-A is a measure of psychomotor speed and visual scanning. Participants were asked to connect a series of 25 numbered dots in ascending order as quickly as they can (e.g., 1-2-3, etc.). TMT-B adds a set-shifting component, and required participants to alternate between numbers and letters in ascending order (e.g., 1-A-2-B, etc.). The times to completion for parts A and B were recorded for each participant; longer times were considered to be indicative of poorer cognitive functioning. The test-retest reliability coefficients for TMT-A and TMT-B are 0.79 and 0.89, respectively (Dikmen, Heaton, Grant, & Temken, 1999).

2. **Ruff 2 & 7 Test (Ruff 2 & 7)**: The Ruff 2 & 7 (Ruff & Allen, 1996) measured sustained attention. The Ruff 2 & 7 consists of a series of 20 trials of a visual search and cancellation task. Participants were asked to detect and mark through all occurrences of the two target digits: "2" and "7." The two target digits were embedded among either other alphabetical letters or other numbers that served as distractors. The number of correct hits and errors were counted for each trial and serve as the basis for scoring the test. The speed score reflect the total number of correctly identified targets (hits). Higher speed scores were considered to be indicative of better cognitive functioning. The Ruff 2 & 7 has demonstrated good test-retest reliability (Lemay, Bedard, Rouleau, & Gilbert Tremblay, 2004).

3. **Letter Number Sequencing (LNS)**: LNS, a subtest of the *Wechsler Adult Intelligence Scale, fourth edition* (Wechsler, 2008), is a test of complex attention...
and working memory. Participants were read a string of numbers and letters in alternating order, and then asked to arrange the numbers and letters in a specific manner—i.e., all the numbers in ascending order followed by all the letters in alphabetical order. For example, if the string were 1-J-A-7, participants have been asked to generate 1-7-A-J as the correct response. Strings increased in length by one item after every three trials. Higher scores were considered to be indicative of better cognitive functioning (Dubois, Slachevsky, Litvan, & Pillon, 2000).

4. **Controlled Oral Word Association Test (COWA):** The COWA (A. Benton, Sivan, Hamsher, Varney, & Spreen, 1994; Eslinger, Damasio, & Benton, 1984) is a test of lexical fluency. Participants were asked to generate as many words as they could for a given letter for 60 seconds. For example, if the letter were B, participants could say boy, bear, bowling, etc. Three trials were performed, each with a different letter (i.e., the letters “C,” “F,” and “L”). A total score was calculated as the sum of performance across the three trials. Lower scores were considered to be indicative of poorer cognitive functioning.

**Use of raw scores.** For all analyses, raw scores of cognitive test performance, as opposed to standardized or scaled scores, were used. It was decided that the cognitive test results would not be converted to a standard metric for three primary reasons. First, conversion of raw scores to standardized or scaled scores would have effectively reduced variability (particularly for scaled scores) and may have resulted in artificially anchoring the scores to a normative sample that is dissimilar from the current sample. Second,
relevant factors (i.e., sex, age, education level), which are typically used to convert raw scores into standardized or scaled scores, were considered for inclusion in the hierarchical multiple linear regression analyses as covariates. Finally, other studies in this area (e.g., Gunstad, et al., 2006; Hoth, et al., 2008) have used raw scores and, thus, current findings are more directly comparable to past work.

**Cognitive test battery order.** Cognitive tests were administered in a fixed order. In theory, counterbalancing the order of administration of the cognitive tests could have helped to minimize any potential effect of participant fatigue. However, practically speaking, participant fatigue was likely very negligible given that the entire battery of cognitive measures administered in the larger study required approximately 45 minutes of time. In addition, the battery of the larger IGNITE Rehab study included measures of memory, which required a more rigid arrangement of measures to ensure that sufficient time elapsed between the learning and delayed recall trials of the memory measures.

**Exercise Stress Testing**

Resting heart rate, maximum heart rate, exercise capacity (also known as functional work capacity), and HR recovery values for each patient were recorded from a symptom-limited, volitional maximum graded treadmill exercise test. The treadmill exercise tests were conducted according to a modified ramp protocol using a Marquette Series 2000 treadmill interfaced with the GE-Case V6.51 monitoring system (General Electric Healthcare, USA). Consistent with American College of Sports Medicine’s (American College of Sports Medicine, 2009) guidelines, an exercise test was terminated at the patient’s request or if the patient showed clinical signs of decompensation,
including ischemia, malignant arrhythmias, severe hypertension (SBP > 250 mmHg and/or DBP > 115 mmHg), hypotension (10 mmHg decrease in SBP), increasing chest pain, fatigue, shortness of breath, wheezing, leg cramps, or claudication. The electrocardiogram (ECG) continuously recorded from rest until 6 minutes following the cessation of exercise using a Burdick ECG management system (Quinton Cardiology, Deerfield, WI). A standard 12-lead ECG measured HR at the following time points: 1) at rest before the initiation of exercise (resting heart rate), 2) at maximum peak of exercise (i.e., the point of exercise termination), 3) within one minute following termination, and 4) at two minutes, four minutes, and six minutes following exercise termination (2-minute, 4-minute, and 6-minute HR recovery, respectively).

**Heart rate recovery.** As noted above, HR recovery was operationally defined as the difference in bpm between peak HR (i.e., HR at exercise termination; also known as volitional max) and HR two minutes after exercise termination (Cole, Foody, Blackstone, & Lauer, 2000; Hughes et al., 2006; Lipinski, et al., 2004).

**Metabolic equivalents.** The metabolic equivalent (MET) of a task is a physiological metric that represents the energy expenditure associated with a given physical activity; it is expressed as a multiple of the resting metabolic rate (Jette, Sidney, & Blumchen, 1990). According to Ainsworth et al. (1993), one MET is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 millimeters of oxygen per kilogram of body weight per minute ($O_2/kg/min$). Accordingly, a two METs activity requires twice the resting metabolic rate (i.e., 7.0 ml $O_2/kg/min$) and a three METs activity requires three times the resting metabolic rate (i.e., 10.5 ml $O_2/kg/min$),
and so on. As an example, a patient who can attain a METs level of 6.0 on his or her exercise stress test can exceed his or her resting oxygen consumption by a multiplier of six.

**Inflammatory Markers**

For each participant, three 4-cc (cubic centimeters) blood samples were drawn by phlebotomists at Summa Health System’s outpatient laboratory. Blood samples were collected via venipuncture into three Vacutainer® tubes (i.e., two sodium heparin tubes and one untreated tube). As noted above, the inflammatory markers assessed in the current study were P-selectin and IL-10.

**Laboratory analysis of P-selectin.**

**Sample preparation.** Whole blood samples were collected in clot-activated Vacutainer® tubes in order to separate the serum from the clot. The vials were then removed and centrifuged at 1000 x g for a period of 5 minutes at room temperature. The supernatant was collected and stored at -80°C until the study was completed.

**Enzyme-linked immunosorbent assay (ELISA) method.** Serum from the samples was allowed to reach room temperature and analyzed using a human soluble P-selectin immunoassay (R&D Systems, Minneapolis, MN) based on the principles of sandwiched ELISA. A monoclonal antibody specific for P-selectin was pre-coated on a 96-well microplate. Standards, samples, and controls were then pipetted into the wells together with a polyclonal antibody specific for P-selectin, which was conjugated to horseradish peroxidase. The unbound horseradish peroxidase was removed and a
substrate with fluorescent dye was added which was proportional to the analyte concentration.

All reagents, standards, controls, and samples were prepared in accordance to the manufacturer’s instructions. Samples were run in duplicate. The following reagents were added to each well of the microplate: 100 µL analyte, 100 µL conjugate, 100 µL substrate, and 100 µL stop solution. The plates were incubated and washed between each addition. The samples were then read on a microplate reader (Bio-Rad® LP 400, Anthos Labtec Instruments, Austria) at 450 nm with a reference filter 550 nm within 30 minutes of the addition of the final reagent. Quantification of P-selectin was interpolated from a standard curve with a linear curve fit of $R^2 \geq 0.90$. The average intra-assay coefficient of variation was 5.38%.

**Laboratory analysis of IL-10.**

**Lipopolysaccharide stimulation.** The whole blood was stimulated with 10 µL lipopolysaccharide (LPS; from Escherichia coli 0111:B4 L 4391, Sigma-Aldrich, St Louis, MO) at a concentration of 250 µg/mL without antibiotics in polypropylene vials under sterile conditions within 24 hours of collection. Control samples containing whole blood without LPS were set-up in parallel to measure for unstimulated production of cytokines. The vials were incubated at 37°C for 24 hours in a CO$_2$ incubator. The vials were then removed and centrifuged at 1000 x g for a period of 5 minutes at room temperature. The supernatant was collected and stored at -80°C until the study was completed.
**Assay method.** Supernatant from the LPS challenged and unchallenged samples were allowed to come to room temperature and analyzed in batches. Bio-Plex Human 8-plex bead kits (BioRad Corporation, Hercules, CA) were used to perform the cytokine assays. These kits are based on the principle of solid-phase sandwich immunoassays. Solid phase sandwich immunoassays bind the antigen of interest to an antibody-coated micro-bead that is infused with varying ratios of two fluorescent dyes. The beads fluoresce when exposed to specialized lasers within the Luminex 200 analyzer (Luminex Corp, Austin, TX).

All reagents, beads, standards, and samples were prepared in accordance to the manufacturer’s instructions using a 96-well. Stimulated samples were diluted and run in duplicate. Plates were coated with 50 µL of the assay beads that were added to each well, which was subsequently washed with Bio-Plex wash buffer and vacuum-filtered. A 50 µL aliquot of standard and/or sample was then added to the designated well. The 96-well plates were then incubated and shaken at room temperature for a period of 30 minutes. The following reagents were then added to each well: 25 µL detection antibody and 50 µL streptavidin-PE. The plate was vacuum-filtered and incubated while shaking in between each addition. Once all the reagents were added, the plate was washed with Bio-Plex wash buffer and the plates were read on a Luminex® 200 cytometer (Luminex, Inc., Austin, TX). Stimulated levels of IL-10 were determined using the Luminex xPONENT® 3.1 software (Luminex Corp, Austin, TX). Quantification of cytokine level was interpolated from a standard curve with a logistic-5PL curve fit of $R^2 \geq 0.90$.

Stimulated cytokine production was quantified by subtracting the cytokine levels in
unstimulated samples from stimulated levels. The average intra-assay coefficient of variation was 10.96%.

**Note.** The assay method for P-selectin (i.e., ELISA) yielded an estimate of each participant’s inflammatory status at the time of blood collection (i.e., cytokine plasma level). The assay method for IL-10 (i.e., LPS stimulation) yielded an estimate of each participant’s response to a challenge. LPS stimulation is a better method for examining ANS regulation. Vagal activity regulates inflammatory response to challenge, which in turn, influences cytokine plasma levels.

**Medical Chart Review**

A medical chart review was conducted to obtain information regarding sociodemographic variables, anthropometric indices, reasons for referral to cardiac rehabilitation, and prescribed medications.

**Sociodemographic information.** Age (in years) was recorded at the date of the initial cardiac rehabilitation intake evaluation and was represented as a continuous variable. Education level (in years) was based on the number of completed years of formal education and was represented as a continuous variable. Sex consisted of two categories: male and female. Race included the categories of Caucasian (non-Hispanic), African-American, Asian, Native Hawaiian or Pacific Islander, Native American or Alaska Native, and Hispanic. Employment status consisted of four categories: employed full-time, employed part-time, unemployed, and retired. Marital status consisted of five categories: married, divorced, widow/widower, single, and in a non-married committed relationship.
Anthropometric information. Data regarding participants’ height, weight, and body mass index (BMI) were extracted from cardiac rehabilitation electronic records. Height was a continuous variable measured in inches. Weight was a continuous variable measured by balance scale in pounds. BMI was a continuous variable calculated on the basis of each participant’s height and weight; the following formula was utilized: \[ \text{BMI} = \left( \frac{\text{weight as measured in pounds}}{\text{height as measured in inches}^2} \right) \times 703. \]

During the prospectus meeting, one of the committee members (M. Zullo) suggested examining the potential associations between anthropometric measures (e.g., weight, BMI, waist circumference, and waist-to-hip ratio) as other potential covariates. Prior research has identified several important relationships among chronic inflammatory states, obesity, insulin resistance, and cognitive function (Balistreri, Caruso, & Candore, 2010; Dandona, Aljada, Chaudhuri, Mohanty, & Garg, 2005; Yaffe, 2007; Yaffe, et al., 2004). As noted above, data regarding participants’ height, weight, BMI, and waist circumference were extracted from cardiac rehabilitation electronic records. However, waist-to-hip ratio data are not routinely collected in Summa Health System’s phase II CR. Inspection of the waist circumference data revealed that several missing values (13 cases), as well as numerous improbable values (e.g., 12 cases with values of zero). Accordingly, waist circumference and waist-to-hip ratio data were not considered as potential covariates in the current study. BMI, as opposed to weight alone, was the anthropometric index selected as a potential covariate because it takes into account both weight and height.
**Medications.** All participants’ prescribed medications were recorded at the time of their cardiac rehabilitation intake evaluation. Medications were then categorized as currently prescribed or not currently prescribed according to the following 10 drug classes: 1) alpha blocker (α-blocker), 2) beta-blocker (β-blocker), 3) diuretic, 4) statin, 5) anti-anxiety, 6) anti-depressant, 7) anti-coagulant, 8) angiotensin-converting enzyme inhibitors (ACE inhibitor), 9) acetylsalicylic acid (ASA, i.e., aspirin), and 10) non-steroidal anti-inflammatory drug other than aspirin (NSAID). The present study did not examine medication dosages, medication adherence, or changes in medication after the cardiac rehabilitation intake evaluation.

**Power Analysis**

Power analyses for the mediation model were conducted using G*Power- Version 3.0 (Faul, Erdfelder, Lang, & Buchner, 2007). Based on previous research (Keary, et al., in press), the effect size between HR recovery and cognitive performance was estimated to be within the medium range. According to Cohen (J. Cohen, 1992), a medium effect size for F-test multiple regression (i.e., $f^2$) is .15.

At the time of the prospectus meeting, it was anticipated that six predictors would have been included in the mediation analyses, including four potential covariates (i.e., sex, age, years of education, and β-blocker usage) and two independent variables (i.e., HR recovery and one of the inflammatory markers). In order to obtain the critical F value, $F(6, 91) = 2.19$, in a multiple linear regression with six predictors, with an alpha of .05, power of .80 and an estimated effect size of .15, a sample size of 98 is required. Consequently, the initially proposed sample size of 100 should have allowed for adequate
power to detect significant associations among the study variables. However, only two covariates (i.e., age, education) and two independent variables (i.e., HR recovery and one of the inflammatory markers) were ultimately included in the mediation regression analyses conducted. In order to obtain the critical F value, $F(4, 80) = 2.49$, in a multiple regression with four predictors, with an alpha of .05, power of .80 and an estimated effect size of .15, a sample size of 85 was required.

**Statistical Analysis Strategy**

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 19 (Chicago, IL, 2010). For all analyses, the criterion for statistical significance was set at $p < .05$. In accordance with Cohen (1992), Cohen’s $d$ values of 0.2, 0.5, and 0.8 were considered to be the minimum thresholds for a small effect, medium effect, and large effect sizes, respectively. In addition, Pearson’s $r$ values of .10, .30, and .50 were considered to be the minimum thresholds for a small effect, medium effect, and large effect sizes, respectively. Moreover, $f^2$ values of .02, .15, and .35 were considered to be the $f^2$ were considered to be the minimum thresholds for a small effect, medium effect, and large effect sizes, respectively.

**Preliminary analyses.** Descriptive data (i.e., frequencies, percentages, means, standard deviations, minimum values, and maximum values) regarding participant demographic characteristics, clinical conditions, cardiovascular indices, and neuropsychological test performance were generated. The following factors with known importance for HR recovery, cognitive test performance, and/or the inflammatory markers were also examined via bivariate correlations and independent samples $t$-tests:
sex, age, years of education, exercise capacity (i.e., METs), \( \beta \)-blocker prescription, and BMI.

**Primary analyses.** In order to determine whether inflammation mediates the relationship between HR recovery and cognitive test performance, a series of hierarchical multiple linear regression analyses were performed according to the method outlined by Baron and Kenny (1986). According to this method, there are four steps in establishing that a variable (i.e., inflammation) mediates the relation between an independent variable (i.e., HR recovery) and a dependent variable (e.g., cognitive functioning; see Figure 2). These four steps can be accomplished with three multiple regression equations, which are discussed in detail in the next paragraph. The first step is to show that there is a significant relationship between the independent variable and the dependent variable (see Path c in Figure 2A). The second step is to show that the independent variable is related to the mediator (see Path a in Figure 2B). The third step is to show that the mediator is related to the dependent variable. This is Path b in Figure 2B, and it is estimated controlling for the effects of the independent variable on the outcome. The fourth and final step is to demonstrate that the strength of the relationship between the independent variable and the dependent is significantly reduced when the mediator is added to the model (compare Path c in Figure 2A with Path c’ in Figure 2B).
A) Direct Pathway

B) Indirect Pathway

*Figure 2.* Diagrams of direct and mediator effects.
Three sets of hierarchical multiple regression analyses were analyzed: (1) regressing the dependent variable (i.e., each of the cognitive test performance variables—separately) on the independent variable (i.e., HR recovery), (2) regressing the mediator variable (i.e., each of the inflammatory markers—separately) on the independent variable (i.e., HR recovery), and (3) regressing the dependent variable (i.e., each of the cognitive test performance variables—separately) on both the independent variable (i.e., HR recovery) and the mediator variable (i.e., each of the inflammatory markers—separately). This third set of regressions provided a test of whether the mediator is related to the dependent variable (Path b), as well as an estimate of the relationship between the independent variable and the dependent variable controlling for the mediator (Path c\textsuperscript{*}).

Because partial (vs. complete) mediation was hypothesized, the relationship between the independent variable and the dependent variable was expected to be significantly smaller when the mediator is included in the model, but would still be greater than zero. The Sobel test could have been conducted in conjunction with all mediation tests to determine if the change in relationship between HR recovery and cognitive test performance was significantly different when the effect of the mediator is included (MacKinnon, Warsi, & Dwyer, 1995). The Sobel test uses unstandardized coefficients and standard errors of the independent variable-mediator and the mediator-dependent variable relationships to determine statistical significance.

Use of a mediation model allowed for the identification of a potential mechanism (i.e., inflammation) to explain the relationship between cardiovascular ANS dysregulation and cognitive functioning (for discussions on establishing causation in
medication analysis, see Frazier, Tix, & Barron, 2004; MacKinnon, Fairchild, & Fritz, 2007). However, it is important to note that temporal precedence could not be established among the variables due to the cross-sectional nature of this study and, thus, true mediation could not be determined. Nonetheless, examining the relationships among cardiovascular ANS dysregulation, inflammation, and cognitive functioning can provide a foundation for a prospective investigation in which true mediation could be examined (i.e., a prospective study with assessments at multiple time points).
RESULTS

Participant Screening and Recruitment

A total of 556 patients entering Summa Health System’s phase II cardiac rehabilitation program between July 2009 and August 2010 were screened according to the study’s eligibility criteria. See Figure 3. The majority of patients screened (i.e., 379/553, 68.5%) were deemed ineligible. Recent history of CABG or other cardiac procedure that required sternotomy (e.g., MVR, AVR) was the most common reason for ineligibility (32.2% of the patients deemed ineligible). History of congestive heart failure (i.e., those with an EF ≤40%) was the second most common reason for ineligibility (17.7% of the patients deemed ineligible). Among the 174 patients who met the eligibility criteria, 29 (16.7%) were not recruited (e.g., underwent cardiac rehabilitation intake evaluation, but did not attend CR), 46 (26.4%) declined the invitation to participate, and 99 (56.9%) agreed to participate. Among the 99 participants who were eligible and agreed to participate, 96 participated in the full IGNITE study protocol, while three patients no-showed for their appointment and could not be rescheduled.
Figure 3. Participant screening and recruitment.
Among the 96 participants who participated in the full study protocol, 10 participants had missing data for at least one of key study variables. More specifically, P-selectin data could not be obtained for two participants because insufficient amounts of blood were drawn. In addition, complete exercise stress test data could not be obtained for an additional eight participants. As a result, 86 participants’ data were initially considered for inclusion in the final sample.

**Examination of Data Distributions**

The distributions of the continuous-level variables were examined for possible violations of univariate normality based on inspection of skewness and kurtosis statistics, histograms and boxplots, and skewness ratios. Both P-selectin and IL-10 values were positively skewed. P-selectin values were effectively corrected with a log_{10} transformation. IL-10 values were effectively corrected with a square root transformation. No other continuous variables required transformation. A total of six participants’ data were excluded from the sample because their data were found to have one or more univariate outliers (as defined by a value greater than ±3 standard deviation from the mean) on the primary variables of interest. Thus, the final sample consisted of 80 participants. See Figure 3.

**Characteristics of Study Participants**

**Sociodemographics.** The sociodemographic characteristics of the final sample (N = 80) are presented in Table 2. Briefly, the majority of participants were male (71.3%), Caucasian (n = 92.5%), and married (80%). The mean age of participants was 62.16 years (SD = 10.27). Participants completed an average of 14.16 years of education
Data regarding the type of event or condition prompting referral to cardiac rehabilitation are also presented in Table 3. The vast majority of the sample was referred following stent insertion (60%), myocardial infarction (MI; 11.3%), or a combination of both (20%). The mean BDI score was 5.95 ($SD = 6.24$), and 19% of the sample reported experiencing elevated depressive symptoms, as defined by BDI score of 10 or greater.
Table 2

Sociodemographic Characteristics of the Sample ($N = 80$)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$M (SD)$</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.16 (10.27)</td>
<td>40-84</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.16 (2.45)</td>
<td>9-20</td>
</tr>
<tr>
<td>MMSE†</td>
<td>28.75 (1.39)</td>
<td>24-30</td>
</tr>
<tr>
<td>BDI-II total score (raw) ‡</td>
<td>5.95 (6.24)</td>
<td>0-34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>% of total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>57</td>
<td>71.3</td>
</tr>
<tr>
<td>Females</td>
<td>23</td>
<td>28.7</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (non-Hispanic)</td>
<td>74</td>
<td>92.5</td>
</tr>
<tr>
<td>African American</td>
<td>6</td>
<td>7.6</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Native Hawaiian or Pacific Islander</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Native American or Alaska Native</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Employment Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed full-time</td>
<td>33</td>
<td>41.3</td>
</tr>
<tr>
<td>Employed part-time</td>
<td>8</td>
<td>10.0</td>
</tr>
<tr>
<td>Unemployed</td>
<td>7</td>
<td>8.8</td>
</tr>
<tr>
<td>Retired</td>
<td>32</td>
<td>40.0</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>64</td>
<td>80.0</td>
</tr>
<tr>
<td>Divorced</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>Single</td>
<td>7</td>
<td>8.8</td>
</tr>
<tr>
<td>Widow/Widower</td>
<td>5</td>
<td>6.3</td>
</tr>
<tr>
<td>In a non-married, committed relationship</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Smoking History</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>4</td>
<td>5.0</td>
</tr>
<tr>
<td>Past smoker</td>
<td>40</td>
<td>50.0</td>
</tr>
</tbody>
</table>

†MMSE data were not available for 8 participants.
‡BDI-II total score datum was not available for 1 participant.
Table 3

*Reasons for Referral to Cardiac Rehabilitation (N = 80)*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% of total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent</td>
<td>48</td>
<td>60.0</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>11.3</td>
</tr>
<tr>
<td>MI &amp; Stent</td>
<td>16</td>
<td>20.0</td>
</tr>
<tr>
<td>CABG and/or AVR†</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Angina</td>
<td>5</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*Abbreviations.* MI = myocardial infarction; CABG = coronary artery bypass graft; AVR = aortic valve replacement.

†Participants with referral diagnoses of CABG and/or AVR underwent their surgical procedure at least three months prior to initiation of cardiac rehabilitation.

**Anthropometric information.** Descriptive data regarding participants’ height, weight, and BMI are presented in Table 4. As noted above, valid data regarding waist circumference and hip-to-waist ratio were not available. The sample mean BMI was 31.78 ($SD = 6.47$), which corresponds to a classification of Obese—Class I (World Health Organization, 2000). Of note, BMI was not significantly correlated with either P-selectin ($r = .14, p = .21$) or IL-10 values ($r = -.20, p = .07$).
Table 4

*Anthropometric Indices*

<table>
<thead>
<tr>
<th></th>
<th>M (SD)</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (inches)</td>
<td>68.11 (3.54)</td>
<td>59-78</td>
</tr>
<tr>
<td>Weight (pounds)</td>
<td>209.50 (43.21)</td>
<td>113-317</td>
</tr>
<tr>
<td>BMI</td>
<td>31.78 (6.47)</td>
<td>19.97-48.04</td>
</tr>
</tbody>
</table>

**BMI Classification†**

<table>
<thead>
<tr>
<th></th>
<th>Principal cut-off points/ ranges</th>
<th>n (% of total sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal</td>
<td>18.50-24.99</td>
<td>10 (12.5%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.00-29.99</td>
<td>26 (32.5%)</td>
</tr>
<tr>
<td>Obese Class I</td>
<td>30.00-34.99</td>
<td>23 (28.7%)</td>
</tr>
<tr>
<td>Obese Class II</td>
<td>35.00-39.99</td>
<td>9 (11.3%)</td>
</tr>
<tr>
<td>Obese Class III</td>
<td>≥40.00</td>
<td>12 (15.0%)</td>
</tr>
</tbody>
</table>


**Medications.** Data regarding the medications participants were prescribed at the time of their cardiac rehabilitation intake evaluation are presented in Table 5. A majority of the sample was prescribed each of the following classes of medication: β-blocker (n = 71, 88.8%), statin (n = 64, 92.5%), anti-coagulant (n = 62, 77.5%), and ACE inhibitor (n = 59, 73.8%).
Table 5

*Medications Prescribed at the time of the Cardiac Rehabilitation Intake Evaluation*

<table>
<thead>
<tr>
<th>Medication Class</th>
<th>n</th>
<th>% of total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-blocker</td>
<td>22</td>
<td>27.5</td>
</tr>
<tr>
<td>β-blocker</td>
<td>7</td>
<td>88.8</td>
</tr>
<tr>
<td>diuretic</td>
<td>2</td>
<td>28.7</td>
</tr>
<tr>
<td>statin</td>
<td>74</td>
<td>92.5</td>
</tr>
<tr>
<td>anti-anxiety</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>anti-depressant</td>
<td>12</td>
<td>15.0</td>
</tr>
<tr>
<td>anti-coagulant</td>
<td>62</td>
<td>77.5</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>59</td>
<td>73.8</td>
</tr>
<tr>
<td>ASA</td>
<td>74</td>
<td>92.5</td>
</tr>
<tr>
<td>NSAID</td>
<td>5</td>
<td>6.3%</td>
</tr>
</tbody>
</table>

*Abbreviations.* α-blocker = alpha-blocker; ACE inhibitors = angiotensin-converting enzyme inhibitors; ASA = acetylsalicylic acid (i.e., aspirin); β-blocker = beta-blocker; NSAID = non-steroidal anti-inflammatory drug.

**Preliminary Analyses**

**Inflammatory markers.** Descriptive data regarding the inflammatory markers are presented in Table 6. The average intra-assay coefficients of variation were 5.38% and 10.96% for P-selectin and IL-10, respectively. As noted above, both P-selectin and IL-10 values were positively skewed; P-selectin values were effectively corrected with a log_{10} transformation, and IL-10 values were effectively corrected with a square root transformation. Although raw descriptive data (i.e., non-transformed mean and standard deviation values) are reported in the following three paragraphs, the inferential statistics reported are based upon the transformed values.
Table 6

*Inflammatory Marker Data*

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin (raw)</td>
<td>5.72</td>
<td>5.02</td>
<td>0.53-20.57</td>
</tr>
<tr>
<td>P-selectin (log(_{10}) transformed)</td>
<td>0.58</td>
<td>0.41</td>
<td>-0.28-1.31</td>
</tr>
<tr>
<td>IL-10 (raw)</td>
<td>1378.05</td>
<td>818.17</td>
<td>82.57-4050.22</td>
</tr>
<tr>
<td>IL-10 (square root transformed)</td>
<td>35.43</td>
<td>11.21</td>
<td>0.09-63.64</td>
</tr>
</tbody>
</table>

Males and females did not significantly differ with respect to P-selectin values (males: \(M = 5.61, SD = 5.15\), females: \(M = 5.97, SD = 4.78\), \(t (78) = -0.26, p = .80\)).

Males and females did not significantly differ with respect to IL-10 values (males: \(M = 1387.04, SD = 842.86\), females: \(M = 1359.11, SD = 775.95\), \(t (78) = 0.02, p = .99\)). Age was not significantly correlated with either P-selectin \((r = -.07, p = .57)\) or IL-10 values \((r = .09, p = .45)\).

Participants who were prescribed an ASA did not have significantly different P-selectin values than those who were not \((M = 5.74, SD = 4.94\) versus \(M = 5.37, SD = 6.38\), respectively, \(t (78) = -0.57, p = .57\)). Participants who were prescribed an ASA also did not have significantly different IL-10 values than those who were not \((M = 1393.27, SD = 835.75\) versus \(M = 1203.15, SD = 608.66\), respectively, \(t (78) = -0.40, p = .69\)).

Participants who were prescribed an NSAID did not have significantly different P-selectin values than those who were not \((M = 4.67, SD = 2.94\) versus \(M = 5.79, SD = 5.13\), respectively, \(t (78) = -0.10, p = .92\)). Participants who were prescribed an NSAID also did not have significantly different IL-10 values than those who were not \((M = \))
1529.07, \( SD = 824.68 \) versus \( M = 1369.00, \ SD = 823.65 \), respectively, \( t (78) = -0.53, p = .60 \).

**Cognitive Functioning Test Performance.** Descriptive data regarding cognitive functioning data are presented in Table 7. As noted above, two Clinical Psychology doctoral students administered cognitive functioning testing; each administrator tested 40 participants. Cognitive test performance did not significantly differ according to test administrator (all \( p \) values > .05). Also of note, participants who were prescribed \( \beta \)-blocker medication did not have significantly different cognitive test performance scores as compared to those who were not (all \( p \) values > .05).

<table>
<thead>
<tr>
<th></th>
<th>( M )</th>
<th>( SD )</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-A time (seconds)( \dagger )</td>
<td>33.94</td>
<td>10.29</td>
<td>17-58</td>
</tr>
<tr>
<td>TMT-B time (seconds)( \dagger )</td>
<td>85.39</td>
<td>34.63</td>
<td>33-180</td>
</tr>
<tr>
<td>Ruff 2 &amp; 7—Speed</td>
<td>238.28</td>
<td>48.59</td>
<td>136-351</td>
</tr>
<tr>
<td>LNS total score</td>
<td>19.15</td>
<td>3.45</td>
<td>10-26</td>
</tr>
<tr>
<td>COWA total score</td>
<td>34.16</td>
<td>10.22</td>
<td>13-57</td>
</tr>
</tbody>
</table>

*Abbreviations.* TMT-A and TMT-B = Trail Making Test, parts A and B, respectively; LNS = Letter-Number Sequencing; COWA = Controlled Oral Word Association. \( \dagger \)Higher values (i.e., longer times) are indicative of poorer cognitive functioning.

As can be seen in Table 8, males and females did not significantly differ in performance on any of the cognitive functioning variables; accordingly, sex was not included as a covariate in the subsequent hierarchical multiple linear regression analyses.
However, as expected, participant age was significantly correlated with performance on all cognitive functioning variables; increasing age was associated with worse cognitive test performance. Also as expected, participant education level was significantly correlated (or demonstrated a trend towards being significantly correlated, as was the case with TMT-A) with performance on all cognitive functioning variables; higher levels of education were associated with better cognitive test performance. Accordingly, participant age and education level were retained as covariates in the subsequent primary analyses.

Table 8

Relationships Between Cognitive Functioning Data and Potential Covariates

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th></th>
<th>Age</th>
<th></th>
<th>Education</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>TMT-A time (seconds)†</td>
<td>-1.24</td>
<td>0.22</td>
<td>0.46$^b$</td>
<td>&lt; .001</td>
<td>-0.19</td>
<td>0.09</td>
</tr>
<tr>
<td>TMT-B time (seconds)†</td>
<td>-0.77</td>
<td>0.44</td>
<td>0.51$^c$</td>
<td>&lt; .001</td>
<td>-0.46$^b$</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Ruff 2 &amp; 7—Speed</td>
<td>1.34</td>
<td>0.18</td>
<td>-0.46$^b$</td>
<td>&lt; .001</td>
<td>0.27$^a$</td>
<td>0.02</td>
</tr>
<tr>
<td>LNS total score</td>
<td>-0.75</td>
<td>0.45</td>
<td>-0.34$^b$</td>
<td>&lt; .01</td>
<td>0.42$^b$</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>COWA total score</td>
<td>0.43</td>
<td>0.67</td>
<td>-0.25$^a$</td>
<td>.03</td>
<td>0.37$^b$</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

*Abbreviations.* TMT-A and TMT-B = Trail Making Test, parts A and B, respectively; LNS = Letter-Number Sequencing; COWA = Controlled Oral Word Association.

*Note.* According to Cohen (1992), Pearson’s $r$ values of 0.10, 0.30, and 0.50 are the minimum thresholds for a small$^a$, medium$^b$, and large effect$^c$ size, respectively.

†Higher values (i.e., longer times) are indicative of poorer cognitive functioning.

**Exercise Stress Test Data.** Exercise stress test descriptive data are presented in Table 9. Males and females did not significantly differ with respect to HR recovery values (males: $M = 37.32$ bpm, $SD = 12.32$, females: $M = 34.61$ bpm, $SD = 11.16$, $t (78)$
Participants who were prescribed β-blocker medication did not have significantly different HR recovery values than those who were not \((M = 36.77, SD = 12.18 \text{ versus } M = 34.67, SD = 10.83, \text{ respectively}, t(78) = -0.49, p = .62)\). Also of note, participants who were prescribed β-blocker medication did not significantly differ from those who were not in terms of resting HR \((M = 69.78, SD = 14.52 \text{ versus } M = 69.68, SD = 10.92, \text{ respectively}, t(78) = 0.03, p = .98)\) or HR at maximum peak of exercise \((M = 125.89, SD = 126.52 \text{ versus } M = 126.52, SD = 22.24, \text{ respectively}, t(78) = -0.08, p = .93)\).

Table 9

<table>
<thead>
<tr>
<th>Exercise Stress Test Data</th>
<th></th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>METs</td>
<td>8.57 (3.54)</td>
<td>3.0-17.50</td>
</tr>
<tr>
<td>Resting HR</td>
<td>69.69 (11.27)</td>
<td>46-101</td>
</tr>
<tr>
<td>HR at maximum peak of exercise</td>
<td>126.45 (21.11)</td>
<td>77-171</td>
</tr>
<tr>
<td>HR at 2 minutes post maximum peak</td>
<td>89.91 (16.90)</td>
<td>53-127</td>
</tr>
<tr>
<td>HR recovery at 2 minutes post maximum HR</td>
<td>36.53 (11.99)</td>
<td>15-72</td>
</tr>
</tbody>
</table>

*Abbreviations.* METs = metabolic equivalent; HR = heart rate.

Participant BMI was not significantly correlated with HR recovery \((r = -.11, p = .34)\). However, participant age was significantly associated with HR recovery \((r = -.32, p = .004, \text{ corresponding to a medium effect size}); increasing age was associated with lower HR recovery values. In addition, METs values were also significantly correlated with HR recovery values \((r = .43, p < .001, \text{ corresponding to a medium effect size}); higher
METs values were associated with higher HR recovery values. Furthermore, HR values at the maximum peak of exercise were also significantly correlated with HR recovery values ($r = .60, p < .001$, corresponding to a large effect size); higher maximum HR values were associated with higher HR recovery values.

Less than half of the current sample (41.3% of the participants) was able to achieve at least 85% of their age-predicted maximum HR [where age maximum predicted HR = 220 - age (years)]. Participants who were able to attain at least 85% of their age-predicted maximum HR had significantly greater HR recovery values than those who were not ($M = 43.70, SD = 11.06$ versus $M = 31.51, SD = 9.96$, respectively, $t (78) = 5.15 p < .05$; Cohen’s $d = 1.2$, corresponding to a large effect size).

**Primary Analyses**

**Covariates.** As noted above, participant age and education level were included as covariates in the series of hierarchical multiple linear regression models examining the partial mediation of inflammation on the relationship between cardiovascular ANS regulation and cognitive functioning. Age and education were intentionally included in the regression analyses in which each the mediator variables (i.e., P-selection and IL-10, separately) are regressed on the independent variable (i.e., HR recovery); refer to Path b of Figure 2B. Although age and education are not theoretically related to the mediator variables, they are retained as covariates because the final set of regressions examining Path c’ include the same covariates.

**Path c: Cardiovascular ANS regulation as a predictor of cognitive functioning.** Five separate hierarchical multiple linear regression analyses were
conducted to examine whether there was a significant relationship between cardiovascular ANS regulation and cognitive functioning. Accordingly, each of the five cognitive test performance variables (i.e., TMT-A time, TMT-B time, Ruff 2 & 7 total speed, LNS total score, and COWA total score) were separately regressed on the independent variable (i.e., HR recovery), after controlling for age and education. The results of these regression analyses are presented in the column entitled “Model 2a” of Tables 10 through 14. As can be seen via inspection of $\Delta R^2$ and F for $\Delta R^2$ values in the column entitled, “Model 2a” of Tables 10, 11, 12, and 14, HR recovery was not significantly associated with performance on TMT-A, TMT-B, Ruff 2 & 7, or COWA, respectively. In contrast, HR recovery was significantly associated with performance on LNS ($\Delta R^2 = .05$, F for $\Delta R^2 = 5.56$, $p < .05$; $f^2 = .05$, corresponding to a small effect size); see Table 13. Of note, the direction of this relationship was in the opposite direction of that which was hypothesized; specifically, higher HR recovery values (i.e., faster HR recovery) were associated with poorer performance on LNS, after controlling for age and education.

**Path a: Cardiovascular ANS regulation as a predictor of inflammatory markers.** Two separate hierarchical multiple linear regression analyses were conducted to examine whether there was a significant relationship between cardiovascular ANS regulation and each of the inflammatory markers. Accordingly, each of the inflammatory markers (i.e., P-selectin and IL-10) was separately regressed on the independent variable (i.e., HR recovery), after controlling for age and education. The results of these regression analyses are presented in Table 15 (re: P-selectin) and Table 16 (re: IL-10),
respectively. As can be seen via inspection of $\Delta R^2$ and $F$ for $\Delta R^2$ values in both Tables 15 and 16, HR recovery values were not significantly associated with either P-selectin or IL-10.

**Paths b and Path c’: Inflammatory markers as predictors of cognitive functioning.** Ten separate hierarchical multiple linear regression analyses were conducted to examine whether there was a significant relationship between inflammation and cognitive functioning. Accordingly, each of the five cognitive test performance variables (i.e., TMT-A time, TMT-B time, Ruff 2 & 7 total speed, LNS total score, and COWA total score) were separately regressed on each of the two mediator variables (i.e., P-selectin and IL-10), after controlling for age, education, and HR recovery. The results of these regression analyses are presented in the columns entitled “Model 2b” (re: P-selectin) and “Model 2c” (re: IL-10) of Tables 10 though 14. As can be seen via inspection of $\Delta R^2$ and $F$ for $\Delta R^2$ values, neither P-selection nor IL-10 were significantly associated with performance on any of the five cognitive test variables.
Table 10

Hierarchical Multiple Linear Regression Analyses Predicting TMT-A from HR Recovery

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2a</th>
<th>Model 2b (P-selectin)</th>
<th>Model 2c (IL-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>11.02 (10.15)</td>
<td>8.02 (11.31)</td>
<td>7.96 (11.50)</td>
<td>7.33 (11.29)</td>
</tr>
<tr>
<td>Age</td>
<td>0.44 (0.11)</td>
<td>0.46 (.11)</td>
<td>0.46 (0.11)</td>
<td>.46*** 0.44 (0.11)</td>
</tr>
<tr>
<td>Education</td>
<td>-0.32 (0.44)</td>
<td>-0.34 (0.44)</td>
<td>-0.34 (0.45)</td>
<td>.08 ** -0.43 (0.44)</td>
</tr>
<tr>
<td>HR Recovery</td>
<td>0.06 (.09)</td>
<td>.06 (0.09)</td>
<td>0.04 (0.09)</td>
<td>.05</td>
</tr>
<tr>
<td>P-selectin†</td>
<td>0.10 (2.57)</td>
<td>.004</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>IL-10††</td>
<td>---</td>
<td>---</td>
<td>0.12 (0.10)</td>
<td>.13</td>
</tr>
<tr>
<td>R²</td>
<td>.216</td>
<td>.220</td>
<td>.220</td>
<td>.236</td>
</tr>
<tr>
<td>F</td>
<td>10.62***</td>
<td>7.15***</td>
<td>5.29**</td>
<td>5.79***</td>
</tr>
<tr>
<td>ΔR²</td>
<td>.004</td>
<td>.004</td>
<td>.020</td>
<td></td>
</tr>
<tr>
<td>F for ΔR²</td>
<td>.374</td>
<td>.185</td>
<td>.97</td>
<td></td>
</tr>
</tbody>
</table>

Notes. Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2a: only HR recovery entered in block 2; Model 2b: HR recovery and P-selectin entered simultaneously in block 2; Model 2c: HR recovery and IL-10 entered simultaneously in block 2; ΔR² refers to change in R² when comparing Model 1 versus either Model 2a, Model 2b, or Model 2c, respectively. Higher values (i.e., longer times) for this dependent variable are indicative of poorer cognitive functioning.

†P-Selectin (log₁₀ transformed)
†† IL-10 (square root transformed)
*p < .05, **p < .01, ***p < .001
Table 11

**Hierarchical Multiple Linear Regression Analyses Predicting TMT-B from HR Recovery**

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2a</th>
<th>Model 2b (P-selectin)</th>
<th>Model 2c (IL-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>67.26 (30.41)</td>
<td>63.54 (33.96)</td>
<td>59.23 (34.23)</td>
<td>63.24 (34.09)</td>
</tr>
<tr>
<td>Age</td>
<td>1.42 (0.31)</td>
<td>.42***</td>
<td>1.44 (0.33)</td>
<td>.43***</td>
</tr>
<tr>
<td>Education</td>
<td>-4.97 (1.31)</td>
<td>-.35***</td>
<td>-4.99 (1.33)</td>
<td>-.35***</td>
</tr>
<tr>
<td>HR Recovery</td>
<td>.05 (0.27)</td>
<td>.02</td>
<td>0.04 (.28)</td>
<td>.01</td>
</tr>
<tr>
<td>P-selectin†</td>
<td>8.52 (7.64)</td>
<td>.10</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>IL-10††</td>
<td>---</td>
<td>---</td>
<td>0.23 (0.29)</td>
<td>.07</td>
</tr>
<tr>
<td>R²</td>
<td>.379</td>
<td>.379</td>
<td>.389</td>
<td>.384</td>
</tr>
<tr>
<td>F</td>
<td>23.49***</td>
<td>15.47***</td>
<td>11.96***</td>
<td>11.70***</td>
</tr>
<tr>
<td>ΔR²</td>
<td>.000</td>
<td>.010</td>
<td>.005</td>
<td>.005</td>
</tr>
<tr>
<td>F for ΔR²</td>
<td>.034</td>
<td>.64</td>
<td>.32</td>
<td></td>
</tr>
</tbody>
</table>

**Notes.** Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2a: only HR recovery entered in block 2; Model 2b: HR recovery and P-selectin entered simultaneously in block 2; Model 2c: HR recovery and IL-10 entered simultaneously in block 2; ΔR² refers to change in R² when comparing Model 1 versus either Model 2a, Model 2b, or Model 2c, respectively. Higher values (i.e., longer times) for this dependent variable are indicative of poorer cognitive functioning.

†P-Selectin (log₁₀ transformed)
†† IL-10 (square root transformed)

*p < .05, **p < .01, ***p < .001
Table 12

Hierarchical Multiple Linear Regression Analyses Predicting 2 & 7 Total Speed

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2a (P-selectin)</th>
<th>Model 2b (P-selectin)</th>
<th>Model 2c (IL-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>314.20 (47.45)</td>
<td>287.31 (52.52)</td>
<td>284.16 (53.32)</td>
<td>290.79 (52.30)</td>
</tr>
<tr>
<td>Age</td>
<td>-1.97 (0.49)</td>
<td>-1.78 (0.51)</td>
<td>-1.77 (0.61)</td>
<td>-1.66 (0.52)</td>
</tr>
<tr>
<td>Education</td>
<td>3.22 (2.05)</td>
<td>3.04 (2.05)</td>
<td>3.03 (2.06)</td>
<td>3.53 (2.07)</td>
</tr>
<tr>
<td>HR</td>
<td>0.51 (0.43)</td>
<td>0.50 (0.43)</td>
<td>0.50 (0.43)</td>
<td>0.60 (0.43)</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-selectin†</td>
<td></td>
<td></td>
<td></td>
<td>5.05 (11.90)</td>
</tr>
<tr>
<td>IL-10††</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| R²        | .232             | .246                  | .248                  | .264             |
| F         | 11.65***         | 8.27***               | 6.18***               | 6.74***          |
| ΔR²       | .014             | .016                  | .032                  |                  |
| F for ΔR² | 1.40             | 0.78                  | 1.63                  |                  |

Notes. Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2a: only HR recovery entered in block 2; Model 2b: HR recovery and P-selectin entered simultaneously in block 2; Model 2c: HR recovery and IL-10 entered simultaneously in block 2; ΔR² refers to change in R² when comparing Model 1 versus either Model 2a, Model 2b, or Model 2c, respectively.

†P-Selectin (log_{10} transformed)
†† IL-10 (square root transformed)
*p < .05, **p < .01, ***p < .001
Table 13

Hierarchical Multiple Linear Regression Analyses Predicting LNS Total Score

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2a</th>
<th>Model 2b</th>
<th>Model 2c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE B)</td>
<td>β</td>
<td>B (SE B)</td>
<td>β</td>
</tr>
<tr>
<td>(Constant)</td>
<td>17.09 (3.37)</td>
<td></td>
<td>20.81 (3.64)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.08 (0.04)</td>
<td>-.24*</td>
<td>-0.11 (0.04)</td>
<td>-.32**</td>
</tr>
<tr>
<td>Education</td>
<td>0.50 (0.15)</td>
<td>.36**</td>
<td>0.53 (0.14)</td>
<td>.375***</td>
</tr>
<tr>
<td>HR Recovery</td>
<td></td>
<td></td>
<td>-0.07 (0.03)</td>
<td>-.24*</td>
</tr>
<tr>
<td>P-selectin†</td>
<td></td>
<td></td>
<td>0.36 (0.82)</td>
<td>.04</td>
</tr>
<tr>
<td>IL-10††</td>
<td></td>
<td></td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>R²</td>
<td>.231</td>
<td>.284</td>
<td>.285</td>
<td>.285</td>
</tr>
<tr>
<td>F</td>
<td>11.58***</td>
<td>10.03***</td>
<td>7.49***</td>
<td>7.48***</td>
</tr>
<tr>
<td>ΔR²</td>
<td>.053</td>
<td>.054</td>
<td>.054</td>
<td></td>
</tr>
<tr>
<td>F for ΔR²</td>
<td>5.56*</td>
<td>2.84</td>
<td>2.83</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2a: only HR recovery entered in block 2; Model 2b: HR recovery and P-selectin entered simultaneously in block 2; Model 2c: HR recovery and IL-10 entered simultaneously in block 2; ΔR² refers to change in R² when comparing Model 1 versus either Model 2a, Model 2b, or Model 2c, respectively.
† P-Selectin (log_{10} transformed)
†† IL-10 (square root transformed)
*p < .05, **p < .01, ***p < .001
### Table 14

**Hierarchical Multiple Linear Regression Analyses Predicting COWA Total Score**

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2a</th>
<th>Model 2b</th>
<th>Model 2c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE B)</td>
<td>B (SE B)</td>
<td>B (SE B)</td>
<td>B (SE B)</td>
</tr>
<tr>
<td>(Constant)</td>
<td>25.41 (10.44)</td>
<td>25.11 (11.66)</td>
<td>24.87 (11.85)</td>
<td>26.18 (11.48)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.17 (0.11)</td>
<td>.17</td>
<td>-0.16 (0.11)</td>
<td>-0.16 (0.11)</td>
</tr>
<tr>
<td>Education</td>
<td>1.35 (0.45)</td>
<td>.32**</td>
<td>1.35 (0.46)</td>
<td>.32**</td>
</tr>
<tr>
<td>HR Recovery</td>
<td>0.01 (0.10)</td>
<td>.01</td>
<td>0.01 (0.10)</td>
<td>.01</td>
</tr>
<tr>
<td>P-selectin†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10‡‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>.160</td>
<td>.160</td>
<td>.161</td>
<td>.199</td>
</tr>
<tr>
<td>F</td>
<td>7.36**</td>
<td>4.84**</td>
<td>3.59*</td>
<td>4.66**</td>
</tr>
<tr>
<td>ΔR²</td>
<td>.000</td>
<td>.001</td>
<td>.039</td>
<td>.003</td>
</tr>
<tr>
<td>F for ΔR²</td>
<td>.003</td>
<td>.01</td>
<td>1.82</td>
<td></td>
</tr>
</tbody>
</table>

**Notes.** Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2a: only HR recovery entered in block 2; Model 2b: HR recovery and P-selectin entered simultaneously in block 2; Model 2c: HR recovery and IL-10 entered simultaneously in block 2; ΔR² refers to change in R² when comparing Model 1 versus either Model 2a, Model 2b, or Model 2c, respectively.

†P-Selectin (log₁₀ transformed)

‡‡ IL-10 (square root transformed)

*p < .05, **p < .01, ***p < .001
Table 15

*Hierarchical Multiple Linear Regression Analyses Predicting P-Selectin*†

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE B)</td>
<td>β</td>
<td>B (SE B)</td>
<td>β</td>
</tr>
<tr>
<td>(Constant)</td>
<td>0.70 (0.46)</td>
<td>-</td>
<td>0.62 (.51)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.002 (0.005)</td>
<td>-0.06</td>
<td>-0.002 (0.005)</td>
<td>-0.06</td>
</tr>
<tr>
<td>Education</td>
<td>0.002 (0.02)</td>
<td>0.01</td>
<td>0.002 (0.02)</td>
<td>0.01</td>
</tr>
<tr>
<td>HR Recovery</td>
<td></td>
<td></td>
<td>0.001 (0.004)</td>
<td>.04</td>
</tr>
</tbody>
</table>

R²                    | .004          |         | .006          |         |
F                      | 0.17          |         | 0.15          |         |
ΔR²                   |               |         | .001          |         |
F for ΔR²             |               |         | 0.11          |         |

†P-Selectin (log₁₀ transformed)

*Notes.* Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2: P-selectin entered in block 2.

Table 16

*Hierarchical Multiple Linear Regression Analyses Predicting IL-10*

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE B)</td>
<td>β</td>
<td>B (SE B)</td>
<td>β</td>
</tr>
<tr>
<td>(Constant)</td>
<td>13.70 (12.23)</td>
<td>.042</td>
<td>5.75 (13.49)</td>
<td>.065</td>
</tr>
<tr>
<td>Age</td>
<td>0.15 (0.13)</td>
<td>.14</td>
<td>0.20 (0.13)</td>
<td>.19</td>
</tr>
<tr>
<td>Education</td>
<td>0.87 (0.53)</td>
<td>.19</td>
<td>0.82 (0.53)</td>
<td>.18</td>
</tr>
<tr>
<td>HR Recovery</td>
<td></td>
<td></td>
<td>0.15 (0.11)</td>
<td>.16</td>
</tr>
</tbody>
</table>

R²                    | .042          |         | .065          |         |
F                      | 1.67          |         | 1.76          |         |
ΔR²                   |               |         | .023          |         |
F for ΔR²             |               |         | 1.89          |         |

†IL-10 (square root transformed)

*Notes.* Model 1: Only control variables (i.e., age and education) entered in block 1 as predictors; Model 2: IL-10 entered in block 2.
**Evaluation of mediation.** Evaluation of the significance of the magnitude of a mediating effect is typically pursued only if the four conditions outlined by Baron and Kenny (Baron & Kenny, 1986) are met. In the current study, the four conditions are not satisfied for any of the five cognitive test variables. Thus, it was not appropriate to conduct Sobel test to determine if the magnitude of relationship between HR recovery and cognitive test performance was significantly different (i.e., lower) when the effect of a mediator variable was included in the model (MacKinnon, et al., 1995).
DISCUSSION

Summary of Major Findings

The present study was designed with the following two aims: 1) examine the associations among cardiovascular ANS regulation, inflammation, and cognitive functioning in patients with CVD, and 2) examine the hypothesis that inflammation was a partial mediator of the relationship between cardiovascular ANS dysregulation and cognitive dysfunction in patients with CVD.

**Aim 1.** HR recovery was significantly associated with performance on only one of the five measures of cognitive functioning considered in the current study, namely, a measure of complex auditory attention and working memory. Of note, however, the direction of this relationship was in the opposite direction of that which was hypothesized, as HR recovery was inversely related to performance on that measure. Thus, the current investigation was unable to replicate previous findings of a positive relationship between HR recovery and cognitive functioning in separate sample of phase II cardiac rehabilitation patients (Keary, et al., in press). Contrary to expectations, HR recovery values were not significantly related to either of the inflammatory makers assessed. Also contrary to expectations, neither inflammatory marker was significantly associated with any of the five measures of cognitive function.

**Aim 2.** There was no support for the hypothesis that inflammation partially mediates the relationship between cardiovascular ANS dysregulation and cognitive impairment in the current study.
Potential Explanations

Some features of the study sample and other design-related issues might have influenced the current findings and may help to partially account for the unexpected results.

Possibility of non-response bias. One methodological consideration pertains to participant recruitment. Although the majority of the cardiac rehabilitation patients who were recruited for the current study agreed to participate (i.e., 99/145 or 68.3%), the possibility of non-response bias cannot be entirely ruled out. Non-response bias occurs when the data of respondents (i.e., those who agree to participate in a study) systematically differ from that data of non-respondents (i.e., those who decline participation). Rosenthal and Rosnow (2008) refer to this issue as the volunteer subject problem. It is possible that the cardiac rehabilitation patients who were cognitively impaired—and perhaps even those who are fearful of being cognitively impaired—may have been more likely to decline participation. Previous work has shown that lower cognitive functioning is a negative factor related to elderly people’s willingness to participate in research (Bhamra, Tinker, Mein, Ashcroft, & Askham, 2008; Launer, Wind, & Deeg, 1994; Norton, Breitner, Welsh, & Wyse, 1994; von Strauss, Fratiglioni, Jorm, Viitanen, & Winblad, 1998).

Theoretically, one way to test the hypothesis that those who were more cognitively impaired were less likely to participate would have been to compare the MMSE scores of responders versus non-responders who met the eligibility criteria. Practically speaking, however, such a comparison could not be conducted because there
was no IRB permission to retain and analyze data used to screen the cardiac rehabilitation patients for eligibility. Regardless, a global cognitive screening measure such as the MMSE may not be sensitive enough to detect incipient decline in specific domains of cognitive function, including attention, executive function, and psychomotor speed (Insel, Morrow, Brewer, & Figueredo, 2006; Meyers & Wefel, 2003). Recent work suggests that the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005), is a brief screening measure that is more sensitive to deficits in such domains (Dong et al., 2010; Pendlebury, Cuthbertson, Welch, Mehta, & Rothwell, 2010).

Previous research has also shown that, compared with responders, non-responders tend to have less education (Bhamra, et al., 2008; Launer, et al., 1994; Miyamoto et al., 2009; Norton, et al., 1994; von Strauss, et al., 1998). Theoretically, one way to test the hypothesis that those who were less educated were less likely to participate would have been to compare the number of years of education of responders versus non-responders. Practically speaking again, however, such a comparison could not be conducted for the same reason identified in the preceding paragraph.

**Potential impact of high cognitive reserve.** Participants in the current study reported that they had received an average of 14.2 years of formal education. It is possible that the relatively high degree of educational attainment characteristic of the current sample may have served to help guard against the cognitive impairment associated with CVD-associated neuropathology (e.g., white matter hyperintensities). This speculative assertion is based upon cognitive reserve theory.

According to cognitive reserve theory (Stern, 2002, 2009), premorbid factors such
as level of education, occupational attainment, or intelligence serve as buffers against cognitive impairment, thus accounting for instances in which individuals with similar neuropathology manifest vastly different cognitive and functional capacities. Cognitive reserve does not protect the brain against the development of neuropathology, but rather moderates the association between neuropathology and the expression of that neuropathology—i.e., cognitive dysfunction (Brickman et al., 2009 in press). Several research studies of normal aging have observed slower cognitive and functional decline in individuals with higher educational attainment (for reviews, see Stern, 2002, 2009). Recent work suggests that cognitive reserve mitigates the impact of white matter hyperintensities on cognitive function, particularly on executive function/speed tasks (Brickman, et al., 2009 in press; Siedlecki et al., 2009).

**Impact of exclusion criteria on the level of cognitive functioning of the sample.** The current study employed a rather rigorous set of exclusion criteria that resulted in more than two-thirds (379 out of 553; refer to Figure 3) of the cardiac rehabilitation patients screened being deemed ineligible to participate. As noted above, history of congestive heart failure (i.e., those with an EF ≤40%) and recent history of CABG or other cardiac procedure that required sternotomy (e.g., MVR, AVR) were the two most common reasons for ineligibility. Only two participants in the sample were referred to cardiac rehabilitation because of history of CABG and/or AVR. Those two participants were deemed eligible to participant because they underwent their surgical procedure at least three months prior to initiation of cardiac rehabilitation.
Previous research has shown that cardiac rehabilitation patients with a low EF (i.e., values < 45%) and those who have undergone CABG surgery evidence significantly greater neuropsychological impairment than those without reduced EF and those who have not undergone CABG surgery, respectively (Moser, et al., 1999). Thus, excluding nearly all such patients (i.e., all CHF and CABG patients except for the two noted in the preceding paragraph) from participation in the present study may have resulted in a substantial decrease in the amount of variability in cognitive test performance. Decreased variability (i.e., range restriction) can, in turn, reduce statistical power (Aguinis & Stone-Romero, 1997). Consideration of the cognitive test performance of the overall sample provides some support for the theory that participants in the current sample were generally intact. For example, refer to Table 17, which presents the qualitative descriptions of the cognitive test performances for a mock participant with the average age (62.16 years) and education level (14.16 years) of the study sample when considered in comparison to normative data from healthy (i.e., non-CVD) samples.
Table 17

Qualitative Interpretation of Cognitive Test Performance for the Average Participant

<table>
<thead>
<tr>
<th>Test</th>
<th>Raw values</th>
<th>Qualitative Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-A time (seconds)†</td>
<td>33.94</td>
<td>normal</td>
</tr>
<tr>
<td>TMT-B time (seconds)†</td>
<td>85.39</td>
<td>low normal</td>
</tr>
<tr>
<td>Ruff 2 &amp; 7 total speed score</td>
<td>238.28</td>
<td>normal</td>
</tr>
<tr>
<td>LNS total score</td>
<td>19.15</td>
<td>normal</td>
</tr>
<tr>
<td>COWA total score</td>
<td>34.16</td>
<td>normal</td>
</tr>
</tbody>
</table>

Notes. “Average Patient” refers to a mock patient with the average age (62.16 years) and education level (14.16 years) of the study sample. “Raw values” refer to the mean values of the entire sample—refer to Table 7. Normative data references (A. L. Benton, Hamsher, Rey, & Sivan, 1994; Ruff & Allen, 1996; Tombaugh, 2004; Wechsler, 2008).

Abbreviations. TMT-A and TMT-B = Trail Making Test, parts A and B, respectively; LNS = Letter-Number Sequencing; COWA = Controlled Oral Word Association.

†Higher values (i.e., longer times) are indicative of poorer cognitive functioning.
**Circadian variation in inflammatory marker production.** Circadian variations can often impact the analysis of blood-derived samples. Abundant research has shown circadian variability in many systemic markers of inflammation in patients with cardiovascular disease (for a review, see Dominguez-Rodriguez, Abreu-Gonzalez, & Kaski, 2009). A small body of literature suggests that there are circadian variations for P-selectin in those who are healthy and those with coronary artery disease, with significantly higher P-selectin levels observed in the evening as compared the morning (Jilma et al., 1997; Kirk, Maple, McLaren, & Belch, 1995; Osmancik, Kvasnicka, Widimsky, & Tarnok, 2004). However, no known studies have identified the specific times of day associated with peak and nadir P-selectin values.

The extant research regarding circadian variation in IL-10 has been notably inconsistent. For example, one study of healthy participants reported that IL-10 levels exhibited a biphasic pattern, with one peak at approximately 7:30AM and a second peak at approximately 7:30PM (Young et al., 1995). In contrast, a second study of healthy participants found no significant circadian variation in IL-10 (Lissoni, Rovelli, Brivio, Brivio, & Fumagalli, 1998). Moreover, another study reported that IL-10 levels peaked at 9PM (Petrovsky, McNair, & Harrison, 1998), while still another indicated that peak IL-10 values were observed during the daytime (Lange, Dimitrov, Fehm, & Born, 2006).

In the current study, blood samples were not drawn at a fixed time of the day. As noted above in the Method section, blood samples were always drawn prior to the cardiac rehabilitation exercise sessions in an effort to minimize the acute effect of exercise on the inflammatory marker levels (Pedersen & Saltin, 2006; Ploeger, et al., 2009).
Consequently, blood samples were drawn as early as 6:30AM and as late as 5:00PM. It is acknowledged that differences in the time of day blood samples were drawn may represent a major confounding factor in the current study.

Ideally, in order to control for any possible circadian rhythm effects, participants’ blood should have been drawn at a fixed time of the day (Domínguez-Rodríguez, et al., 2009; Petrovsky & Harrison, 1998), prior to exercise or on a day that participants are not planning to exercise. The specific time of day selected for blood draws should be contingent upon the particular inflammatory marker of interest. In addition to consideration of the circadian rhythm effects of cytokine production, it may also be wise to consider the effects of plasma cortisol on cytokine production since cortisol serves as an immune-suppressant (Petrovsky & Harrison, 1998; Petrovsky, et al., 1998).

**HR recovery as a cardiovascular ANS index**

A consideration of the use of HR recovery as an index of cardiovascular autonomic functioning is that HR recovery cannot be easily separated from other HR variables. For example, HR recovery is computationally dependent on maximum HR. Consistent with previous research (e.g., Desai, De La Pena-Almaguer, & Mannting, 2001; Hughes, et al., 2006), HR recovery values were significantly correlated with HR values at the maximum peak of exercise in the current study. In addition, HR recovery is positively correlated with exercise capacity (Cole, Blackstone, Pashkow, Snader, & Lauer, 1999; Hughes, et al., 2006) and is influenced by the participants’ ability to increase HR during exercise (Hughes, et al., 2006; Lauer, 2001). As noted above, HR recovery values in the present study were significantly positively correlated with both HR
values at the maximum peak of exercise and maximum exercise capacity (as measured in METs) achieved. Even with these considerations, it is important to emphasize that HR recovery independently predicts mortality and unique information regarding cardiovascular autonomic functioning as compared to other HR-related indices (Camerini, 2001; Cheng et al., 2003; Cole, et al., 1999; Cole, et al., 2000; Desai, De La Peña-Almaguer, & Mannting, 2001; Diaz, Brunken, Blackstone, Snader, & Lauer, 2001; Lipinski, et al., 2004; Nishime, Cole, Blackstone, Pashkow, & Lauer, 2000; Nissinen et al., 2003; Vivekananthan, Blackstone, Pothier, & Lauer, 2003; Watanabe, Thamilarasan, Blackstone, Thomas, & Lauer, 2001).

**Beta-Blocker Usage**

Beta-blockers (β-blockers; also known as beta-adrenergic blocking agents or beta-adrenergic antagonists) represent a class of drugs that are frequently prescribed to individuals with cardiac arrhythmias, hypertension, and history of myocardial infarction. Beta-blockers increase cardiac vagal efferent activity and reduce sympathetic efferent activity, resulting in reductions in heart rate and blood pressure (Frenneaux, 2004; Frishman, 2003; Goldsmith et al., 1997; Lin et al., 1999; Mortara et al., 2000; Niemela, Airaksinen, & Huikuri, 1994; Pousset et al., 1996; Sanderson et al., 1999; Sandrone et al., 1994).

**Beta-blocker usage and HR recovery.** The majority of the participants in the present study (88.8%) were prescribed beta-blocker medication as part of their routine medical management. Participants in the current study who were prescribed β-blocker medication did not have significantly different HR recovery values than those who were
In addition, participants who were prescribed β-blocker medication did not significantly differ from those who were not in terms of resting HR or maximum HR (i.e., HR at maximum peak of exercise).

Research regarding the relationship between beta-blocker usage and HR recovery following exercise stress testing has been mixed, leading some to conclude that there is no clear association between beta-blocker usage and abnormal (i.e., slower) HR recovery (e.g., Lauer, 2001). Some research suggests that HR recovery is indirectly affected by the use of beta–blockers by blunting the chronotropic response, the change in HR in response to exercise (Desai, De La Peña-Almaguer, et al., 2001; Desai, De La Peña-Almaguer, et al., 2001; Pavia, Meyers, & Rusconi, 2002). However, other research indicates that exercise stress testing in the presence of beta-blockers has no significant effect on the reinstitution of vagal tone, and therefore, no effect on HR recovery (Arena et al., 2010; Karnik, Lewis, Miles, & Baker, 2008).

**Beta-blocker usage and cognitive function.** Research regarding the potential effect of beta-blocker usage on cognitive functioning has also been mixed. Although some studies have reported beneficial effects of beta-blocker therapy and others have reported detrimental effects, the majority of studies have found no significant effect (for a review, see Dimsdale, Newton, & Joist, 1989). Recent work suggests that the duration of beta-blocker therapy is an important moderating factor, with chronic beta-blocker use associated with significantly lower risk of reduced cognitive function (Torres et al., 2008). In the current study, participants who were prescribed beta-blocker medication did not have significantly different cognitive test performance scores as compared to
those who were not. Of note, however, the present study did not examine duration of medication therapy or medication adherence.

**Limitations and Future Directions**

A few limitations of the current study warrant brief mention and suggestions for future research are offered. First, there are several factors which limit the generalizability of the current findings to all patients with CVD, including the study’s eligibility criteria (see above), physician cardiac rehabilitation referral practices, and incomplete adherence to cardiac rehabilitation referral recommendations (Brown et al., 2009; Cortes & Arthur, 2006; Daly et al., 2002; Hagan, Botti, & Watts, 2007; Johnson, Fisher, Nagel, Inder, & Wiggers, 2004). Second, as noted above, blood samples were drawn throughout the day, and thus, circadian variations of the inflammatory may have influenced the results. In order to address this potential confound, future studies should draw blood samples at a fixed time of day. Finally, the present study investigated inflammation as assessed by two inflammatory markers, P-selectin and IL-10. These two markers were selected on the basis of their potential contribution to the literature—that is, their relationships with cognitive functioning and cardiovascular ANS functioning had not yet been described in the literature. Future research may wish to consider other inflammatory markers within the context of the mediation model proposed in the current study. CRP may be a particularly good candidate pro-inflammatory marker to consider, as its relationship to cardiovascular morbidity and mortality has been extensively studied (Blake & Ridker, 2001) and previous work has established an association between high CRP and declines
in attention-executive-psychomotor performance in older adults with CVD (Hoth, et al., 2008).
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APPENDIX A

IRB CONSENT FORM
INFORMED CONSENT

Project Title: IGNITE Rehab (Integrating Growth, Neurocognitive, and Inflammation research To Enhance Rehabilitation)

Investigators: Joel Hughes, Ph. D, Thomas Alexander, Ph.D., Elizabeth Casey, M.A., John Gunstad, Ph.D., Tracy Hammonds, B.A., Therese Anne Keary, M.A., James Rosneck BSN, MS, Donna Waechter, Ph.D.; Angela Roberts Miller, MPH, Colleen Cole Mattson, BS.

We are asking you to be in a research study. The following information is provided to inform you about the study and your participation in it. Please read it carefully and feel free to ask any questions.

PURPOSE OF STUDY:
You are being asked to participate in a research study. We want to understand mental and physical factors that are related to inflammation in people with heart problems. Inflammation means that there are high levels of certain blood markers throughout the body that could increase a person’s risk of medical complications and death. Mental factors (such as mood, stress, sleep difficulties, memory or thinking problems) and physical factors (such as fitness level) may be related to higher levels of inflammation. We would like to do this study to find out what specific mental and physical factors are related to inflammation in people with heart problems. We plan to enroll approximately 120 participants from cardiac rehabilitation in this study.

PROCEDURES:
Your total time commitment to this study is about 1 hour, including an interview, cognitive functioning tests, a blood draw, and completing questionnaire packets.

If you decide to take part in this project, you will be asked to arrive 1 hour early to one of your regularly scheduled sessions during your first two weeks of cardiac rehabilitation. You will participate in (1) a blood draw, (2) an interview, (3) cognitive tests, and (4) a questionnaire packet. There will also be a chart review to obtain certain information from your medical chart.

Blood draw: The blood draw will follow standard procedures and be administered in the outpatient lab next to cardiac rehabilitation. A small amount of
blood (approximately ½ tablespoon) is needed for this study and will be drawn in three tubes. The blood draw will take place before you participate in your cardiac rehabilitation session. A study investigator will accompany you to the outpatient lab prior to completing the interview and cognitive test.

Interview and cognitive test: You will also complete an interview with a study investigator who will ask you questions about your mood and other aspects of your life. In addition, you will complete a series of tasks, such as memory, word, and problem solving tests, assessing your cognitive functioning. The interview and cognitive testing will take approximately 35 minutes.

Questionnaire packet: You will be asked to complete paper-and-pencil questionnaires asking you about your health history, mood, anxiety, sleep habits, alcohol use, and smoking status. You can take the questionnaire packet home and return it during your next session in cardiac rehabilitation. The questionnaire will likely take you approximately 30 minutes to complete.

Medical chart review: The chart review will involve looking in your medical chart to record information including name, date of birth, your medical conditions, medications you are taking, medical problem or procedure that prompted your participation in rehabilitation (e.g., heart attack, surgical heart procedure), and results from your treadmill exercise stress test.

VOLUNTARY PARTICIPATION:
Your participation in this study is strictly voluntary and confidential. If you wish to drop out of the study at any time, you may do so without any penalty by simply telling us that you do not wish to continue participating in this study. Refusal to participate in this study will not effect your treatment at Summa Health System. If you have any questions or concerns during the study, you are encouraged to call Joel Hughes (330-672-7721), Elizabeth Casey (330-221-8491), or Therese Anne Keary (330-378-8199).

If findings emerge during this research that might affect your willingness to continue to participate, this information will be provided to you so that you can make an informed decision.

If it becomes apparent during the course of the study that you are severely depressed or pose a risk to yourself, your physician will be notified.

CONFIDENTIALITY:
As soon as possible, your personal identifying information (e.g., your name and date of birth) will be removed from your file. The information you provide us with will then be identified only by a participant number, and will be examined only by the primary investigators and qualified members of our research team. The only copies of the data will remain inside a locked file cabinet in a locked office, and confidentiality of your records will be maintained within the limits of the law. However, Federal law
authorizes representatives from the Office of Human Research Protection and the Summa Health Systems Institutional Review Board to inspect the research records. The results of the study may be published in scientific journals, but data will not be published in any manner that can identify you.

**COMPENSATION:**
You can receive $40 (check) or a $40 gas card for participating in this study. We will give you your check or gas card at cardiac rehabilitation within two weeks of your completion of participation. If you choose to discontinue your participation in this study, you will be compensated for the portions of the study that you complete. The table below explains the payments.

<table>
<thead>
<tr>
<th>Study Participation</th>
<th>Payment Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood draw</td>
<td>$10</td>
</tr>
<tr>
<td>Interview</td>
<td>$10</td>
</tr>
<tr>
<td>Cognitive test</td>
<td>$10</td>
</tr>
<tr>
<td>Questionnaire Packet</td>
<td>$10</td>
</tr>
<tr>
<td><strong>Total Monetary Compensation:</strong></td>
<td><strong>$40</strong></td>
</tr>
</tbody>
</table>

**POTENTIAL BENEFITS:**
You will not receive any direct medical benefit from being in this study. You should be aware that this is a research study and is not a medical assessment; therefore, you will not receive any feedback regarding your personal health condition. If you have any concern about your health, please contact your physician or the Cardiac Rehabilitation staff.

**CRITERIA THAT WILL EXCLUDE YOU FROM PARTICIPATION:**
You must be between 40 and 85 years of age and be able to communicate in English in order to participate in this study.

You will **not** be able to participate in this study if we find in the initial screening that you are unable to do the procedures of the study. For example, you would **not** be eligible if you have a medical condition that renders you unable to complete the blood draw, questionnaires, or participate in an interview. You will **not** be eligible if you were unable to participate in a treadmill exercise stress test as part of your intake procedures in cardiac rehabilitation.

**POTENTIAL RISKS:**
We are not aware of any risks in this study beyond those risks normally encountered in participating in a blood draw and cardiac rehabilitation. For example, there is a chance
that participants may have an adverse reaction to the blood draw (possible swelling, pain, bruising, or infection). Immediate necessary care is available if an individual is injured by participation in the research project. However, there is no provision for free medical care or for monetary compensation for such injury. There may also be some discomforts that you may experience in this study. For example, some people may find that questions about depression are upsetting or that thinking and memory tasks are stressful. We are unaware of any health risks that might be caused by your participation, although it may be inconvenient and it does take some of your time.

If you experience a medical problem or any feelings of discomfort, medical care and mental health services are available at Summa Health System. Medical care and mental health services are also available outside the Summa Health System. However, you would have to make arrangements to pay for any services, and medical or mental health care would not be provided for free because you were in this research project.

ALTERNATIVES TO PARTICIPATION:
If you do not want to participate in this study, the alternative is to not participate.

FUTURE STUDIES:
Your consent participation in the present study gives us permission to contact you in the future regarding additional studies. For example, near completion of cardiac rehabilitation, you may be invited to participate in a separate follow-up study. You have the right to accept or decline the invitation to participate, and your decision will not affect your participation in the present study. Check “yes” below if you give us permission to contact you about participating in future studies and check “no” below if you do not want to be contacted about participating in future studies. Please also write your initials next to the box that you checked.

Do you give us permission to contact you about participating in future studies?

_____ □Yes  _____ □No

SPONSORS:
This research involves collaboration between researchers at Summa Health System and researchers at Kent State University.

QUESTIONS OR PROBLEMS:
If you want to know more about this research project, please call Joel Hughes (330-672-7721), Elizabeth Casey (330-221-8491), or Therese Anne Keary (330-806-7340). The procedures for this project have been reviewed by the Human Subject’s Committee.
You acknowledge that you have read the above information and have had any questions regarding the risks and benefits of this study satisfactorily answered, and you are voluntarily consenting to participate in this study. You have been told that you will receive a copy of this consent form. Further, you realize that by signing this form you do not waive any of your legal rights, and that you can choose to stop your participation at any time.

You will get a signed copy of this consent form for your records.

________________________________________  Date: _____________
Participant printed name

________________________________________  Date: _____________
Participant signature

________________________________________  Date: _____________
Signature of person obtaining consent
APPENDIX B

HIPAA CONSENT FORM
HIPAA Authorization Agreement – Authorization to Use and Disclose (Release) Health Information for a Research Study

IGNITE Rehab
(Integrating Growth, Neurocognitive, and Inflammation To Enhance Rehabilitation)

State and Federal laws, including the Health Insurance Portability and Accountability Act (HIPAA), require researchers to protect your health information. This form describes how researchers, with your authorization (permission), may use and release (disclose or share) your protected health information for this research study. Please read this form carefully.

You have been asked to take part in a research study. The study is described to you in a separate consent form. By signing this form you are permitting Joel Hughes, PhD, and his/her research team to create, get, use, store, and share protected health information that identifies you for the purposes of this research. Protected health information may include results of tests, procedures or surveys that are part of the research, as well as information that can identify you, such as your name, date of birth and medical record number.

You have the right to see and copy your protected health information related to the study for as long as the study doctor holds this information. However, to make sure the scientific findings of this study are accurate, you may not be able to review some of your records related to this study until after the study is completed.

Research use of your protected health information

If you sign this form, the researchers may use or share your health information during the conduct of the research with:
• Each other and with other researchers involved in the study
• Law enforcement or other agencies, when required by law
• Summa Health System’s Institutional Review Board (a group of people who protect the rights of research subjects)
• Representatives of government agencies (i.e. Food and Drug Administration and the Office of Human Research Protection)

Description of protected health information that may be used and released (disclosed or shared)

Health information includes all information created and/or collected during the research as described in the research study consent form entitled IGNITE Rehab (Integrating Growth, Neurocognitive, and Inflammation research To Enhance Rehabilitation). Health information in your medical record may be used and released if it is needed for the research; for example, past medical

Version Number: __________
Date: ______________
Health information includes: names, initials, address, telephone number, date of birth, age, gender, race, height, weight, body mass index, education, marital status, employment status, smoking status, medical diagnoses and procedures, medications, treadmill exercise results, and entry questionnaires to cardiac rehabilitation (Beck Depression Inventory, SF-36, and Mini-Mental Status Exam). A subject number (code) will be linked to your name. Only the Principal Investigator and authorized research personnel will have access to the code.

Protection of your health information

The researchers agree to protect your health information and will only share this information as described in this Authorization and the research consent form. Once you give your permission to Summa Health System (SHS) to release the information needed for this study, SHS can no longer guarantee your privacy.

Expiration of Authorization

This Authorization does not have an expiration date but can be canceled sooner if you decide to withdraw your permission.

Withdrawal or removal from the study

You may change your mind and cancel this Authorization at any time. To cancel this Authorization, you must write to:

Joel Hughes, Ph.D.
Dept. of Psychology
Kent State University
P.O. Box 5190
Kent, OH 44242
jhughes1@kent.edu

If you cancel this Authorization, you may no longer be allowed to take part in the research study. Even if you cancel this Authorization, the researchers may still use and disclose health information they have already obtained to maintain the integrity and reliability of the research, and to report any adverse (bad) effects that may have happened to you.

Contact information for questions about my rights under HIPAA

If you have questions or concerns regarding your privacy rights under HIPAA, you should contact the Privacy Officer at 330-375-6665.

Version Number: __________
Date: ____________
Right to refuse to sign this Authorization

You do not have to sign this Authorization. However, because your health information is required for research participation, if you decide not to sign this Authorization form, it will only mean you cannot take part in this research. Not signing this form will not affect your non-research related treatment, payment or enrollment in any health plans, or your eligibility for other medical benefits.

Signature of subject

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions, and my questions have been answered to my satisfaction. I authorize the use and disclosure of my protected health information for this research. I will be given a signed copy of this Authorization form.

______________________________
Printed Name of Subject

______________________________    _________________
Signature of Subject                Date

______________________________    _________________
Signature of Person Obtaining Authorization Agreement    Date

Version Number: __________
Date: __________