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Pathways between Sleep Disordered Breathing and Adverse Clinical Outcomes in Heart Failure Patients: An Exploration of Proinflammatory Effects and Depressive Symptomatology

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Public Health (Health Behavior)

by

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The Dissertation of Jessica Ann Jiménez is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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San Diego State University

2012

EPIGRAPH

If we follow our heart

We are bound

To Succeed.

--Sri Chinmoy, Service Trees

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LIST OF ABBREVIATIONS

Sleep Disorder Breathing (SDB)
Obstructive Sleep Apnea (OSA)
Heart Failure (HF)
Cardiovascular Disease (CVD)
B-type Natriuretic Peptide (BNP)
Central Sleep Apnea (CSA)
Intercellular Adhesion Molecules (ICAM-1)
Interluekin-6 (IL-6)
Tumor Necrosis Factor-alpha (TNF-α)
C-reactive Protein (CRP)
Continuous Positive Air Pressure (CPAP)
Apnea- Hypopnea Index (AHI)
Obstructive Apnea- Hypopnea Index (OAHI)
Central Apnea- Hypopnea Index (CAHI)
Mixed Apnea- Hypopnea Index (MAHI)
Beck Depression Inventory (BDI)
Social Security Death Index (SSDI)
Electronic Medical Record (EMR)
Left Ventricular Ejection Fraction (LVEF)

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VITA

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ABSTRACT OF THE DISSERTATION

Pathways between Sleep Disordered Breathing and Adverse Clinical Outcomes in Heart Failure Patients: An Exploration of Proinflammatory Effects and Depressive Symptomatology

by

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The study was to explored potential mechanisms underlying the association between sleep disordered breathing (SDB) and clinical outcomes in a convenience sample heart failure (HF) patients (n=66) and controls (n=53).

Subjects participated in a sleep study, and physical assessments and blood draws were performed. Longitudinal outcomes were extracted from the HF patient medical records, and deaths were confirmed using the Social Security Death Index. Depressive symptoms were assessed using the Beck Depression Inventory (BDI). Mediation models

were performed, and a test of product coefficients (PRODCLIN) was used to assess statistical significance of compound paths.

Regression analyses showed disease status, but not SDB severity, predicted BDI scores and inflammation levels ($p<.01$). There was a significant second-order effect for two measures of hypoxia (average % oxygen saturation and lowest % oxygen saturation) by disease status for somatic BDI scores ($p<.05$), and a significant second-order effect for indices of obstructive and mixed apnea-hypopnea by disease status for C-Reactive Protein (CRP; $p<.05$). Simple slopes analyses revealed that these interaction effects were only significant for controls. Controls evidenced an expected association where worse SDB related to higher somatic scores and higher resting mean levels of CRP, respectively. In HF patients somatic symptom scores remained relatively constant, and CRP levels decreased in the presence of higher SDB severity.

During the 3 year follow-up, HF patients experienced an average of 3 (SD=3.9) hospital admissions and 18% (n=12) died. There was a moderated-mediated effect of cognitive-affective depressive symptomatology and resting levels of circulating CRP on the association between total minutes oxygen saturation fell below 90% and mortality ($p<.05$). Among patients with lower cognitive-affective BDI scores, higher resting levels of CRP corresponded with more minutes that oxygen saturation was below 90%. Among those with higher cognitive-affective scores, lower resting levels of CRP corresponded with more minutes oxygen saturation was lower than 90% ($p<.05$). In those with lower cognitive-affective scores, higher resting CRP levels corresponded with greater percentage of mortality. Among those with higher cognitive-affective scores, higher resting mean levels of CRP corresponded to fewer deaths. Simple slopes analyses were not significant.

The patterns seen in HF patients may be evidence of dysregulation of inflammatory responses resulting from HF syndrome and/or cognitive-affective aspects of depression. Inflammatory mechanisms may mediate associations between SDB and morbidity and mortality, although this association appears to vary by levels of cognitive-affective depressive symptoms.

Chapter 1: Introduction and Purpose

Sleep disorder breathing (SDB) is a general term referring to a group of disorders characterized by abnormalities in respiratory patterns or the quantity of ventilation during sleep. Sleep apnea is a common, yet under-diagnosed, problem that is characterized by chronic intermittent hypoxia, oxygen desaturation and sleep fragmentation (1). There is compelling epidemiological evidence to support that obstructive sleep apnea (OSA) is a significant risk factor for the initiation and progression of cardiovascular disease (CVD) (2-8), particularly heart failure (HF) (1). HF patients have a disproportionately high prevalence of sleep apnea compared to the general population (9-12). Untreated sleep apnea in HF patients has been associated with increased mortality (13-14). Emerging data from clinical trials suggests that treating OSA with continuous positive airway pressure (CPAP) may improve cardiac prognosis in HF patients (13, 15-18).

Although the deleterious effects of SDB conditions on HF have been well established, studies testing the underlying pathophysiological and psychological mechanisms accounting for this association have been slow to accumulate (1). SDB has been associated with increased circulating inflammatory products (1, 19), which is one of the proposed mechanisms explaining the complex link between OSA and cardiac prognosis, and when OSA is treated with CPAP, inflammation profiles are lowered (20).

Studies have shown that increased severity of sleep apnea, related hypoxia and sleep deprivation have been closely linked to declines in psychological outcomes, including increases in depressive symptomatology (21). Depression may exert its influence on HF outcomes via behavioral mechanisms. For example, poor sleep may be related to lack of medication adherence in HF patients (22). Approximately 20% of HF

patients have co-morbid depression (23), regardless of sleep apnea. The study of depression in HF has garnered scientific interest, in part, due its tendency to worsen cardiac prognosis. Inflammation has also been implicated as one of the underlying mechanisms linking co-morbid depression to the pathogenesis of HF (24). The current study seeks to empirically test the proposed mediated effects of inflammation and depression on the association between SDB and adverse clinical outcomes in HF patients.

Heart Failure

Epidemiology of heart failure

In the United States, the prevalence of HF is 2.42%, with higher rates found in older, male adults (25-26). Despite significant advances in treatment, the prognosis for patients remains grim: 20% to 30% of HF patients die within a year of diagnosis, and 45%-60% die within five years (27). Among older adults, HF is the most common condition for hospitalization (28), with 990,000 hospitalizations per year in the US at an approximate cost of $39.2 billion (26).

Definition and Classification of Heart Failure

The American College of Cardiology (ACC) and American Heart Association (AHA) define HF as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricles to fill with or eject blood (29). The upper chambers of the heart are composed of the right and left atria, and the lower chambers include the right and left ventricles. The ventricles are muscular chambers that contract to pump blood (systole). After systole, the ventricle muscles normally relax during diastole, allowing blood from the atria to fill the ventricles. The heart's ability to pump can be compromised via two mechanisms: 1) reduction in the

volume of oxygenated blood ejected from the left ventricle as a result of diminished myocardial contractility; and 2) inadequate venous return to heart, resulting from impaired ventricle filling and relaxation.

Although HF varies in its etiologies and clinical features, it can be broadly classified into two categories: HF with systolic dysfunction [also known as 'HF with reduced ejection fraction' (HFrEF)], characterized by a reduced left ventricle ejection fraction (LVEF), which is a measure of the percentage of blood that is ejected from the heart into the aorta with each systole; HF with preserved ejection fraction [HFpEF] is a complex disorder, where LVEF is normal or mildly abnormal. Other left ventricle (LV) abnormalities include abnormal relaxation and filling, concentric remodeling, hypertrophy, increased extracellular matrix, abnormal relaxation and filling, decreased diastolic distensibility ,and abnormal calcium handling.

There are two primary scales that are used to classify HF. The New York Heart Association (NYHA) functional scale, which is based on symptoms, classifies HF in categories from I to IV (see Table 1.0). The other scale is the American College of Cardiology/American Heart Association (ACC/AHA) scale, a newer classification that stages patients as either A, B, C, or D (29). The ACC/AHA staging system classifies HF as a progressive disease, and once a particular stage is reached there is no opportunity to transition to a lower stage (e.g., a stage C HF patient cannot return to stage B). This system is often complemented by the NYHA functional classification system. In contrast, ACC/AHA Stage C patients can shift between Functional Classes I - IV at any given time. Movement up and down NYHA classes is common, depending on the clinical status of the patient during the time of assessment.

Table 1.0: Functional Classifications and Disease Progression Stages of Heart Failure

New York Heart Association Functional (NYHA) Classes		
	Definition	Examples
NYHA Class I	No limitation of physical activity	Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath)
NYHA Class II	Slight limitation of physical activity	Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea
NYHA Class III	Marked limitation of physical activity	Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea
NYHA Class IV	Unable to carry out any physical activity without discomfort	Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased
American College of Cardiology/American Heart Association Stages of Heart Failure		
	Definition	Examples
Stage A	High risk for developing HF, but without structural heart disease or symptoms of HF	Hypertension, diabetes mellitus, CAD, family history of cardiomyopathy
Stage B	Structural heart disease, but asymptomatic	Previous myocardial infarction, left ventricular dysfunction, valvular heart disease
Stage C	Structural heart disease with previous or current symptoms, but managed with medical treatment	Structural heart disease, dyspnea and fatigue, impaired exercise tolerance
Stage D	Marked symptoms at rest despite maximal medical therapy	Advanced disease requiring hospital-based support, a heart transplant or palliative care

HF is characterized by structural changes in the heart, resulting, in part, from structural changes in cardio mycotes. Within the past 10 years, a host of biomarkers have been studied with relational to their clinical relevance and diagnostic predictive value in HF. Perhaps the most well established biomarker in this regard is B-type natriuretic peptide (BNP) and its amino-terminal fragment (NT-proBNP). Both BNP and NT-proBNP have been shown to be useful in diagnosis and risk stratification, showing that there is incremental value in measuring BNP levels and that for every 100 ng/l increase in BNP level, there is a corresponding 35% relative increase in the risk of mortality (30-32). The Systolic Heart Failure Treatment Supported by BNP trial compared 220 outpatients being treated to a target BNP of less than 100 ng/l with usual clinical care (33). Those patients in the BNP-guided strategy group had a 50% reduction in death related to HF or HF-hospitalization compared with the usual care group. However, larger trials of BNP guided therapy have failed to show a clear benefit. The generalizability of these trial results has been questioned due to small sample size and the exclusion of patients with renal failure or hypotension. Regardless, testing of BNP and NT-proBNP has been reinforced by cardiology societal guidelines (34).

Pathophysiology of Heart Failure

HF is characterized by activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS), as well as inflammatory pathways. Regardless of its etiology and classification, HF begins with injury to the myocardium (e.g., myocardial infarction, sustained hypertension), which reduces cardiac output. In response, the body engages in a series compensatory mechanisms, including: 1) maintaining perfusion pressure by increasing the circulation of blood volume; 2)

activating immune and inflammatory pathways; and 3) restructuring cardiac muscle cells and reshaping the ventricle chamber. This systematic response involves complex interactions between the RAAS and SNS, which are collectively referred to as neurohormonal responses. Neurohormonal activation and inflammatory cytokine activation are designed for acute responses to injury, but prolonged activation of these compensatory mechanisms eventually leads to further declines in cardiac functioning. Currently, the most successful pharmacological therapies for HF block aspects of the body's compensatory responses to myocardial injury (29); thus, there is increasing scientific interest in understanding neurohormonal and cytokine activation in the context of HF.

Sleep Disordered Breathing in Heart Failure

Perhaps one of the least recognized risk factors for HF is obstructive sleep apnea (OSA) (35). OSA is caused by a blockage of the airway, usually when the soft tissue in the rear of the throat collapses and closes during sleep. HF is the most common cause of central sleep apnea (CSA) (14, 36). CSA is characterized by inconsistent breathing patterns, where the breathing repeatedly starts and stops. CSA in HF occurs as a result of the slower circulation of blood and a consequent unmasking of the apneic threshold due to the decrease in the partial pressure of carbon dioxide (PaCO2) in the blood resulting in cessation of breathing. 'Mixed apnea' refers to when a combination of CSA and OSA are present. Patients with systolic HF have a prevalence of 35% for OSA and of 35% to 66% for CSA (9-10). The overall prevalence of OSA in otherwise healthy adults is 3% to 7% (11). CSA in the general population is uncommon (12).

A hallmark of apenic events is hypoxia, a pathological condition where the body is deprived of an adequate oxygen supply to maintain healthy functioning. A recent study in stable HF patients found that it was not the frequency of apneic events that produced deleterious effects, but rather the duration hypoxic events (37). There is an increasing body of literature to suggest that hypoxia is a key part of the pathophysiology linking sleep apnea and poor health outcomes in HF (37). As noted earlier, SDB is a general term to refer a group of disorders, including OSA and CSA, characterized by abnormalities of respiratory pattern or the quantity of ventilation during sleep.

Sleep apnea has been associated with poor quality of life (38) as well as a two to three fold increase in mortality in HF patients (12).While there is still no direct evidence to indicate that treating OSA will reduce incident HF, there is evidence to suggest that proper treatment of sleep apnea may significantly reduce risk of death and hospitalization (17).

Pathphysiology of Sleep Disordered Breathing: The role of Systematic Inflammation

Systematic inflammation has been purported to be a part of the underlying physiological processes linking SDB to worse outcomes in HF, and CVD in general (39). Cytokines regulate and mediate immune and other inflammatory-related responses (20). The functions of proinflammatory cytokines are diverse, including developing and proliferating immune cell subsets, activating adhesion molecules and blood coagulation pathways, and altering neurochemical and neuroendocrine processes. Cellular adhesion molecules (CAMs) can reflect generalized inflammation as well as specific activation of numerous cell types, including endothelial and immune cells. CAMs play a central role in

mediating leukocyte migration and homing, as well as cell-to-cell interactions and adhesion.

Although this link has not been explicitly studied in HF patients, several studies have shown that Soluble Intercellular Adhesion Molecule-1 (sICAM-1), Interluekin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) are elevated in individuals with OSA (40). Higher levels of C-Reactive Protein (CRP) have been found in OSA patients (41), and increased CRP levels result in the direct as well as indirect (by monocytes activation) reduction of nitric oxide (NO) synthase (30,69), which is associated with endothelial dysfunction, another proposed pathophysiological mechanism of SDB in HF. NO is a vasodilator secreted by endothelium, and a reduction in NO is associated with endothelial dysfunction (42), with an impairment of quantifiable endothelial mediated vasodilatory response (43-45). OSA patients also have reduced NO levels as well as impaired endothelial mediated vasodilatation (46). Since the endothelium keeps a balance between several mediators and hormones, imbalances caused by inflammation could result in adverse effects . OSA increases levels of various adhesion molecules, including the soluble form of sICAM-1 (47) as well as other vascular cell adhesion molecules (48), thus contributing to further downstream endothelial dysfunction.

Hypoxia and sleep deprivation also modulate the expression of inflammatory mediators, such as interleukins and TNF-α (40). CPAP therapy, the gold standard for treating apnea, reduces inflammation, evident by its reduction of various circulating inflammatory mediators such as sICAM-1, Interleukin-8 (47) and CRP (49). These biomarkers have also been correlated with carotid intima-media thickness, suggesting

that apnea-related systemic inflammation is associated with progression of atherosclerosis and increased risk of cardiovascular morbidity (40).

Depression, Sleep Disordered Breathing, and Heart Failure

Although depression has not been explicitly identified in the literature as an underlying mechanism of the association between SDB and adverse outcomes, it is a robust predictor of poor clinical outcomes in HF patients. Poor sleep quality is a somatic symptom of depression. Depression has been correlated with SDB (50-51), as well as heightened inflammatory states (20).

HF patients experience disproportionately high rates of depression compared to the general population. The point prevalence of depression in HF patients is 21.5% (23), whereas the general population is estimated at 6.6% (52). Prevalence increases with the severity of HF diagnosis, ranging from 11% in patients with New York Heart Association (NYHA) functional class I to 42% in patients with NYHA class IV (23). Depressed HF patients have worse clinical outcomes than their non-depressed counterparts, including greater functional decline (53-54), increased hospitalization (55-56), poorer quality of life (57-58), and increased mortality (56, 59).

Significance

HF is a major public health concern, affecting 58 million Americans (25). HF is the leading cause of hospitalization among older adults (28), and accounts for a large proportion of health care spending in the US (60). HF has a high case fatality rate, with approximately one-quarter of patients dying within the first year of diagnosis (27). HF patients have disproportionately high rates of SDB and depression, both of which are associated with worse clinical outcomes. Inflammation has been identified as a possible

mechanism between SDB and HF (9, 20); however, there have been no longitudinal studies to demonstrate this. Understanding if heightened inflammatory states and depressed mood underlie the relationship between SDB and adverse health outcomes is the first step in identifying points of intervention in the progression of HF. The findings from this study may provide clinically meaningful data, which could be used to guide treatment and management in HF.

Introduction to Study

The purpose of this secondary data analysis is to explore potential mechanisms linking SDB and adverse clinical outcomes in a convenience sample of 66 stable HF patients and 53 non-HF controls. The data are from an ancillary sleep study, which was a part of a larger study of neuroimmune characteristics of depression and HF.

The proposed aims and corresponding hypotheses have been developed to test each of the theoretical relationships depicted in Figures 1.0 and 1.1 The primary aims of the study investigate the relationship between SDB and possible mechanisms of heightened inflammatory states and greater depressive symptom severity, which may underlie the association between SDB and poor clinical prognosis in HF patients. The primary aims use cross-sectional data to study both HF patients and controls (longitudinal data were only collected for HF), assessing the extent to which associations between SDB and inflammation and depression, respectively, vary by disease status.

The secondary aims of the study investigate the mechanistic roles of heightened inflammatory states and depressive symptom severity on the relationship between SDB and adverse clinical outcomes in HF patients.

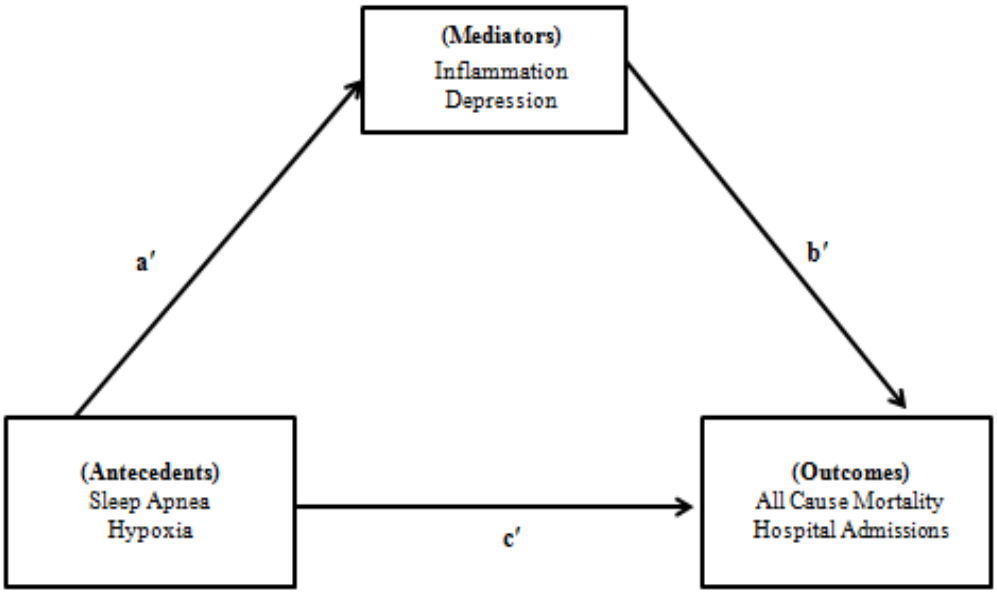

Figure 1.0: Mediated Effects of Depressive Symptoms and Inflammation on Sleep Disordered Breathing and Clinical Outcomes in Heart Failure Patients

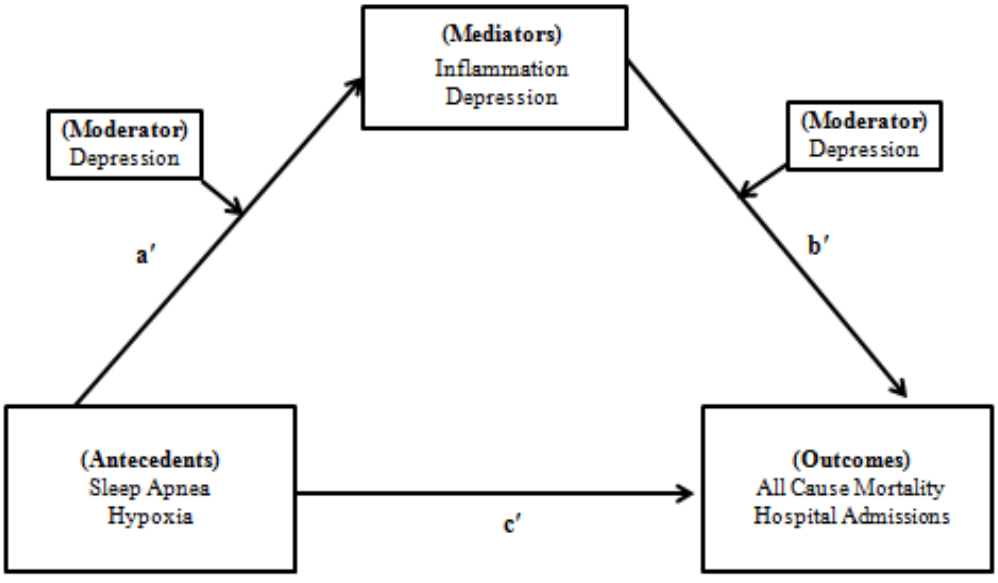

Figure 1.1: Moderated-Mediated Effects of Depressive Symptoms and Inflammation on Sleep Disordered Breathing and Clinical Outcomes in Heart Failure Patients

Finally, the tertiary aims explore the synergistic effects of depressive symptoms on the proposed mediated effects of inflammation on the association between SDB and adverse outcomes in HF (i.e., moderated-mediation).

Primary Aims

AIM 1: To assess if SDB is associated with increased levels of inflammation

Hypothesis 1.1: Greater frequency of apneic events in HF patients will be associated with greater increases in circulating levels of proinflammatory cytokines and cellular adhesion molecules compared to non-HF controls.

Hypothesis 2.2: Greater oxygen desaturation (hypoxia) in HF patients will be associated with greater increases in resting circulating levels of proinflammatory cytokines and cellular adhesion molecules compared to non-HF controls.

AIM 2: To assess if SDB is associated with depressive symptomatology

Hypothesis 2.1: Greater frequency of apneic events in HF patients will be associated with more depressive symptoms compared to non-HF controls.

Hypothesis 2.2: Greater oxygen desaturation (hypoxia) in HF patients will be associated with more depressive symptoms compared to non-HF controls.

Secondary Aims

AIM 3: To assess if circulating levels of proinflammatory and cellular adhesion molecules mediate the relationship between SDB and all-cause mortality and/or hospitalization in HF patients.

Hypothesis 3.1: Circulating levels of proinflammatory and cellular adhesion molecules will mediate the association between frequency of apneic events and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 3.2: Circulating levels of proinflammatory and cellular adhesion molecules will mediate the association between measures of hypoxia and all-cause mortality and number of hospital admissions in HF patients.

AIM 4: To assess if depressive symptoms mediate the relationship between SDB and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 4.1: Depressive symptoms will mediate the relationship between the frequency of apneic events and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 4.2: Depressive symptoms will mediate the relationship between oxygen desaturation and all-cause mortality and number of hospital admissions in HF patients.

Tertiary Aims

AIM 5: To assess if depressive symptoms moderate the path between sleep and inflammation and/or the path between inflammation and all-cause mortality and number of hospital admissions in HF patients

Hypothesis 1.1: Depressive symptoms will moderate the proposed mediating effects of inflammation on the association between SDB and all-cause mortality and number of hospital admissions in HF patients.

Chapter 2: Methods

Sample and Recruitment

The data are from an ancillary sleep study, which was a part of a larger study of neuroimmune characteristics of depression and HF. A convenience sample of stable HF patients with LVEF ≤ 40% were recruited from the University of California, San Diego (UCSD) Heart Failure Program from September 2005-August 2010. Clinic nurses identified HF status from patient medical records, and compiled a list of potential participants for the study coordinator. The study coordinator then contacted the patients by phone to screen for eligibility, and invited them to participate in the study. Eligible participants came to the UCSD General Clinic Research Center (GCRC) to be re-screened and consented into the study.

Study inclusion criteria specific to HF patients included New York Heart Association (NYHA) classes II through III, symptoms of HF for at least 3 months which had been optimally treated with diuretics, angiotensin converting enzyme (ACE) inhibitors, and/or beta-blockers, and an ejection fraction ≤ 40%. Other study inclusion criteria included ages between 30–85 years, blood pressure < 180/110 mm Hg and men and women of all ethnicities and races. Exclusion criteria included myocardial infarction (1 month), recent stroke or significant cerebral neurological impairment, severe chronic obstructive pulmonary disease and psychiatric illness other than depression.

All persons who had enrolled in the main study were invited to participate in a sleep monitoring study the night before their baseline visit. Of 82 HF patients who agreed to participate in the larger study, 66 participated in the volunteer sleep monitoring sub-study. The HF sleep study participants had a mean age of 55.37 years (*SD*=13.4), mean

BMI of 31.23 kg/m^2 (*SD*=6.8), 74.2% were male, 62.1% white, 80.3% non-Latino, BNP levels 222.74 pg/mL (*SD*=262.1), LVEF 30.3% (*SD*=12.5) and completed 370.3 (*SD*=79.1) meters during the six minute walk test (a test for functional capacity in HF). The larger HF sample from the University of California, San Diego site had a mean age of 55 years (*SD*=12.77), mean BMI of 30.98 kg/m^2 (*SD*=7.60), 73.2% were male, 61% were white, 80.2% non-Latino, BNP levels 207.37 pg/mL (*SD*=257.4), LVEF 30.41% (*SD*=11.59) and completed 341.48 (*SD*=130.75) meters during the six minute walk test.

A convenience sample of non-HF controls (ages 30-85 years) was recruited from the local community via advertisement and referrals. Of the 68 non-HF controls who agreed to participate in the study, 53 participated in the volunteer sleep monitoring sub-study. The non-HF controls who participated in the sleep study had a mean age of 52.11 years (*SD*=10.72), mean BMI of 28.80 kg/m^2 (*SD*=5.87), 45.3% were male, 88.7% were non-Latino and 50% were white. The total control sample had a mean age of 52 years (*SD*=11.01), mean BMI kg/m^2 of 28.67 (*SD*=4.36), 42.9% were male, 88.6% were non-Latino and 51.4% were white.

The investigation conformed with the principles outlined in the Declaration of Helsinki. The study was approved by the University of California, San Diego Institutional Review Board. All subjects gave informed written consent.

Procedures

Initial Screening: Eligible participants came to UCSD GCRC for an initial screening appointment, where the research assistant collected demographic information and an echocardiogram was conducted for HF patients.

Sleep Study: Seventy-nine percent (n=119) of the sample recruited from the UCSD Hillcrest Medical Center site participated in the ancillary study. Participants were scheduled for one night of sleep monitoring at the Gillin Laboratory of Sleep and Chronobiology at the UCSD GCRC. Participants arrived at 6 pm. Sleep setup began at 8 pm and took approximately 1 hour. Lights were turned off at 10 pm. Sleep monitoring ended at 7:30 AM the next morning.

Testing Day (baseline visit): Anthropometric measures were taken by a trained staff member. Then the study nurse took baseline measurements, including blood pressure readings using an using an automated BP monitor (Dinamap Compact BP® monitor, Critikon, Tampa, FL), and a baseline blood draw via an intravenous catheter was used to assess resting levels of neuroimmune and cellular adhesion molecule biomarkers. Study participants then completed a battery of psychosocial questionnaires.

Follow-up Assessments: Abstractions from participants' electronic medical records (EMR) were performed to obtain information on the number of all-cause hospital admissions and mortality between the date of the baseline visit until February 2012. Date and primary causes of hospitalizations were noted. Social Security Death Index (SSDI) searches were performed on cases, and once a death record was confirmed via SSDI, the date of death was noted. Longitudinal follow-up was only conducted on HF patients. These patients were receiving treatment at UCSD prior to referral into the study, whereas controls were recruited from the larger San Diego area (e.g., not necessarily patients at the UCSD Medical Center).

Measures

All-Cause Mortality and Number of Hospital Admissions: Number of hospital admissions and mortality data were abstracted from the patients' EMR starting from the patient's baseline visit until February 2012. Thus patients were followed for varying lengths of time depending on the date of their baseline visit [77 days (2.5 months) to 2237 days (6 years)], and length of follow-up time was a covariate in all analyses using morbidity and mortality endpoints. The date and primary cause was noted for each hospital admission, which was then classified as HF-related, cardiovascular or non-cardiovascular by a cardiologist. However, approximately 5% of the hospitalizations were not able to be classified . All-cause hospital admissions was used in the current study (e.g., as noted in the original dissertation proposal).

If cases did not have a contact/record (e.g., visit, phone call) on their EMR within the past week or if the last contact noted a severe decline (e.g., referred to hospice), the SSDI was searched using social security numbers. Date of death was obtained via SSDI. Mortality was coded as '0' alive, and '1' dead, and number of hospital admissions was modeled as a continuous variable.

Sleep Disordered Breathing: Sleep data was recorded using standard polysomnography (PSG). Electroencephalography (EEG), electrooculography, chin electromyography (EMG), and thoracic and abdominal respiration were recorded on a Grass model PSG36-2 (Grass Technologies, West Warwick, RI) or Embla model A10 polysomnograph (Broomfield, CO). Oxyhemoglobin saturation was monitored using a pulse oximeter (Biox 3740; Ohmeda, Louisville, CO) and analyzed by software from PROFOX (Escondido, CA). Anterior tibialis EMG was used to rule out periodic limb

movements during sleep. Records were scored with the Rechtshaffen and Kales31 criteria by technicians with inter-rater reliabilities >90%.

Apneas and Hypopneas: Respiratory analysis consisted of defining 'apneas' as a -≥10s cessation of airflow, and 'hypopneas' as a ≥50% reduction in airflow (detected by an oro-nasal airflow/pressure signal) that is associated with a ≥4% fall in SaO_2 or terminated by an arousal from sleep. Obstructive events were defined by either: (a) the cessation of airflow and the presence of thoracoabdominal movements, or by (b) the presence of inspiratory airflow limitation on visual inspection of the nasal pressure signal. The latter is defined by the occurrence of an early peak in inspiratory flow followed by a plateauing of inspiratory airflow and negative effort dependence, and is often accompanied by paradoxical movements of the thorax and abdomen. In contrast, central apneas were defined by absent or reduced thoracoabdominal movements during apneas, and by the absence of inspiratory flow limitation. Mixed apneas and hypopneas were defined when events contain both a central and obstructive component.

A general apnea-hypopnea index (AHI) was calculated by summing the total number of sleep apnea and hypopnea events per hour of non-REM and REM sleep. Sleep apnea specific indices were also created for obstructive (OAHI), central (CAHI) and mixed (MAHI) sleep apnea by replacing total number of sleep apnea events, with specific sleep apnea events (e.g., total number of obstructive sleep apnea and hypopnea events per hour of sleep). The AHI can also be used to stratify the severity of the disease; an AHI of 5-15 is classified as mild, 15-30 is considered moderate, and greater than 30 is considered severe. AHI variables were modeled as continuous variables in the analyses. Categorical descriptions were provided as part of the descriptive analyses.

Hypoxia: Significant transient oxyhemoglobin desaturations were defined as drops in oxyhemoglobin saturation of ≥ 3% from baseline lasting more than 10 seconds but less than three minutes. The average percentage of oxygen saturation, the lowest percentage of oxygen saturation and total minutes oxygen saturation level fell below 90% were derived from pulse oximeter data.

Respiratory Arousals: Arousals were defined as a sudden rises in EEG frequency to theta or alpha lasting at least three seconds but less than 15 seconds.

Inflammatory Markers: Biomarkers were chosen based on their importance in explaining the relationship between SDB and clinical outcomes in HF, which was previously discussed on p. 7. Resting levels of proinflammatory biomarkers were obtained via blood draw during the baseline visit.

Proinflammatory Cytokines: Cytokines are relatively small molecular weight protein molecules that are secreted by a variety of different cell types and tissues. 'Proinflammatory' cytokines is a term that is used to describe a group of cytokines that are responsible for initiating acute phase reaction to bacterial infection as well as repair of tissue following injury (61). Acute phase reaction refers to complex endocrine, metabolic and neurological changes that occur in an organism shortly after injury or onset of infection. Specifically, TNF-α, IL-6 and IL-1β are known as vascodepressor proinflammatory cytokines, and are expressed by all nucleated cell types residing in the myocardium (61). These cytokines influence the release of other cytokines and inflammatory products, but also affect cardiac performance when expressed at high levels (61).

TNF-α is secreted in macrophages, but may also be secreted by other cells and tissues (e.g., lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue). TNF-α acts as an autocrine regulator of leukocytes and endothelial cells, regulating inflammatory responses to microbes and facilitates tissues repair. However when TNF-α production exceeds the number of TNF receptors in a given tissue, TNF-α spills over into the blood stream where it acts as an endocrine hormone and may lead to metabolic wasting, microvascular coagulation and hypotension (61).

IL-1ra, as well as TNF-α, is often thought of as a prototypical proinflammatory cytokine. It is secreted in macrophages as a proprotein, and is known to depress myocardium contractility (61). It is involved a variety of cellular activities, including cell proliferation, differentiation and apoptosis. Since IL-1β is difficult to reliably assay due to low circulating levels, its receptor IL-1ra, which mirrors IL-1ra levels, is typically assayed in proxy (62).

IL-6 is also a part of the acute phase response, and can act as a proinflammatory and anti-inflammatory cytokine. IL-6 is secreted by T cells, macrophages as well as muscle. IL-6 is released in direct response to TNF-α. Elevated concentrations of IL-6 have been associated with myocyte hypertrophy, myocardial dysfunction and muscle wasting (63).

Circulating TNF-α, IL-6, and IL-1ra levels were determined by enzyme-linked immunosorbent assay (ELISA). For IL-6 the intraassay coefficient variability (CV;%) is 2.2, the inter-assay CV (%) is 3.9, and the assay sensitivity <0.71pg/ml; for TNF-α the intraassay CV (%) is 8.0, the inter-assay CV (%) is 16.3, and the assay sensitivity <0.18

pg/ml; for IL-1ra the intraassay CV (%) is 6.8, the inter-assay CV (%) is 8.3, and the assay sensitivity <0.1 pg/ml.

C-Reactive Protein (CRP): CRP is an acute phase protein that is produced exclusively in the liver. CRP binds to specific molecular configurations that are generally associated with cell death or found on the surface of pathogens (61, 64). It rapidly increases in synthesis within hours of tissue injury (64), and for this reason it is thought to play a key part in the acute phase reaction.

Circulating CRP levels were determined in plasma using the High Sensitive CRP Reagent Set (DiaSorin; Stillwater, MN) using the Roche Cobas Mira Plus analyzer (Roche, Palo Alto, CA). Intra- and inter-assay coefficients of variation are < 5%. The precision and sensitivity performance values of this assay are excellent: intra-assay CV (%) < 1.0, inter-assay CV (%) = 1.6, sensitivity < 0.05 mg/l.

Adhesion molecules: Adhesion molecules are cell-surfaced receptors involved in binding of leukocytes to each other, to endothelial cells and extracelluar matrix. There are three different families of adhesion molecules: immunoglobulin super family, and integrins, and selectins. sICAM-1 is a member of the immunoglobulin super family. It is induced by TNF-α and IL-1β, and is expressed by the vascular endothelium, macrophages and lymphocytes(61). sICAM-1 plays a key role in cell signaling, helping to stabilize cell-cell interactions and facilitating leukocyte-endothelial transmigration. sP-selectin is involved in recruiting the leukocytes onto the endothelial surface, as well as recruiting and aggregating platelets at areas of vascular injury (61).

sICAM-1 and sP-selectin were determined by ELISA. The precision and sensitivity performance values are as follows: sICAM-1 [intra-assay CV (%) = 4.6, inter-

assay CV (%) = 6.6, sensitivity < 0.35 ng/ml]; sP-selectin [intra-assay CV (%) = 5.1, inter-assay CV (%) = 8.8, sensitivity < 0.5 ng/ml].

Depressive Symptomatology

The 21-item self-administered BDI (version –IA) assessed the extent to which patients experienced depressive symptoms (65). Scores of 0-9 indicated minimal or no depression, 10-18 indicated mild-moderate depression, 19-29 moderate-severe depression, and 30-63 severe depression. The reliability of this measure in our sample was α=.913. Two sub-scales were created: 1) the cognitive-affective subscale assessed symptoms such as sadness and dissatisfaction (13 items, score range 0-39; α=.87); and 2) the somatic subscale assessed features such as changes in appetite and feelings of fatigue (7 items, score range 0-21;α=.83)(65). A clinical psychologist administered a modified Structured Clinical Interview for DSM-IV (SCID) to evaluate for possible Major Depressive Disorder (MDD) if the participant who scored ≥ 10. If participants were suspected of having MDD and were not currently being treated for depression, they were provided with information on depression as well as a referral to mental health services.

Covariates

Covariates were selected for possible inclusion in the analyses based on theoretical relationships with the outcome or mediator. Different covariates were used depending on the time point (e.g., length of follow-up was a covariate in longitudinal analyses). For a list of variables, please consult Table 2.0, p. 26.

Demographics: Age, gender (coded as ‘0’ male and ‘1’ female), ethnicity (coded as ‘0’ non-Latino, and ‘1’ Latino), and race (coded as ‘0’ white, ‘1’ black, and ‘2’ other) were obtained via self-report during the initial screening process.

Advancing age has been associated with declines in cardiovascular functioning, and psychological well-being, as well as increased inflammation. Men are more susceptible to HF (25) and OSA (66) than women. Women often report greater depression and emotional distress, relative to men (67). African Americans have a higher prevalence of HF as well as higher rates of HF hospital admissions compared to whites (68-70). These disparities may be attributable, in part, to poorer outpatient management and greater dependence on emergency medical services for HF exacerbations (71). Some studies have shown depression severity to be greater, and treatment for depression to be less likely in underserved and minority communities (72-73). Although there is limited data on racial and ethnic disparities in SDB, there is evidence to suggest that African Americans experience more OSA (74), while Asian populations experience less OSA compared to Caucasians (75). These disparities have been attributed, in part, to racial and ethnic differences in Body Mass Index (BMI). Heavier BMI has also been associated with greater SDB (76) and increased inflammatory load (77).

Health Behaviors: Increased alcohol consumption as well as smoking has been associated with greater depressive symptomatology (78) and CVD severity (79). A brief questionnaire assessing past and current smoking and alcohol consumption was administered. Participants were asked if they currently consumed alcohol or smoked tobacco products, respectively. The responses were coded dichotomously (coded as '0' for not a current smoker or drinker, respectively, and '1' for current smoker or drinker, respectively).

Body Mass Index: Heavier BMI is associated with OSA (40), heightened inflammatory load (80), depression (81) as well as greater mortality and morbidity

outcomes in HF (82). Weight was measured to the nearest pound using a Health-o-Meter standard scale with the participant standing without shoes and heavy objects removed from pockets. Height was measured to the nearest 1/4 inch, using a standard portable stadiometer with shoes removed. These measures were converted into metric equivalents to calculate BMI. BMI was calculated using the Quetelet index (kg/m2).

Comorbities: Information on comorbities was self-reported for controls, and study nurses collected this information from HF patient EMRs, including a history of hypertension (only collected for HF patients), diabetes and coronary artery disease (coded '0' for no responses, and '1' for yes responses).

Hypertension: Several large epidemiological studies have shown that OSA is an independent risk factor for high mean systolic and diastolic blood pressures (3, 5-6, 83). Current hypertension management guidelines have acknowledged OSA as an identifiable and independent cause of hypertension, and recommend blood pressure screenings amongst OSA patients (84). Hypertension is a leading risk factor for the development of HF (25). Overtime hypertension increases pressure in the blood vessels, and as a result the heart must pump harder, and heart begins to thicken.

Diabetes: There has been increasing research that shows that type 2 diabetics have a high prevalence of OSA, which ranges from 86% (85) to 53% (86). It is well-known that severity of sleep apnea is correlated with degree of insulin resistance (1). Type 2 diabetes mellitus and insulin resistance are established risk factors for HF (87-88) . Many patients with type 2 diabetes have diastolic dysfunction (89) , which can lead to HF with preserved ejection fraction. Diabetes has been a consistent, and powerful risk factor for

the development of post-MI in HF patients, accounting for 66% of mortality during the first year (90).

Coronary artery disease (CAD): Epidemiological studies have consistently reported a close and strong presence of CAD in OSA patients, regardless of other risk factors (91). Although the exact mechanisms are unknown, OSA related hypoxia causes sympathetic overactivation, and an acute imbalance of vasoactive hormones. These changes can provoke acute coronary syndromes. CAD is the most common cause of HF, accounting for approximately 70% of HF cases (92). CAD causes obstruction to the coronary arteries, which prevents blood flow and oxygen delivery to the heart. The heart must exert more effort to maintain blood flow, and overtime this may lead to reduced contractility of the heart and reduced left ejection fraction.

HF Disease Severity: Brain (B-type) natriuretic peptide (BNP) is a polypeptide that is secreted by the ventricles in response to cardiomyocycte stretching (37). BNP levels are routinely obtained at clinic visits and are used as a marker of HF severity (30-32). BNP levels ≥100 pg/ml constitute high-risk. Baseline BNP was collected via blood draw the morning of the test day. BNP was measured on the ADVIA Centaur (Siemens Healthcare Diagnostics, Tarrytown, NY) instrument. This system determines BNP concentrations by using a fully automated two-site sandwich immunoassay using direct chemiluminescent technology. The assay measures BNP concentrations up to 2500 pg/ml with a minimum detectable concentration of < 1.0 pg/ml. The intra-assay CV (%) = 2.2 and the inter-assay CV (%) = 1.8. To assess functional capacity, the number of meters that the participants could walk in 6-minutes was assessed, which is a well-known measure of functional capacity in cardiovascular patient populations. (24).

HF-Specific Covariates: The following covariates were only collected in HF patients. LVEF % was calculated via echocardiography. Researchers have cited that some medications routinely prescribed for HF patients exacerbate or attenuate sleep apnea (93). Patients' medication lists were collected at baseline via EMRs. Each medication name, dose, and reason it was prescribed were noted qualitatively in the database. A study nurse identified if the patient was prescribed medications within particular classes (coded as '0' no, and '1' yes), including angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), diuretics, and beta-blockers.

Table 2.0: Study Variables

Antecedent (Collected night one)	
Sleep Apnea	Apnea-Hypopnea Index, events/hr
	Obstructive Apnea-Hypopnea Index, events/hr
	Central Apnea-Hypopnea Index, events/hr
	Mixed Apnea-Hypopnea Index, events/hr
Hypoxia	Time Oxygen Saturation Below 90 (%), M (SD)
	Average Oxygen Saturation (%) , M (SD)
	Lowest Oxygen Saturation (%), M (SD)
Mediators (Collected at baseline visit, day after night one)	
Inflammation	C-Reactive Protein, mg/L, M (SD)
	Interleukin-1ra, pg/mL, M (SD)
	Interleukin-6 ,pg/mL,M (SD)
	Soluble P-selectin, ng/mL, M (SD)
	Soluble Intercellular Adhesion Molecule-1,ng/mL, M (SD)
	Tumor Necrosis Factor-α , pg/mL, M (SD)
Depressive Symptomatology	Beck Depression Inventory, Total Scores, M (SD)
	Beck Depression Inventory, Somatic Subscale, M (SD)
	Beck Depression Inventory, Cognitive-Affective Subscale, M (SD)
Outcomes (Follow-up, collected from Patient's Electronic Medical Records, Primary Aims 1 and 2)	
	Number of Hospital Admissions, M (SD)
	Mortality, n (%)
Covariates (Collected at baseline visit)	
Demographics and Behaviors	Age, M (SD)
	Male, n (%)
	Body Mass Index, kg/m^2,M (SD)

Table 2.0: Study Variables Continued

Covariates (Collected at baseline visit)	
	Current Smoker, n (%)
	Current Drinker, n (%)
	Ethnicity, n (%)
	Race, n (%)
Diagnosis/ Treatment Related to the Mediator	Current Antidepressant Use, n (%)
	Sleep Disordered Breathing diagnosis, n (%)
Heart Failure Severity	BNP pg/mL, M (SD)
	Six Minute Walk test (meters), M (SD)
Comorbidity	History of Hypertension, n (%)
	History of Diabetes, n (%)
	History Coronary Artery Disease, n (%)
Heart Failure Specific	Heart Failure Medications, n (%)
	Implantable Cardioverter Defibrillator, n (%)
	Follow-up Time, M (SD)
	Systolic Dysfunction, n (%)
	Diastolic Dysfunction, n (%)
	Left Ventricular Ejection Fraction (%), M (SD)
	Heart Failure Etiology, n (%)

Chapter 3: Results

Primary Aims

AIM 1: To assess if SDB is associated with increased levels of inflammation

Hypothesis 1.1: Greater frequency of apneic events in HF patients will be associated with greater increases in circulating levels of proinflammatory cytokines and cellular adhesion molecules compared to non-HF controls.

Hypothesis 2.2: Greater oxygen desaturation (hypoxia) in HF patients will be associated with greater increases in resting circulating levels of proinflammatory cytokines and cellular adhesion molecules compared to non-HF controls.

AIM 2: To assess if SDB is associated with depressive symptomatology

Hypothesis 2.1: Greater frequency of apneic events in HF patients will be associated with more depressive symptoms compared to non-HF controls.

Hypothesis 2.2: Greater oxygen desaturation (hypoxia) in HF patients will be associated with more depressive symptoms compared to non-HF controls.

Analytical Plan for Primary Aims

Model assumptions were assessed graphically and analytically. Suspected outliers were assessed for biological plausibility. Due to disparities in levels of SDB in controls versus cases (e.g., HF patients had worsened, more extreme SDB values), biologically plausible values for cases were winsorized, replacing these values with a value that was three standard deviations away from the mean (94). Three standard deviations from the mean was chosen based on the empirical rule, which states that in a normal distribution approximately 68% of observations will lie within plus or minus one standard deviation from the mean. Approximately 95% of observations will lie within two standard

deviations from the mean and 99.7% of the observations will lie within three standard deviations from the mean. Apnea-hypopnea index (AHI), obstructive apnea-hypopnea index (OAHI), central apnea-hypopnea index (CAHI), average oxygen saturation (%) and lowest oxygen saturation (%) were winsorized. For variables where overdispersion (a high number of zero values) was present, and winsorizing extreme values could not adequately transform the distribution, a median split was performed. Total time oxygen saturation fell below 90%, was dichotomized (≤15mins was coded as '0' and >15 minutes were coded as '1'). All proinflammatory markers were positively skewed, and were normalized via natural-log transformation. Bivariate associations between the predictors and outcomes were assessed using correlations (Pearson's *r*), t-tests, chi-square and ANOVA as appropriate. Ordinary least squares hierarchical multiple linear regression was used to test relationships between SDB, disease status (HF or control), and their interaction with each outcome (e.g., depressive symptomatology and inflammation levels). Model 1 included the first-order effects of SDB, disease status and their interaction. Each model assessed one predictor, one moderator and their interaction (e.g, multiple SDB variables and interaction terms were not assessed simultaneously). If the interaction term was statistically significant or approached significance ($p<.01$) then covariates that had a statistically significant bivariate relationship with the outcome variable ($p<.05$) were added to the model. Statistically significant SDB by disease status effects ($p<.05$) were probed via simple slope analyses by computing simple regression lines for the relationship between SDB and outcome for HF participants and non-HF controls (95). For example, if an interaction effect was no longer statically significant after adjusting for covariates then simple slope analyses were performed on the

unadjusted model. Regression analyses were conducted using Predictive Analytics SoftWare, Version 18.0 (PASW, Chicago, Illinois, USA). Statistical significance was set at p=.05.

Results for Primary Aims

Sample Characteristics (see Table 3.0)

Analyses were conducted on 66 HF and 53 non-HF controls. Most of the sample was Caucasian (56.2%) and non-Latino (84.0%). On average the sample was 54.14 (*SD*=12.39) years of age and obese (*M* BMI=30.13 kg/m^2, *SD*=6.48). Although only 13% reported that they currently smoked tobacco products, 44.5% reported that they drank alcohol. Only a small percentage of participants reported that they were currently taking antidepressants (5.9%), and only one non-HF control reported antidepressant use.

On average, HF participants were older [55.37 years (*SD*=13.4), 52.11 years (10.72)] and heavier than controls [31.23 kg/m2 (*SD*=6.8), 28.80 kg/m2 (SD=5.87)] and most reported comorbidities (See Table 3.0). HF patients had significantly higher BNP levels compared to controls [222.74 pg/mL (*SD*=262.1), 17.50 pg/mL (SD=12.96)] a marker of HF severity, and walked a shorter distance during the six minute walk test compared to controls [370.3meters (*SD*=79.1), 489.85 meters (SD=110.34);*ps*<.01].

HF patients had statistically higher mean levels of sleep apnea indices (AHI, OAHI, MAHI; *ps*<.01), and numerically higher levels of CAHI. HF patients spent more time at an oxygen saturation less than 90% [186.52 minutes (199.32), 132.23minutes (183.64); p<.05] and their oxygen saturation percentages dropped lower than controls [72.97% (21.84), 81.17% (13.22); p<.01]. HF patients has received approximately 25 minutes less sleep than controls (p<.01; See Tables 3.0-3.1).

Although SDB variables were modeled continuously, it may be helpful assess the sample's level of sleep apnea relative to clinical cut off measurements. With regard to frequency and severity of apneas in HF patients, 10.6% had no sleep apnea (AHI < 5.00), 22.7% had mild sleep apnea (AHI =5.00-15.00), 25.8% had moderate sleep apnea (AHI=15.01-30.00), and 40.9% had severe sleep apnea (AHI>30.01; categorical data not shown). With regard to specific sleep apnea types, 19.7%, 19.7%, and 27.3% (OAHI, CAHI and MAHI, respectively) had no sleep apnea, 34.8%, 37.9% and 36.4% had mild sleep apnea, 24.2%, 27.3%, and 24.2% had moderate sleep apnea, and 21.2%, 15.2, and 12.1% had severe sleep apnea. Whereas for controls, 45.3 % had no sleep apnea (AHI < 5.00), 32.1% had mild sleep apnea (AHI =5.00-15.00), 13.2% had moderate sleep apnea (AHI=15.01-30.00), and 9.4% had severe sleep apnea (AHI>30.01; categorical data not shown). With regard to specific sleep apnea types, 47.2%, 56.6%, and 62.3% (OAHI, CAHI and MAHI, respectively) had no sleep apnea, 30.2%, 28.3% and 22.6% had mild sleep apnea, 15.1%, 13.2%, and 11.3% had moderate sleep apnea, and 9.4%, 7.5%, and 3.8% had severe sleep apnea.

HF patients displayed more depressive symptomatology compared to controls, albeit this finding was only significant for somatic (not cognitive-affective) and total depressive symptomatology scales (*p*s<.01).With the exception of IL-6, HF patients had significantly higher resting levels of all proinflammatory cytokines and cellular adhesion molecules (*ps*<.05).

Table 3.0: Sample Characteristics and Bivariate Associations by Cases and Controls

	M (SD) or n (%)			M (SD) or n (%)
	Heart Failure (n=66)	Controls (n=53)	Bivariate Associations	Total (n=119)
Age, years, M (SD)	55.37 (13.4)	52.11 (10.72)	*t*=-1.61	54.14 (12.39)
Male, n (%)	49 (74.2%)	24 (45.3%)	X^2=10.39**	73 (61.3%)
Body Mass Index, kg/m^2,M (SD)	31.23 (6.8)	28.80 (5.87)	*t*=-2.05*	30.15 (6.48)
Current Smoker, n (%)	9 (13.2%)	6 (11.3%)	X^2=*.705*	15 (12.6%)
Current Drinker, n (%)	27 (40.9%)	26 (49.06%)	X^2=*.790*	53 (44.5%)
Non-Latino Ethnicity, n (%)	53 (80.3%)	47 (88.7%)	X^2=*1.54*	100 (84%)
Race, n (%)			X^2=*2.84*	
White	41 (62.1%)	26 (50.%)		68 (56.2%)
Black	20 (30.3%)	24 (45.3%)		45 (37.2%)
Other	5 (7.6%)	2 (3.8%)		8 (6.6%)
Current Antidepressant Use, n (%)	6 (9.1%)	1 (1.9%)	--------	7 (5.9%)
B-type Natriuretic Peptide (pg/mL), M (SD)	222.74 (262.1)	17.50 (12.96)	*t*=-5.58**	130.11 (219.15)
Six minute walk test (yards), M (SD)	370.3 (79.1)	489.85 (110.34)	*t*=7.08**	429.75 (113.37)
History of diabetes, n (%)	13 (19.7%)	1 (1.9%)	---------	14 (11.8%)
History Coronary Artery Disease	22 (33.3%)	0 (0%)	---------	22 (18.5%)
Sleep Disordered Breathing (Antecedents)				
Apnea-Hypopnea Index (events/h), M (SD)[a]	32.01 (28.98)	12.23 (17.31)	*t*=-4.52**	23.24 (26.32)
Obstructive Apnea-Hypopnea Index (events/h), M (SD) [a]	20.53 (21.38)	10.27 (13.15)	*t*=-3.22**	15.96 (19.82)

Table 3.0: Sample Characteristics and Bivariate Associations by Cases and Controls Continued

	M (SD) or n (%)			M (SD) or n (%)
	Heart Failure (n=66)	Controls (n=53)	Bivariate Associations	Total (n=119)
Central Apnea-Hypopnea Index (events/h), M (SD)[a]	18.60 (19.09)	6.56 (7.55)	*t*=-5.21	13.24 (16.18)
Mixed Apnea -Hypopnea, (events/h), M (SD)	13.46 (10.66)	6.58 (8.13)	t=-3.98**	10.64 (10.72)
Total Time Oxygen Saturation Fel Below 90% (min), M (SD)[c]	186.52 (199.32)	132.23 (183.64)	X^2=3.79*	160.67 (193.40)
Average Oxygen Saturation % , M (SD)[a]	93.43 (2.71)	95.14 (2.51)	*t*=3.80	94.19 (2.75)
Lowest Oxygen Saturation%, M (SD) [a]	72.97 (21.84)	81.17 (13.22)	*t*=2.46**	76.62 (18.88)
Total Sleep Time (hours), M (SD)	5.34 (1.49)	6.01 (1.01)	*t*=2.93**	5.63 (1.35)
Beck Depression Inventory Scores (Mediators)				
Cognitive-Affective Score, M (SD),range:0-39	4.55 (4.46)	3.44 (4.46)	*t*=-1.22	4.48 (4.54)
Somatic score, M (SD), range: 0-21	6.48 (3.49)	2.96 (2.54)	*t*=-6.04**	4.89 (3.55)
Total Score, M (SD), range:0-63	10.05 (7.21)	6.40 (6.42)	*t*=-3.52**	8.98 (7.20)
Inflammatory Markers (Mediators)				
C-Reactive Protein mg/L, M (SD)[c]	3.42 (3.54)	1.65 (2.01)	*t*=-3.53**	2.61 (3.05)
Interleukin-1ra pg/mL, M (SD)[c]	579.61 (518.39)	289.32 (154.40)	*t*=-4.32**	446.75 (424.04)
Interleukin-6, pg/mL, M (SD) [c]	5.70 (4.75)	4.51 (3.58)	*t*=-1.30	5.18 (4.29)

Table 3.0: Sample Characteristics and Bivariate Associations by Cases and Controls Continued

	M (SD) or n (%)			M (SD) or n (%)
	Heart Failure (n=66)	Controls (n=53)	Bivariate Associations	Total (n=119)
Soluble P-selectin, ng/mL, M (SD)[c]	82.20 (47.88)	65.29 (26.56)	*t*=-1.99*	74.57 (40.44)
Soluble Intercellular Adhesion Molecule-1 ng/mL, M (SD)[c]	290.14 (129.14)	232.89 (68.43)	*t*=-2.66**	264.75 (109.94)
Tumor Necrosis Factors-α pg/mL, M (SD) [c]	2.31 (1.89)	2.06 (2.55)	*t*=-2.33**	2.20 (2.19)

Note: In cases where the variable was transformed, the M (SD) of the untransformed variable are presented and transformed version of the variable was used to test bivariate associations; [a]winsorized extreme cases; [b]median split; [c] natural log; [§]$p < .10$ *$p<.05$ **$p<.01$

Bivariate Associations between Beck Depression Inventory Scores, Inflammatory Markers and Sleep Disordered Breathing

Beck Depression Inventory Scores: The BDI total score and subscales were highly correlated. The BDI total score was highly correlated with somatic and cognitive-affective subscales (*r*=.920, *r*=.865, *ps*<.01; data not shown). The somatic and cognitive-affective subscales were strongly correlated (*r*=.600, *p*<.01). All BDI measures were highly correlated with screening positive on the Structural Clinical Interview Diagnostic and Statistical Manual of Mental Disorders (SCID), a screening tool for Major Depressive Disorder [*r=.532* cognitive-affective ,*r=.465* somatic, *r=.545* total score *p*<.01; data not shown].

Beck Depression Inventory Scores and Sleep Disordered Breathing (see Table 4.0): Among the BDI scales, higher somatic scores were significantly correlated with more SDB, including more central and mixed sleep apnea events (*p*<.05) as well as lower

average oxygen saturation ($p<.01$), and more total time oxygen saturation was below 90%, and lowest oxygen saturation percentage ($ps<.05$). Higher total scores were correlated with more time oxygen saturation was below 90%, and the lowest average oxygen saturation level ($p<.01$). Higher cognitive-affective scores were significantly correlated with lower average oxygen saturation levels ($p<.05$).

Beck Depression Inventory Scores and Covariates (see Tables 4.0): Higher depressive symptomatology (all scales) was significantly correlated with higher BMI ($ps<.05$) .Higher cognitive-affective scores were associated with younger age ($p<.05$). Latino participants, on average, had higher somatic scores ($p<.01$). Higher somatic scores were also correlated with increased HF severity, higher BNP and fewer meters walked in the six minute walk test ($ps<.05$). Fewer meters walked in the six minute walk test was also associated with higher total scores ($ps<.01$).

Table 4.0: Bivariate Associations between Sleep Disordered Breathing, Covariates and Beck Depression Inventory Scores

	Cognitive –Affective Score	Somatic Score	Total Score
Apnea-Hypopnea Index (events/h), M (SD)	*r*=.021	*r*=-.167§	*r*=.098
Obstructive Apnea -Hypopnea Index (events/h), M (SD)	*r*=-.019	*r*=.116	*r*=.047
Central Apnea -Hypopnea Index (events/h), M (SD)	*r*=.019	*r*=.206*	*r*=.117
Mixed Apnea-Hypopnea, (events/h), M (SD)	*r*=.021	*r*=.239*	*r*=.132
Total time Oxygen Saturation is Below 90% (min), M (SD)	*t=-2.507*	*t*=-2.046*	*t*=-2.57**
Average Oxygen Saturation % , M (SD)	*r*=-.185*	*r*=-.247**	*r*=-.236**
Lowest Oxygen Saturation%, M (SD)	*r*=-.099	*r*=-.182*	*r*=-.109
Age, years, M (SD)	r=-.177*	*r*=-.078	*r=.-146*
Male, n (%)	*t=-1.45*	*t=.285*	*t=-.941*
Body Mass Index, kg/m^2,M (SD)	*r=.216**	*r*=.256**	*r*=.259**
Current Smoker, n (%)	*t=-.980*	*t=-.879*	*t=-.988*
Current Drinker, n (%)	*t=.083*	*t=-.356*	*t=-.098*
Non-Latino Ethnicity, n (%)	*t=-1.02*	*t=.292***	*t=-1.92*§
Race, n (%) White Black Other	*F=.606*	*F=.195*	*F=.136*
Current Antidepressant Use, n (%)	*t=-.557*	*t=-1.64*	*t=-.897*
B-type Natriuretic Peptide (pg/mL), M (SD)	*r=-.013*	*r=.213**	*r=.099*
Six minute walk test (meters), M (SD)	*r=-.171*§	*r=-.447***	*r=-.325***
History of Diabetes, n (%)	*t=-.589*	*t=-1.78*§	*t=-1.23*
History Coronary Artery Disease	*t=.356*	*t=.098*	*t=.267*

Inflammation: The inflammatory markers were weakly to moderately correlated with each other (r=.033, .446, p>.10; data not shown).

Inflammation and Sleep Disordered Breathing (see Table 5.0): With regard to sleep apnea, higher OAHI was associated with higher levels of IL-6 (p<.05), and higher CAHI was associate with higher levels of CRP (p<.01). More total time where oxygen saturation was below 90% was correlated with lower levels of TNF-α and sP-selectin, and higher levels sICAM-1 and IL-1ra (ps<.05). Lowest percentage of oxygen saturation was correlated with higher levels IL-1ra and IL-6 (ps<.05).

Inflammation and Covariates (see Table 5.0): Heavier BMI was associated with higher levels of CRP, IL-1ra and sP-selectin (ps<.05). Higher CRP was correlated with Latino ethnicity (p<.05). Lower IL-1ra was correlated with antidepressant use (p<.01). Higher levels of IL-6 were correlated with higher levels of BNP (p<.05). Higher levels of IL1-ra, sP-selectin and TNF-α were associated with fewer meters walked during the six minute walk test (ps<.05). Higher levels of CRP and IL-1ra were associated with a positive history of diabetes and coronary artery disease (p<.05).

Table 5.0: Bivariate Associations between Sleep Disordered Breathing, Inflammation, and Covariates

	CRP	IL-1ra	IL-6	sP-selectin	sICAM-1	TNF-α
Apnea-Hypopnea Index (events/h), M (SD)	*r*=.101	*r*=.087	*r*=-.086	*r*=.135	*r*=.085	*r*=.046
Obstructive Apnea-Hypopnea Index (events/h), M (SD)	*r*=.064	*r*=.089	*r*=-.191*	*r*=.138	*r*=.009	*r*=.076
Central Apnea-Hypopnea Index (events/h), M (SD)	*r*=.255**	*r*=.094	*r*=.017	*r*=.230*	*r*=.184	*r*=.032
Mixed Apnea-Hypopnea, (events/h), M (SD)	*r*=.034	*r*=.063	*r*=-.008	*r*=.100	*r*=.109	*r*=.034
Total time Oxygen Saturation is Below 90% (min), M (SD)	*t*==-.211	*t*=1.97*	*t*=-1.41	*t*=2.62**	*t*=2.73**	*t*=-2.00*
Average Oxygen Saturation % , M (SD)	*r*=-.125	*r*=-.171[§]	*r*=-.034	*r*=-.149	*r*=-.107	*r*=-.012
Lowest Oxygen Saturation%, M (SD)	*r*=-.125	*r*=-.210*	*r*=-.221*	*r*=.059	*r*=-.139	*r*=.057
Age, years, M (SD)	*r*=-.084	*r*=.036	*r*=.141	*r*=-.014	*r*=.001	*r*=.009
Male, n (%)	*t=1.19*	*t=.057*	*t=.109*	*t=1.25*	*t=2.76*	*t=-.880*
Body Mass Index, kg/m^2,M (SD)	*r*=.310**	*r*=.361**	*r*=.-044	*r*=.203*	*r*=.055	*r*=.060
Current Smoker, n (%)	*t=.911*	*t=.364*	*t=-.901*	*t=-1.40*	*t=.487*	*t=.432*
Current Drinker, n (%)	*t=.893*	*t=-1.29*	*t=-.419*	*t=1.93*[§]	*t=.098*	*t=1.34*
Non-Latino Ethnicity, n (%)	*t=-2.08**	*t=-.150*	*t=.495*	*t=.115*	*t=-.958*	*t=.167*
Race, n (%) White Black Other	*F=2.711*[§]	*F=2.658*[§]	*F=2.028*	*F=.675*	*F=3.699**	*F=.798*

Table 5.0: Bivariate Associations between Sleep Disordered Breathing, Inflammation, and Covariates Continued

	CRP	IL-1ra	IL-6	sP-selectin	sICAM-1	TNF-α
Current Antidepressant Use, n (%)	*t=.285*	*t=-2.97***	*t=-1.07*	*t=-.933*	*t=-1.23*	*t=-.141*
B-type Natriuretic Peptide (pg/mL), M (SD)	*r=.040*	*r=-.050*	*r=.225**	*r=-.024*	*r=.063*	*r=.015*
Six minute walk test (meters), M (SD)	*r=-.174*[§]	*r=-.315***	*r=-.008*	*r=-.236**	*r=-.070*	*r=-.216**
History of Diabetes, n (%)	*t=-2.39**	*t=-2.99***	*t=-1.76*[§]	*t=-.171*	*t=-.340*	*t=-1.37*
History Coronary Artery Disease	*t=-2.66***	*t=-2.06**	*t=-.368*	*t=-1.13*	*t=-.329*	*t=-.275*

[§]$p < .10$ *$p < .05$ **$p < .01$

Beck Depression Inventory Scores and Inflammation (Data not shown): BDI scores and the proinflammatory markers were weakly to moderately correlated, despite this there was a significant association between cognitive-affective scores and IL1-ra (r=.233, p<.05), somatic scores and CRP, IL-1ra, sICAM-1 and TNF-α (r= .193,.503, ps<.05) and total BDI scores with CRP, IL-1ra and sICAM-1 (r=.366,-.190 p<.05).

Regressing Beck Depression Inventory Scores on Sleep Disordered Breathing and Disease Status

Significant first-order effects were observed for disease status (p<.05, data not shown), but not for SDB. An average oxygen saturation by disease status interaction effect was observed in relation to somatic scores (p<.01), however this associated was further attenuated after controlling for BMI, ethnicity and six minute walk test (p=ns). HF patients with higher somatic scores also had higher average oxygen saturation

percentages (see Figure 2.0 for unadjusted association). In controls, higher somatic scores related to lower mean percentages of oxygen saturation. The simple slope analyses were only statistically significant for controls ($p<.05$).

A significant lowest oxygen saturation percentage by disease status interaction was also observed for somatic scores ($p<.05$), however this association was attenuated after controlling for BMI, ethnicity and six minutes walk test (p=ns). Similar to the patterns seen in Figure 2.0, somatic BDI scores remained relatively constant regardless of oxygen saturation percentage for HF patients (p=ns), while controls with higher oxygen saturation percentages reported fewer somatic symptoms ($p<.05$; Figure 3.0 shows the unadjusted association).

A lowest oxygen saturation percentage by disease status interaction approached significance for total BDI scores ($p<.10$), and this association became stronger after controlling for BMI ($p<.05$), but was no longer significant after controlling for meters walked during the six minute walk test ($p>.01$). Higher BDI scores related to lower oxygen saturation percentages in non-HF controls, and in HF patients, BDI scores remained relatively constant regardless of oxygen saturation percentage (data not shown). Simple slope analyses were not significant for either group.

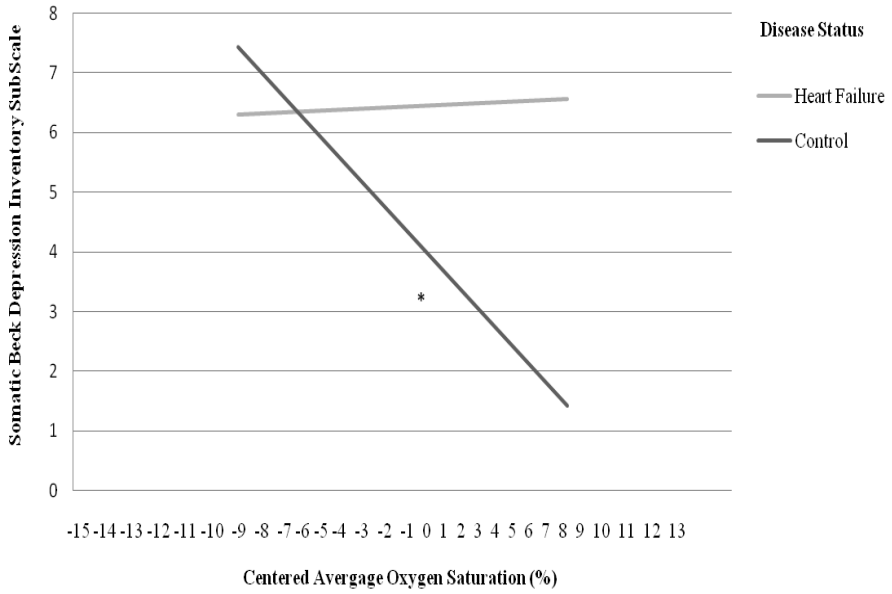

Figure 2.0: Somatic Depression Scores Regressed on Average Oxygen Saturation (%) and Disease Status

*$p \leq .05$

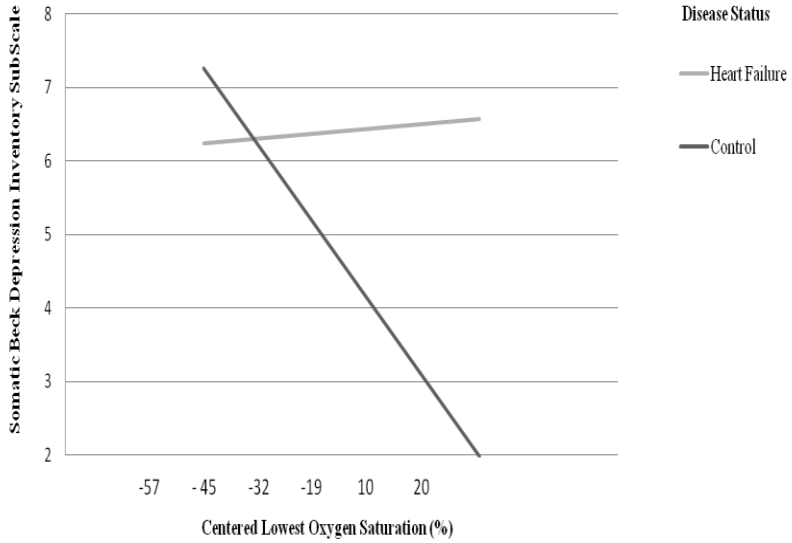

Figure 3.0: Somatic Depression Scores Regressed on Lowest Oxygen Saturation (%) and Disease Status

*$p \leq .05$

Regressing Inflammation on Sleep Disordered Breathing and Disease Status

Significant first-order effects were observed for disease status ($p<.05$, data not shown), but not for SDB variables. The relationship between CRP and markers of sleep apnea varied by disease status. For example, there was a significant interaction effect for OAHI by disease status related to resting levels of CRP, which remained significant after controlling for BMI, ethnicity and history of diabetes and coronary artery disease ($p<.05$; adjusted analyses are shown in Figure 4.0). There was also a significant interaction effect for MAHI by disease status related to resting levels of CRP, which remained significant after controlling for BMI, ethnicity and history of diabetes and coronary artery disease ($p<.05$; adjusted analyses are shown in Figure 5.0). A significant AHI by disease status interaction effect as well as an interaction effect for CAHI by disease status was observed in relation to resting levels of CRP ($p<.05$), however this associated was further attenuated after controlling for BMI, and history of diabetes and coronary artery disease ($p>.01$; data not shown). Simples slopes analyses were not significant for AHI or CAHI.

As depicted in Figures 4.0 and 5.0, higher resting levels of CRP were associated with higher levels of AHIs in for controls. In the HF patients, CRP levels were lower among those with more sleep apnea. Simple slopes analyses were only significant for controls in OAHI and MAHI analyses ($p<.05$).

There was also a significant interaction effect of OAHI and MAHI by disease status with resting levels of sICAM-1, after controlling for race ($p<.05$). Controls with higher OAHI and MAHI also had higher levels of sICAM-1; however, in HF, lower

levels of sICAM-1 were associated with higher OAHI and MAHI (simple slopes analyses were not significant for either group; data not shown).

An interaction effect for lowest percentage of oxygen saturation by disease status was observed for resting levels of IL-6 ($p<.01$), however this association was attenuated after controlling for covariates. In controls, IL-6 levels were lower among those with a lower percentage of oxygen saturation, whereas in HF patients, those with higher IL-6 levels also had lower percentages of oxygen saturation. Simple slopes analyses were not significant for either group, data not shown.

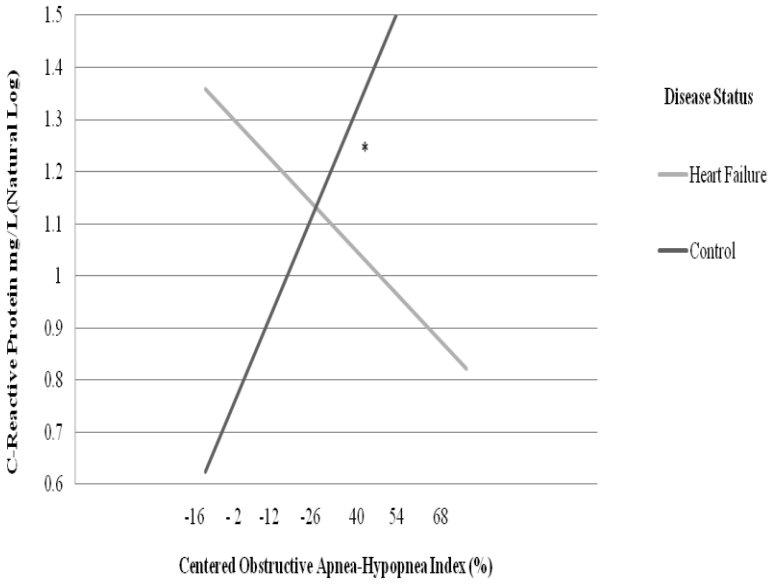

Figure 4.0: Mean C-Reactive Protein Levels Regressed on Obstructive Apnea-Hypopnea Index and Disease Status

*$p \leq .05$

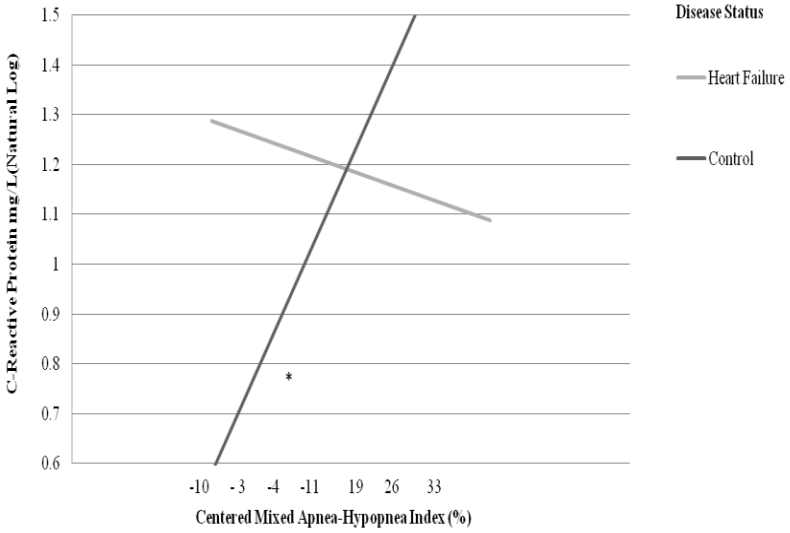

Figure 5.0: Mean C-Reactive Protein Levels on Mixed Apnea-Hypopnea Index and Disease Status

*$p<.05$

Secondary Aims

AIM 3: To assess if circulating levels of proinflammatory and cellular adhesion molecules mediate the relationship between SDB and all-cause mortality and/or hospitalization in HF patients.

Hypothesis 3.1: Circulating levels of proinflammatory and cellular adhesion molecules will mediate the association between frequency of apneic events and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 3.2: Circulating levels of proinflammatory and cellular adhesion molecules will mediate the association between measures of hypoxia and all-cause mortality and number of hospital admissions in HF patients.

AIM 4: To assess if depressive symptoms mediate the relationship between SDB and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 4.1: Depressive symptoms will mediate the relationship between the frequency of apneic events and all-cause mortality and number of hospital admissions in HF patients.

Hypothesis 4.2: Depressive symptoms will mediate the relationship between oxygen desaturation and all-cause mortality and number of hospital admissions in HF patients.

Analytical Plan for Secondary Aims

Follow-up data were only collected for HF patients. The mediation analyses were limited to 66 participants. Model assumptions were assessed graphically and analytically. Suspected outliers were assessed for biological plausibility. Biologically plausible values for cases were winsorized, replacing these values with a value that was three standard

deviations away from the mean. This was conducted for number of hospital admissions, AHI, OAHI, CAHI, average oxygen saturation (%) and lowest oxygen saturation (%). For variables where overdispersion (a high number of zero values) was present and winsorizing extreme values could not adequately transform the distribution, a median split was performed. Total minutes oxygen saturation fell below 90%, was dichotomized (≤36 minutes was coded as '0' and >36 minutes were coded as '1'). All inflammatory markers were positively skewed and were normalized via natural-log transform.. Bivariate associations between the predictors and outcomes were assessed using correlations (Pearson's *r*), t-tests, chi-square and ANOVA as appropriate. In order to assess the paths between the antecedents (SDB), mediators (depression and inflammation) and outcomes (mortality and number of hospital admissions), regression coefficients were standardized prior to estimating mediated effects (96). Firstly, the direct effects between the antecedent (SDB) and the outcome were tested using ordinary least squares multiple regression for number of hospital admissions, and multiple logistic regression for mortality (coded as '0' alive, and '1' dead), while controlling for the mediator (depression or inflammation) and length of follow-up time. Secondly, the a′ (the path between the antecedent and mediator) was tested using ordinary least squares regression. Thirdly, the b′ was tested to assess relationships between the mediators and outcomes, while controlling for the antecedent and length of follow-up time. For mediated effects, simulation studies have shown that standard errors and confidence limits based on a normal distribution are often inaccurate [38]. MacKinnon's asymmetrical confidence intervals were calculated to determine if the mediation effects was statistically significant (97). A mediated effect was supported if the 95% confidence

interval did not contain zero. Due to the small sample size, the relationship with each outcome variable was tested with one antecedent and one mediator per mediation model. Covariates were only entered into the model if a mediated effect was present and if they had a significant bivariate relationship with either the mediator or the outcome ($p<.05$). Regression analyses were performed in MPLUS (version 6).

Results for Secondary Aims

Sample Characteristics (see Table 6.0)

HF participants were followed on average for three years. Over which time each HF patient experienced an average of 3 (*SD*=3.9) hospital admissions and 18% died. Participants were mostly non-Latinos (80.3%), Caucasian (62.1%) and male (74.2%). On average, participants were middle-aged 55.37 (*SD*=13.4), and obese (*M* BMI kg/m^2=31.23, *SD*=6.8). Only 13.2% reported that they currently smoked tobacco products, however more 40.9% reported that they drank alcohol. Although 12% of participants screened positive for major depressive disorder (data not shown), only 9% reported taking antidepressants. With regard to BDI scores, on average, patients were experiencing mild-moderate depressive symptoms [*M* BDI=10.05 (SD=7.21)]. With regard to types of HF, 89.4% had systolic dysfunction, and 74.2% had diastolic dysfunction, and more than half of participants had an HF etiology that was classified as nonischemic cardiomyopathy (54.5%).

With regard to SDB, 4.5% had a prior diagnosis, and/or were currently being for sleep apnea via CPAP at baseline. Frequency and severity of SBD was previously reported in findings of primary aims.

Table 6.0: Baseline Characteristics of Heart Failure Patients and Bivariate Associations with Mortality and Hospital Admissions

	M (SD) or n (%)	**Mortality**	**Hospital Admissions**
Age, years, M (SD)	55.37 (13.4)	*t*=-1.68§	*r*=.041
Male, n (%)	49 (74.2%)	X^2=.440	*t*=-.1.07
Body Mass Index, kg/m^2,M (SD)	31.23 (6.8)	*t*=.093	*r =-.116*
Current Smoker, n (%)	9 (13.2%)	X^2=.294	*t*=2.58**
Current Drinker, n (%)	27 (40.9%)	X^2=1.84	*t*=.012
Non-Latino Ethnicity, n (%)	53 (80.3%)	X^2=.085	*t*=-.061
Race, n (%)		X^2=2.87	*F*=2.17
White	41 (62.1%)		
Black	20 (30.3%)		
Other	5 (7.6%)		
Current Antidepressant Use, n (%)	6 (9.1%)	X^2=1.02	t=-.744
Sleep Disordered Breathing Diagnosis, n (%)	3 (4.5%)	X^2=4.96*	t=.601
Heart Failure Etiology		X^2=.080	t=-.488
Systolic Dysfunction, n (%)	59 (89.4%)		
Diastolic Dysfunction, n (%)	49 (74.2%)	X^2=1.73	t=-.845
Left ventricular ejection fraction, (%), M (SD)	30.3 (12.5)	*t*=.766	*r*=-.271*
B-type Natriuretic Peptide (pg/mL), M (SD)	222.74 (262.1)	*t=-3.88***	*r*=.241§
Six minute walk test (meters), M (SD)	370.3 (79.1)	*t*=1.16	*r*=.045

Table 6.0: Baseline Characteristics of Heart Failure Patients and Bivariate Associations with Mortality and Hospital Admissions Continued

	M (SD) or n (%)	**Mortality**	**Hospital Admissions**
HF Etiology		X^2=.981	*t*=.576
Ischemic cardiomyopathy,n (%)	30 (45.5%)		
Nonischemic cardiomyopathy, n (%)	36 (54.5%)		
History of Hypertension,n (%)	18 (27.3%)	X^2=3.82**	*t*=.124
History of Diabetes, n (%)	13(19.7%)	X^2=1.72	*t*=.327
History Coronary Artery Disease, n (%)	22 (33.3%)	X^2=.458	*t*=1.90§
Medications and Devices, n (%)			
Diuretics	60 (90.9%)	X^2=1.47	*t* =-1.28
Beta-blockers	62 (93.9%)	X^2=2.89§	*t* =.542
Angiotensin Converting Enzyme Inhibitors	51 (77.3%)	X^2=6.21**	*t* =.908
Aldosterone Inhibitors	26 (39.4%)	X^2=.691	*t* =-1.16
Angiotensin II Receptor Blockers	11 (16.7%)	X^2=2.93§	*t* =-1.97
Digoxin,	35 (53.0%)	X^2=2.84§	*t* =-1.87§
Statins	41 (62.1%)	X^2=1.03	*t* =.439
Implantable Cardioverter Defibrillator	8 (12.1%)	X^2=.198	*t* =-1.04
Outcomes			
Follow-up time (days), M (SD)[a]	1102.71 (695.3)	*t=4.04***	*r*=.267*
Deceased, n (%)	12 (18.2%)	-------	--------
Number of Hospital Admissions, M (SD)[a]	2.56 (3.9)	*t*=-1.85§	---------

Table 6.0: Baseline Characteristics of Heart Failure Patients and Bivariate Associations with Mortality and Hospital Admissions Continued

	M (SD) or n (%)	Mortality	Hospital Admissions
Apnea-Hypopnea Index (events/h), M (SD)[a]	32.01 (28.98)	*t*=*-.149*	*r*=.021
Obstructive Apnea-Hypopnea Index (events/h), M (SD) [a]	20.53 (21.38)	*t*=4.52**	*r*=-.125
Central Apnea-Hypopnea Index (events/h), M (SD)[a]	18.60 (19.09)	*t*=-.685	*r*=-.010
Mixed Apnea-Hypopnea, (events/h), M (SD)	13.46 (10.66)	*t*=.976	*r*=.004
Total time Oxygen Saturation is Below 90% (min), M (SD)[b]	186.52 (199.32)	X^2=6.51**	*t=-1.62*
Average Oxygen Saturation, % , M (SD)[a]	93.43 (2.71)	*t*=-.500	*r*=-.227[§]
Lowest Oxygen Saturation%, M (SD) [a]	72.97 (21.84)	*t*=1.61	*r*=-.204
Total Sleep Time (hours)	5.34 (1.49)	*t*=1.69[§]	*r*=-.016
Mediators			
Cognitive-Affective Score, M (SD), range: 0-39	4.55 (4.46)	*t*=.537	*r*=.292*
Somatic Score, M (SD), range:0-21	6.48 (3.49)	*t*=.156	*r*=.237[§]
Total BDI score, M (SD), range:0-63	10.05 (7.21)	*t*=.408	*r*=.296*
C-Reactive Protein, mg/L, M (SD)[c]	3.42 (3.54)	*t*=-.312	*r*=-.028
Interleukin-1ra, pg/mL, M (SD)[c]	579.61 (518.39)	*t*=-.205	*r*=.022
Interleukin-6, pg/mL, M (SD) [c]	5.70 (4.75)	*t*=-2.76**	*r*=.073

Table 6.0: Baseline Characteristics of Heart Failure Patients and Bivariate Associations with Mortality and Hospital Admissions Continued

	M (SD) or n (%)	Mortality	Hospital Admissions
Soluble P-selectin*ng/mL, M (SD) [c]	82.20 (47.88)	t=1.87[§]	r=-.229[§]
Soluble Intercellular Adhension Molecule-1* ng/mL, M (SD) [c]	290.14 (129.14)	t=.067	r=.125
Tumor Necrosis Factor-α, pg/mL, M (SD)[c]	2.31 (1.89)	t=.716	r=.090

Note: In cases where the variable was transformed , the M (SD) of the untransformed variable are presented and transformed version of the variable was used for to test bivariate associations; [a]winsorized extreme cases; [b] median split was performed; [c]natural log; [§]p< .10 *p<.05 **p<.01

Bivariate Associations for Mortality and Hospital Admissions (see Table 6.0)

On average HF patients who died during the follow-up period had a higher number of hospital admissions (M =3.77, SD =1.09) compared to those who were alive at the completion of the study (M=2.07, SD=.40, p<.05; M (SD) not shown). Shorter follow-up was associated with mortality (p<.01), and longer follow-up time was associated with more hospital admission (p<.05). Mortality was significantly associated with a positive diagnosis of, and/or treatment for sleep apnea (p<.05) as well as with a positive history of hypertension (among the 12 patients with hypertension, six died compared to the 42 patients without hypertension and six died; p<.01). Among those patients taking Angiotensin Converting Enzyme (ACE) inhibitors (n=45) only 6 died, however among the nine patients not taking ACE inhibitors, six died (p<.01). Higher BNP values were associated with a higher percentage of mortality (p<.01). Higher mean OAHI was associated with a lower percentage of mortality (e.g., those who lived longer had fewer OAHIs; p<.01). Greater minutes spent at an oxygen saturation less than 90% was

associated with higher percentage of mortality ($p<.01$). Higher mean levels of sP-selectin were associated with greater percentage of mortality ($p<.01$). Greater cognitive-affective, and total (somatic and cognitive-affective) BDI scores were associated with a higher number of hospital admissions ($p<.05$).

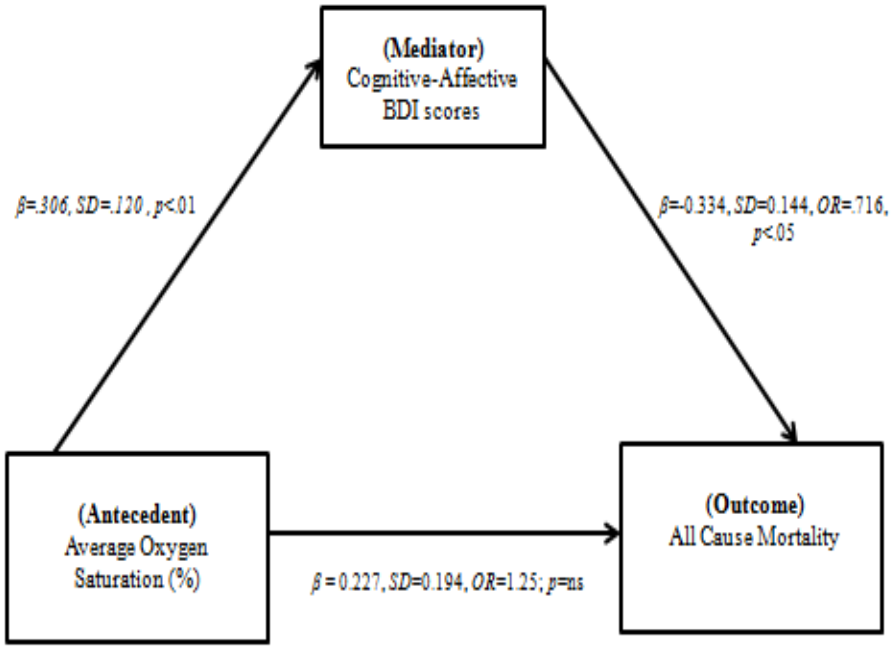

Figure 6.0: Mediated Effects of Cognitive-Affective BDI Scores on Average Oxygen Saturation and All-Cause Mortality

Mediation Analyses

A series of regression models tested the relations between SDB, BDI scores, inflammation and clinical outcomes in HF patients. The target model specified indirect relations from SDB to mortality and hospital admissions via depression and inflammation mediating variables, respectively. Given the high number of analyses performed (126), only mediation analyses with significant mediated effects are described in detail below. Please see Appendix A for tables detailing mediation analyses with non-significant mediated effects.

A mediation model was performed to explore the associations between average oxygen saturation (%), cognitive-affective BDI scores and mortality (see Figure 6.0). The target model specified indirect relations from average oxygen saturation (%) to mortality via the mediating variable of cognitive-affective BDI scores. With respect to the relations specified within the model, the direct effect from average oxygen saturation (%) to mortality was not statistically significant (β = 0.227, *SD*=0.194, *OR=1.25*; *p*=ns). The first indirect path between lowest oxygen saturation and cognitive-affective scores was statistically significant (β=-.*306*, *SD=.120 p*<.01). Those with greater average saturation (%) had higher cognitive-affective scores. The second indirect path between cognitive-affective scores and mortality, after controlling for the effects of average oxygen saturation and length of follow-up time was statistically significant (β=-0.334, *SD*=0.144, *OR*=.716, *p*<.05). Lower cognitive-affective scores were associated with increased log odds of mortality. The compound (ab) path was statistically significant, suggesting that cognitive-affective scores mediate the association between average oxygen saturation and

mortality. However these associations were no longer significant after adjusting for covariates.

Tertiary Aim

AIM 5: To assess if depressive symptoms moderate the path between SDB and inflammation, and the path between inflammation and all-cause mortality and number of hospital admissions, respectively, in HF patients.

Hypothesis 5.1: Depressive symptoms will moderate the mediated effects of inflammation on the association between SDB and all-cause mortality and the number of hospital admissions, respectively, in HF patients.

Analytical Plan for Tertiary Aim

The tertiary aim builds upon the mediation analyses performed for the secondary aims. Regression analyses were performed to test if the antecedent to mediator was moderated by BDI scores, and if the mediator to outcome was moderated by BDI scores (e.g., moderated-mediation of a′ and b′). All continuous variables were centered prior to entering the models, and the interactions terms were created from the centered variables. Interaction terms were created for the antecedent (SDB) and moderator (BDI scores) to assess the moderated effects of depressive symptoms on the association between the antecedent (SDB) and the mediator (inflammation). Another interaction term was created to assess the moderated effects of BDI scores on b′ by multiplying the mediator (inflammation variable) by the moderator (BDI scores). Multiple ordinary least squares and logistic regression analyses were performed as previously stated in the analytical plan for the secondary study aims, with the addition of the second-order terms. The moderated effect for a′ was tested by entering the antecedent (SDB variables), mediator

(inflammation variable), moderator (BDI score) and the interaction term for the antecedent by moderator (SDB*BDI score). b′ was tested by entering the mediator, moderator and their interaction term (inflammation* BDI), while controlling for the antecedent, the interaction of antecedent by moderator and length of follow up time. Regression coefficients were standardized prior to estimating mediated effects (96). Moderated-mediated effects were assessed by calculating MacKinnon's asymmetrical confidence intervals for the βs and standard deviations of the two interaction terms. A moderated-mediated effect was supported if the 95% confidence interval did not contain zero (98). Covariates were only entered into the model if a mediated effect was present, and if they had a statistically significant bivariate relationship with either the mediator or the outcome (p<.05). Models testing associations of total minutes oxygen saturation fell below 90%, total BDI scores and mortality were not performed due to a correlation of r=1.0 between total minutes oxygen saturation fell below 90% and total BDI scores. Regression analyses were performed in MPLUS (version 6).

Results for Tertiary Aim

Of the models tested, there was a statistically significant moderated-mediated effect for cognitive-affective BDI scores on the mediated effects of CRP on total time oxygen saturation fell below 90% and mortality as seen in Figure 7.0 (a′ by z: β=-.544, SD=.254, p<.05; and b′ by z: β=-.986, SD=.017, OR=.373, p<.01).

For a′, among those with lower cognitive-affective BDI scores, denoted as – 1 SD below the mean, higher resting levels of CRP corresponded with more minutes that oxygen saturation was below 90%. However, among those with higher cognitive-affective scores, denoted as + 1 SD above the mean, lower resting levels of CRP

corresponded with more minutes oxygen saturation was lower than 90%. Simple slopes analyses were statistically significant for both high and low cognitive-affective score groups. The association was no longer statistically significant after controlling for BMI, ethnicity and history of diabetes and coronary artery disease ($p<.10$).

In those with lower cognitive-affective scores, denoted as -1 SD, higher resting CRP levels corresponded with greater odds of mortality (Figure 9.0). However, among those with higher cognitive-affective scores, denoted as + 1 SD, higher resting mean levels of CRP corresponded to fewer deaths. Simple slopes analyses were not significant either regression line, +1 SD and -1 SD, and the overall model was no longer statistically significant after covarying for age, diagnosis of sleep disorder breathing, BNP, and Angiotensin Converting Enzyme (ACE) inhibitors. Although there was a statistically significant interaction effect for CRP and cognitive-affective scores, this mostly likely is not clinically significant as higher levels of CRP appear to have a protective effect for both those with high and low cognitive affective scores (as seen from the negative log odds for mortality, depicted in Figure 8.0).

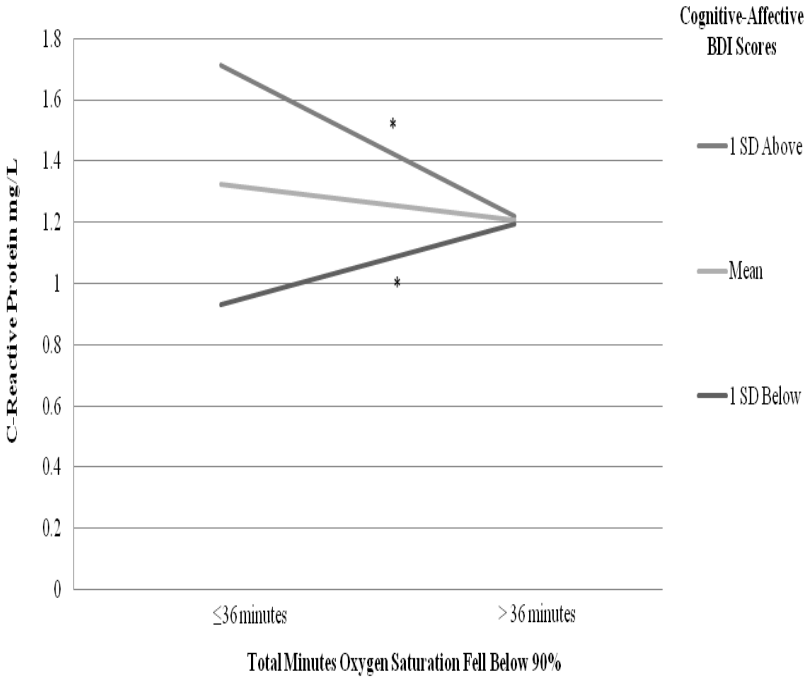

Figure 7.0: Moderated Effects of Cognitive-Affective Beck Depression Inventory Scores on Total Minutes Oxygen Saturation Fell Below 90% and C-Reactive Protein Levels

*$p<.05$

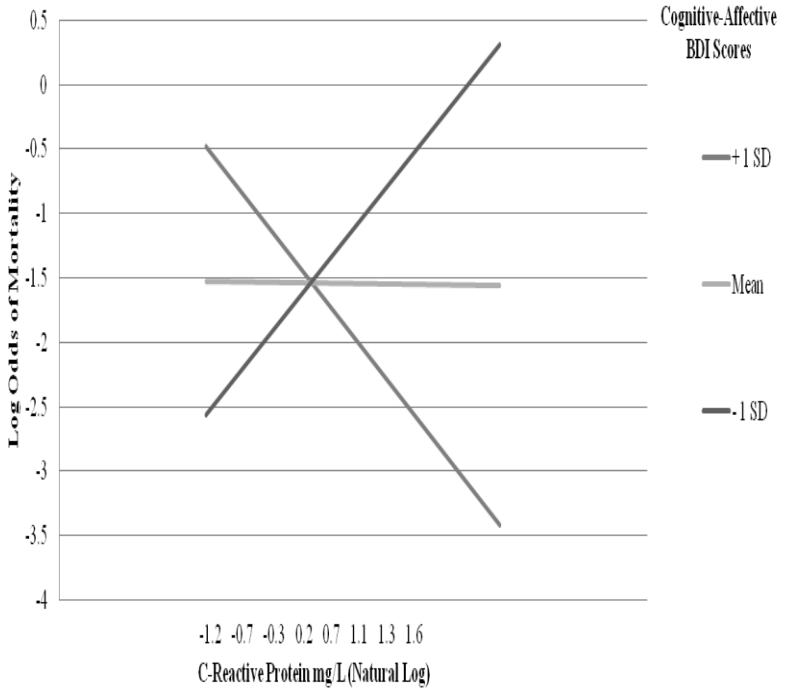

Figure 8.0: Moderated Effects of Cognitive-Affective Beck Depression Inventory Scores on C-Reactive Protein Levels and Mortality

*$p<.05$

Chapter 4: Discussion

Although the association between SDB and adverse cardiovascular events is well-known, the biological and psychological mechanisms underlying this association have remained relatively unknown (1). Most of the literature detailing the mechanistic pathways of this association has been conceptual (1, 99), and has not been subjected to empirical tests of mediation. Thus the current study adds to the existing literature by testing these conceptual models, and establishing if there is an empirical basis for two of the hypothesized pathways linking SDB to morbidity and mortality outcomes in HF patients. A detailed discussion of the findings for each aim is found herein.

Primary Aims

Overview Findings for the Primary Aim

The focus of the primary aims was to establish if the association between SDB and the proposed mediators, depressive symptoms and inflammation, varied by disease status. The original hypotheses stated that the association between SDB, depression and inflammation, respectively, would be similar for HF patients and controls (e.g., worse SDB would relate to higher depressive symptomatology and inflammatory load). However, the magnitude of the effects were expected to be greater in HF patients. It was hypothesized that a physiological stressor, such as sleep apnea and related hypoxia, would further exacerbate the already high levels of depression (23) and inflammation (100) that are characteristic to HF syndrome. However, this was not the case. HF patients' BDI scores and inflammatory markers either remained constant or slightly decreased in response to SDB stressors. Whereas, in general, controls showed the expected relationships: greater SDB related to greater depressive symptomatology and

heightened resting levels of inflammatory markers. Below is a more detailed discussion of the findings for depressive symptomatology and inflammatory outcomes, respectively.

Sleep Disordered Breathing, Disease Status and Inflammation

As anticipated, we found that HF patients had worse SDB profiles (e.g., greater sleep apnea and hypoxia) compared to controls. Although it has been well-established that SDB, especially obstructive sleep apnea (OSA), is associated with cardiovascular disease (CVD) risk and progression (101), tests of the first-order effects showed that disease status, but not SDB predicted inflammation levels. However, several sleep apnea by disease status interaction effects were found for CRP. Controls evidenced the expected relationship, where higher resting levels of CRP were associated with higher sleep apnea indices (OAHI, MAHI). Lower resting levels of CRP related to higher indices of sleep apnea in HF patients. However, the simple slopes analyses were only statistically significant for controls. Other SDB by disease effects were found for sICAM-1 and IL-6, however the simple slopes analyses were not significant for either group.

CRP is arguably the most widely-researched cytokine with relation to CVD, relative to other commonly studied markers (102). CRP is an acute-phase protein produced mainly by the liver, but also by adipocytes and vascular smooth muscle cells in response to rises in IL-6 and TNF-α (102-103). CRP levels increase 6 hours after an acute stimulus and peak within 48 hours (104). Although CRP levels fall to baseline within one to two days, its half-life is long, approximately 19 hours (105). One of the potential explanations for why CRP has been widely associated with CVD outcomes is its long half life. Cytokines with longer half lives remain stable long enough to make random measurements clinically relevant (106). CRP has multiple proinflammatory and

proatherogenic properties. It is thought that CRP influences cardiovascular prognosis by contributing to atherosclerotic processes (102), in part, by binding to low and very low-density lipoprotein as well as platelet activation factors (107).

Upregulation of proinflammatory cytokines, such as CRP, are proposed mechanisms underlying the association between sleep apnea and worse cardiac functioning (1, 99). Most of the literature testing the path between SDB and proinflammatory responses has been conducted in healthy or OSA patients, who are otherwise healthy. For example, the independent influence of OSA on data generally healthy adolescents showed a progressive increase in CRP levels with increases in AHI, suggesting that in the absence of significant comorbidities even modest levels of OSA may result in elevations in inflammatory markers (108). Guven et al. (109) found that elevated CRP levels were associated with AHI, independent of obesity, in newly diagnosed OSA patients. Another study found that elevations in soluble IL-6 receptors varied with OSA severity (110). In the current study, the results for controls were in-line with the state of literature.

However, research on the effects SDB on inflammatory responses in HF patients is lacking. The current study found the HF patients exhibited a blunted inflammatory response when exposed to SDB stressors. Findings from literature in psychoneuroimmunology may help to explain the patterns seen in HF patients. Under conditions of chronic stress, biological pathways become habituated to the influence of stress hormones and inflammatory cytokines, resulting in a loss of negative feedback loops (111). Baseline inflammation develops, as indicated by increases in CRP, IL-1, IL-6 and TNF-α (111-112) and overtime a dysregulation in the overall inflammatory

response occurs. During stress, cortisol receptors on immune cells are activated, triggering an acute-phase inflammatory response characterized by increases in serum levels of proinflammatory cytokines (113-114). This upregulation of inflammatory products is maintained over time, and negative feedback loops are damaged (115-116). Thus, the effect appears like a 'blunted' response or' ceiling effect', where proinflammatory cytokines show limited responsivity to stressors (117). Thus damage to negative feedback loops and dysregulation of the overall inflammatory response may partially explain the patterns seen in HF patients.

Sleep Disordered Breathing, Disease Status and Depressive Symptomatology

In the present study, somatic and total depressive symptoms were associated with hypoxia [e.g., average oxygen saturation (%) and lowest oxygen saturation (%)]. Similar to the patterns observed for CRP and sleep apnea, higher somatic scores were associated with lower mean levels of oxygen saturation in controls. In HF patients, depressive symtomatology remained unchanged or slightly increased with higher percentages of oxygen saturation. The simple slopes analyses were statistically significant for controls in models where somatic depression was the outcome. The results suggest that SDB is not an influential predictor of depressive symptomatology in HF patients, but may predict low mood in healthier populations.

SDB, most notably OSA, and psychiatric disorders, especially depression, have been studied for decades in non-CVD patients (118). These studies show that high rates of depressive symptoms are evident in the presence of OSA (29, 119). With regard to hypoxia, Deldin and colleagues reported significant increases of oxygen desaturations during sleep in a group of 19 patients with MDD, compared to controls (120). The

authors postulated that respiratory-related sleep fragmentation may induce prefrontal dysfunction, thus predisposing individuals with sleep-related hypoxia to mood disorders (120). Studies have found significant reductions in BDI scores in OSA patients treated with continuous positive airway pressure (CPAP) (121-122). A placebo-controlled study assessing improvement in psychological symptoms following CPAP demonstrated that psychological symptoms improved with both CPAP and with oxygen therapy versus a 'mock' therapy (123). The pathophysiology linking SDB, particularly hypoxia and depression, remains unclear (118), yet there is evidence to suggest that SDB and depressive symptoms are associated with OSA in relatively healthy populations.

Secondary Aims

The focus of the secondary aims and hypotheses was to perform formal tests of mediation on the association between SDB and morbidity and mortality, while accounting for the mediated effects of depressive symptomatology and inflammation, respectively. A mediator is defined as an intermediate in the casual process between the antecedent and outcome (124). Although other variables may explain the association between the antecedent and outcome, these other variables are treated as confounders; whereas a formal test of mediation models a causal process between variables, as opposed to treating the mediator as a statistical control. A common misunderstanding of mediation analyses is that there must be a statistically significant relationship between the antecedent and outcome in order for mediation to exist (124). However, this is not the case. There may not be a statistically significant association between the antecedent and outcome because the mediated effect has more statistical power than the overall

association of the antecedent on the outcome (124). It is for this reason that mediated effects were interpreted even in the absence of a direct effect.

To the author's knowledge, this is the first time that pathophysiological and psychological mechanisms of SDB on clinical outcomes in a HF sample have been subjected to formal tests of mediation. Among the 126 mediation models tested, only one was statistically significant, however these results were no longer significant after controlling for covariates. The findings from the mediation model ran contrary to the hypothesized mediated effects of depression on SDB and the outcomes. The results suggest that there is a mediated effect for cognitive-affective depressive symptomatology on the association between hypoxia and mortality in HF patients. No statistically significant direct effects were observed. The individual and compound paths were statistically significant. Those with greater average saturation (%) had higher cognitive-affective scores. Those with greater cognitive-affective depressive symptomatology had lesser odds of dying. It is the author's opinion that these results should be interpreted with caution considering the following: 1) only one mediation model out of 126 performed was statistically significant; 2) the findings were no longer significant after covarying for standard statistical controls; and 3) the direction of findings was contrary to the literature on SDB, depression and cardiovascular health.

Tests for mediation used for the current study were the most appropriate given the small sample size. Statistical tests for multiple mediators may reduce Type I error, but they require more power (125). Thus, it is possible that the aforementioned effect is a product of Type I error, falsely rejecting a true null hypothesis. Type I error is inherent in mediation analyses. However, product coefficient methods, such as the one used for the

current analyses, have more accurate estimates of Type I error and greater statistical power, relative to other tests of mediation (e.g., Baron and Kenny's methods and SOBEL tests) (97, 125).

It is well documented that HF patients have a high prevalence of depression, and that depressed HF patients suffer worse clinical outcomes than their non-depressed counterparts, including greater functional decline (53-54), increased hospitalization (55-56), poorer quality of life (57-58), and increased mortality (56, 59). However, the precise mechanism by which depression influences HF outcomes remains unclear. Depression may contribute to poor HF prognosis, in part, via lack of adherence to medications and other self-care behaviors (22). HF is a complex syndrome, which requires polypharmacy and sometimes includes behavioral therapies, such as exercise regimens and dietary restrictions. Depression and HF also appear to share a similar biological pathogenesis, which complicates scientific understanding of the etiology of co-morbid depression in HF (100). Thus, there was strong scientific rational for testing for mediated effects of depressive symptoms. Overall the models produced null or weak results, suggesting that depressive symptoms may not mediate the association between SDB and morbidity and mortality outcomes in HF.

There are several potential explanations for why mediated effects for inflammation and depressive symptomatology were not found in the current study. Perhaps the most poignant are proximal and distal mediation and power. Formal mediation analyses are hinged on the premise that there is specific temporal order between the variables under examination. The effect of the antecedent on the outcome is transmitted through a third intervening variable, a mediator. That is the antecedent causes

the mediator, and the mediator causes the outcome. For example, I hypothesized that increased sleep apnea and related-hypoxia would trigger upregulation of inflammatory products, and overtime damage negative feedback loops causing chronic elevation of proinflammatory cytokines and cellular adhesion molecules, which in turn would exacerbate HF. However, the model itself does not comprehensively test all the biological, psychological or behavioral mediators at each step in the hypothesized 'causal' pathway between SDB and mortality in HF patients.

Mediation effects may not have been found in the current study because of proximal mediation, which is when a mediator is 'causally nearer' to the antecedent than the outcome (126). From a biological perspective, systematic inflammation is a catalyst of several, adverse downstream processes such as endothelial dysfunction and atherosclerosis (1). These processes are also proposed mechanisms in the underlying association between SDB and cardiovascular decline, and may be more distal mediators of this association. A distal mediator is closer to the outcome in the causal chain or temporal order. This logic can also extend to the models testing the mediated effects of depressive symptomatology, which are thought to impact HF outcomes, in part, via behavioral mechanisms. Perhaps lack of adherence to medication is a more distal mediator to mortality and hospital admissions then the depressive symptoms themselves.

Similarly related to proximal and distal mediation, the lack of mediation effects may have occurred due to measurement. For example, the measurement of sleep apnea and hypoxia took place only a few hours before blood was drawn and the BDI was adminstered. Thus, the measurement of the antecedent and mediator were proximal, relative to the outcome which was collected from patient's electronic medical records.

Ideally, the mediators should have been collected at multiple time points over the follow-up period, or at a time point further from baseline to more accurately capture mediated effects.

Although the deleterious effects of OSA on cardiovascular outcomes have been well established for decades, the underlying pathophysiological mechanisms linking sleep apnea to worse cardiac prognosis have only begun to unfold (1). Several factors, other than inflammation and depression, have been implicated as possible mediators of this complex association. However, it is unclear which biological system or process plays the most central role. Dysregulation of systematic nervous activity (SNA) is one of the most widely discussed mechanisms linking SDB and worse HF prognosis. Intermittent hypoxia and carbon dioxide retention stimulate central and peripheral chemoreceptors, which augment SNA (32). Reductions in stroke volume and blood pressure during sleep apnea events unload carotid sinus baroreceptors and reflexively augment SNA, and this response is exaggerated in HF patients (127). Elevated SNA has been associated with increased mortality in HF patients (128). Chronically elevated levels of SNA may lead to desensitization of cardiac β-adrenoreceptors, myocyte injury and necrosis, and hypertension (129). Evidence suggests that long-term treatment with CPAP results in a significant improvement of autonomic indices in OSA patients (130). Thus, it is also possible that inflammation and depression are not central mediators in this complex chain of associations. Other processes, such dysregulation SNA, may have stronger mediated effects on SDB and CVD outcomes.

Large sample sizes are often needed to detect mediated effects. Fritz and McKinnon (125) conducted a series of simulation studies to empirically compute samples

sizes needed for different tests of mediation at .8 power. For each simulation, the point estimates for a′, b′ and Y′ were varied to reflect small, medium, and large effect sizes (131-132). All possible combinations of effect sizes for a′, b′ and Y′ (e.g., small-small-small, small-small-medium) were investigated. They found that for product coefficients tests using PRODCLIN, which were used in the current study, that in order to detect significant differences at .8 power in a sample n=63, medium to large effects for both the a′ and b′ are needed. To detect small effect sizes for a′ and b′ at .8 power, a sample of 539 is needed. Still PRODCLIN was more powerful than traditional tests of mediated effects. For example, Baron and Kenny's test (133) would require a sample size of 20,886 to detect small effects for the a′ and b′ at.8 power .Although the simulation studies reinforced that PRODCLIN was the most appropriate method for testing mediated effects in the current study, the small sample size may be the main reason that mediated effects were not found.

Tertiary Aim

The tertiary aim built upon the hypotheses and findings from the secondary aims. The focus of tertiary aim was to statistically test if depressive symptoms moderated the mediating effects of inflammation on SDB and morbidity and mortality, respectively, in HF patients. Although there was no direct effect, there was a statistically significant moderated-mediated effect for cognitive-affective BDI scores on the mediating effects of CRP on total minutes oxygen saturation fell below 90% and mortality. The first indirect effect showed that among those with lower cognitive-affective depression scores, higher resting CRP levels corresponded with more minutes oxygen saturation was below 90%. However, among those with higher cognitive-affective scores, lower resting CRP levels

corresponded with more minutes oxygen saturation was lower than 90%. With regard to the second indirect effect, among those with lower cognitive-affective scores, higher resting CRP levels corresponded with greater percentage of mortality. However, among those with higher cognitive-affective scores, higher resting mean levels of CRP corresponded to fewer deaths.

A common misunderstanding of moderated-mediated analysis is that a statistically significant mediated effect must exist in order to assess moderated-mediation. In mediation analysis, the mediated effects are averaged across the sample. In the case of cognitive-affective depressive scores, if those with high and low BDI scores have opposing effects, these may cancel each other out when averaged, producing a null mediated effect.

Interestingly the pattern for the moderated-mediated analyses is similar to those seen for the primary aim, assessing if the association between SDB and depression and inflammation, respectively, varied in HF patients versus controls. Perhaps one of the reasons that those with higher cognitive-affective depression did not experience the expected association was due dysregulation of inflammatory responses, producing a 'ceiling effect' or a blunted response. Physiological explanations for these responses were previously explained in the discussion of the findings for the primary aims (p. 56)

It is well documented that HF patients experience disproportionately high rates of depression compared to the general population, with a point prevalence of 21.5% (23) compared to 6.6% in the general population (52). However, the etiology of depression in HF remains unclear. Some scholars have postulated that the 'true' prevalence of depression in HF has been overestimated due overlapping symptoms experienced in HF

and depression, respectively (100). Screening for depressive symptoms includes both cognitive-affective and somatic symptoms. Depressive symptomatology, such as fatigue, loss of energy, problems concentrating, weight loss or gain, and sleep disturbance may be the result of underlying cardiac dysfunction as opposed to psychiatric illness (100). More recent studies have recognized this overlap, and now it is favored to report somatic and cognitive-affective symptomatology separately. Therefore it is interesting that moderated-mediated effect was found with cognitive-affective symptoms, and not somatic depressive symptoms. This provides further evidence for the hypothesis that HF and depression share a similar neurohormonal and inflammatory pathogenesis. HF and depression are both accompanied by high neurohormonal activation and increased inflammation. In particular, depression is characterized by overstimulation of the hypothalamic-pituitary-adrenal (HPA) axis, and HF is characterized by increased norepinephrine, renin–angiotensin–aldosterone, arginine vasopressin and endothelin-1 (100). Several proinflammatory cytokines (e.g., TNF-α, IL-1 and IL- 6) are elevated in both HF and depression. Cytokines may also act on the HPA axis stimulating responses such as elevated blood pressure (134).

Limitations

The main strength of the current study is that it tested the mediated effects of pathophysiological and psychological mechanisms linking SDB to morbidity and mortality outcomes in HF. Until now only the individual paths linking SDB and inflammation/depression, and inflammation/depression to clinical endpoints have been tested. Thus, the mediated effects of inflammation and depression have been hypothsized, but not subjected to empirical tests of mediation. In the current study, the mediated

effects were weak or null, suggesting that inflammation and depression, respectively, are not statistically significant mediators of the association between SDB and mortality and hospital admissions in HF patients. As discussed for the findings under the secondary aims, the current study was under-powered to find mediated effects (125). Inherently mediation analyses are vulnerable to Type I error because the analyses require estimating multiple associations in the causal pathway between the antecedent and the outcome. The current study tried to minimize the effect of Type I error by using PROCLIN (97). Still 378 unadjusted, regression analyses were performed due to the large number of predictors (7) and mediators (9) being assessed. As previously discussed, proximal and distal mediation effects related to the time points that data was collected may also account for the null mediated effects.

The current study used a convenience sample of HF patients, and it is difficult to assess the extent to which this sample can be generalized to other HF populations. In the current study, patients tended to be younger, and perhaps healthier than HF samples previously studied in the literature (25). For example, 20% to 30% of HF patients die within the first year (27), however this sample was followed on average for three years and only 18% died. It is estimated that 50% of HF have sleep apnea (36), but in the current study 98% had AHI scores ranging from mild to severe. Despite the high indices of AHI (over 40% had an AHI over 30, indicting severe sleep apnea), only three HF patients had a diagnosis of SDB in their electronic medical record (EMR) at baseline, and seven more had a diagnosis by the end of the study. Treatment method and adherence were not noted uniformly in the EMR, and thus could not be accurately assessed. The current study did not account for time-dependent covariates, such as antidepressant use

and depression diagnosis past baseline, and thus it is plausible that the current findings may be confounded by treatment effects for SDB or depression.

Conclusions

Although the association between SDB and adverse cardiovascular events is well-known, their mechanisms remain relatively understudied (1). Most of the literature detailing mechanistic pathways of this association has been conceptual (1, 99), and has not been subjected to empirical tests of mediation. Thus the current study is the first to empirically testing the hypothesized mediated effects of depressive symptoms and inflammation on SDB and morbidity and mortality outcomes in HF. Overall the results appear to suggest the SDB may be more predictive of inflammatory load and somatic depressive symptomatology in healthier populations. SDB does not appear to significantly predict depressive symptoms or inflammatory load in HF patients. This may be due in part to dysregulation of immune and inflammatory responses resulting from chronic stressors associated with HF syndrome. Also inflammatory mechanisms, particularly CRP, may mediate the association between SDB and morbidity and mortality, but this association appears to vary by levels of cognitive-affective depressive symptomatology. In the short, these associations are complex and challenging to model statistically. The current analyses attempted to model causal pathways, but the sample size was insufficient to detect small effects at .8 power. Successful future studies may want to include multiple mediators, including measures endothelial dysfunction, oxidative stress, autonomic activity, as well as more proximal outcomes such as stoke or arrhythmias. Finally, the current study could have been strengthened by incorporating multiple assessments of the antecedent and mediators over time in order to assess trajectories inflammatory load and depression

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