Association Between Obesity, Depression, and Inflammation Among Seventh-day Adventists in the Biopsychosocial Religion and Health Study

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ASSOCIATION BETWEEN OBESITY, DEPRESSION, AND INFLAMMATION AMONG SEVENTH-DAY ADVENTISTS IN THE BIOPSYCHOSOCIAL RELIGION AND HEALTH STUDY

By

Michael Paalani

A Dissertation Proposal in Partial Fulfillment of the Requirements for the Degree of Doctor of Public Health in Preventive Care

June 2010
Each person whose signature appears below certifies that this dissertation, in his/her opinion, is adequate in the scope and quality as a dissertation for the degree of Doctor of Public Health.

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ABSTRACT OF THE DISSERTATION

Association between Obesity, Depression, and Inflammation among Seventh-day Adventists in the Biopsychosocial Religion and Health Study

by

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Loma Linda University, 2010

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The effects of obesity and depression on morbidity and mortality may be mediated by inflammatory processes. Homeostasis within the immune system depends on a balance of pro-inflammatory and anti-inflammatory cytokine molecules, so chronic inflammatory diseases may result from cytokine dysregulation. The target population consisted of 508 Seventh Day Adventists (SDAs) who participated in the Biopsychosocial Religion and Health Study (BHRS), a sub-study of the Adventist Health Study-2. The study was a cross-sectional analysis of the association between obesity, depression, and inflammation after controlling for demographic, socioeconomic, health behavioral, and health status variables among BHRS participants.

Obesity was assessed by body mass index (BMI) and waist circumference (WC) measurements, while the Center for Epidemiological Studies Depression Scale 11-item short form was used to measure depression. Enzyme linked immunosorbant assays were
utilized to measure the serum concentrations of c-reactive protein (CRP), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α). Statistical analysis was accomplished by multiple linear regression using SPSS version 17. Obesity measurements and depression scores served as predictor variables, while inflammatory marker concentrations functioned as dependent variables in linear regression equations. Control variables included age, gender, ethnicity (Blacks and Whites), education, degree of difficulty meeting family expenses, exercise frequency, diet (vegetarian or non-vegetarian), and inflammatory conditions.

Results showed that CRP was positively associated with age, female gender, BMI, WC, and non-vegetarian diet. IL-6 was positively associated with age, BMI, WC, and Black ethnicity, while IL-10 was positively associated with exercise frequency after controlling for all other covariates. In addition, depression scores were positively associated with female gender, BMI, WC, increased difficulty meeting family expenses in the last year, and the diagnosis of an inflammatory condition after controlling for all other factors. However, depression scores were not associated with any of the inflammatory markers based on the regression analysis.

In conclusion, the risk for inflammation varied according to age, gender, ethnicity, and overall body mass among SDAs. Lifestyle factors such as exercise and vegetarian diet were associated with reduced inflammation. Therefore, health behavior variables may serve as important components of therapeutic lifestyle interventions and should be studied among distinct sociodemographic populations.
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CHAPTER 1
INTRODUCTION

A. Statement of the Problem

The increase in obesity continues to be a significant public health problem in the United States, where the prevalence has doubled among adults between 1980 and 2004 (Ogden et al., 2006). Obesity is associated with an increased risk for chronic health problems such as diabetes mellitus, cardiovascular disease, hypertension, and some cancers (Blanck et al., 2006; Haslam & James, 2005; Must et al., 1999; Pi-Sunyer, 1999). Moderate amounts of weight loss help to reduce the risk for many of these diseases, but many studies of dietary and behavioral treatments have shown that maintaining weight loss is difficult (Ogden, Yanovski, Carroll, & Flegal, 2007). The prevalence of depression also increased significantly among US adults between 1992 and 2002 (Compton, Conway, Stinson, & Grant, 2006), often leading to severe physical and psychological impairments, and increasing the risk of health complications including coronary heart disease, myocardial infarction, heart failure, osteoporosis, and increased mortality (J. Abramson, Berger, Krumholz, & Vaccarino, 2001; Robbins, Hirsch, Whitmer, Cauley, & Harris, 2001; Schulz et al., 2000).

Given the fact that both obesity and depression are common disorders, it is possible that the two conditions may co-exist among many individuals. For instance, the negative thoughts and attitudes characteristic of depression may contribute to weight management problems due to low levels of self-efficacy (Markowitz, Friedman, & Arent, 2008) and obesity may contribute to a poor self-image and, hence, increased risk of depression (O'Dea, 2006). Studies have demonstrated a positive association between
obesity and depression (McElroy et al., 2004; Rosmond, 2004), yet some discrepancies exist in the literature which may be a result of variations in the study populations related to age, gender, ethnicity, and degree of obesity (Greenman & Stern, 2007). Therefore, the association between obesity and depression may depend on population specific factors. McElroy et al (2004) have speculated that obesity and mood disorders (such as depression) are separate but related disorders with distinct but overlapping pathophysilogies. According to this model, obesity and depressive mood disorders are both heterogeneous and complex illnesses that may share pathogenic and genetic factors. There may be forms of these conditions that are pathogenically similar, and other forms that are not related. For instance, typical or melancholic depression is often associated with anorexia and decreased food intake, while atypical depression is primarily characterized by increased appetite and weight gain (Dipietro, Anda, Williamson, & Stunkard, 1992). This model may help to explain why obesity and depression may be associated with one another under some conditions, but not others.

In addition to determining the relationship between obesity and depression, it is important to understand the mechanisms by which these conditions function in order to determine the effect of obesity and depression on overall health. For instance, inflammation may result from an inability of the central nervous system and the immune system to adapt to the physiological needs of the body for individuals with increased body weight and depressive symptoms. Homeostasis can be disrupted by changes in the levels of cytokines and other important inflammatory molecules. This may lead to problems associated with well-being, affecting cognitive ability as well as metabolic and
cardiovascular health which can influence human disability and life expectancy (Elenkov, 2008).

Inflammation has been associated with cardiovascular disease (CVD) which is the main cause of death in western societies (Greaves & Channon, 2002). The main underlying cause of CVD is atherosclerosis, which is a pathological process that begins in the first decade of life and gives rise to fatty streak lesions within arteries. Growth of atherosclerotic plaques can cause significant narrowing of the arterial lumen, leading to chronic problems such as stroke, myocardial infarction, and angina (Libby, 2001). A previous study by Ross (1993) found that no classic risk factor for atherosclerosis could be determined in approximately 50% of coronary disease events among a normal, healthy population. Yet in the last 15 years, a growing body of evidence supports the notion that inflammation plays a pivotal role in the onset and progression of atherosclerosis (Doria, Sherer, Meroni, & Shoenfeld, 2005; Libby, 2002).

Inflammation has also been shown to play an important role in insulin resistance, a condition in which normal amounts of insulin are inadequate to effectively transport glucose into the tissues of the body. In fact, there is epidemiological evidence that inflammatory markers may predict the development of diabetes and glucose disorders (Barzilay et al., 2001; Schmidt et al., 1999). Insulin resistance often serves as a precursor to the onset of diabetes which is a major cause of morbidity and mortality in the United States (Cowie et al., 2006). In addition, insulin resistance has been associated with other metabolic problems such as glucose intolerance, hyperlipidemia and hypertension, and consistent results have shown that cytokines are involved in the pathophysiology of insulin resistance and atherosclerosis as well as the complications
associated with them (Black, 2003; Festa et al., 2000). Therefore, inflammation may function as an important mediating factor that puts obese and depressed individuals at increased risk for atherosclerosis and CVD as well as other metabolic problems associated with insulin resistance.

B. Theoretical Framework

1. Effects of the HPA Axis and Depression

Lupien et al. (1998) found that a significant relationship exists between depression and hypothalamic pituitary adrenal (HPA) axis dysregulation which can lead to chronic elevations in cortisol levels in the body. Disruption of the HPA axis may result from an inability to manage stress, and may lead to chronic inflammation (Lozovaya & Miller, 2003). Miller, Stetler, Carney, Freedland, & Banks (2002) determined that correlations exist between depression and increased levels of inflammatory markers, including C-reactive protein (CRP) and interleukin-6 (IL-6). It is therefore possible that these proteins mediate the relationship by which depression increases the risk for chronic health problems such as coronary heart disease events (Carney, Freedland, Miller, & Jaffe, 2002). The increased inflammation from depression may result from an elevated pro-inflammatory to anti-inflammatory cytokine ratio within the immune system, in which there is a predominant Th1 lymphocyte response. (Myint, Leonard, Steinbusch, & Kim, 2005). The use of antidepressants has also been shown to reduce pro-inflammation by decreasing the Th1/Th2 ratio, further supporting the idea that cytokine secretion may be involved in the pathophysiology of depressive disorders (Leonard, 2001).
2. **Inflammatory Effects of Adipose Tissue**

Adipose tissue is considered to be an active organ that secretes molecules regulating appetite, energy expenditure, insulin sensitivity, endocrine systems, bone metabolism, and immunity (Fantuzzi, 2005). Obesity results from excessive caloric intake that may lead to an inflammatory response due to the production and stimulation of cytokine molecules from adipocytes (Musaad & Haynes, 2007). Specifically, obesity has been associated with increased levels of pro-inflammatory proteins such as CRP, TNF-α, IL-1, IL-6, and IFN-γ (I. Lemieux et al., 2001; Mahadik, Deo, & Mehtalia, 2008). In addition, there is evidence that plasma TNF-α and IL-6 are further elevated in patients with visceral obesity, and are potent stimulators of the HPA axis during inflammation. Elevations in TNF-α and IL-6 can lead to increased concentrations of glucocorticoids and catecholamines (Chrousos, 1995). Both glucocorticoids and catecholamines are involved in the stress response to protect an individual from a systemic increase in pro-inflammatory cytokine production by inducing a Th2 shift. However, stimulation of the Th1 axis may occur under conditions of extreme stress which may activate macrophage molecules that will release various pro-inflammatory cytokines. Many of these molecules are associated with increased risk for coronary heart disease by increasing inflammation through a systemic response that is possibly mediated at the genetic level. (Y. H. Lee et al., 2005; Maachi et al., 2004). These changes in the body may lead to physiological damage due to high concentrations of reactive oxygen molecules and other harmful substances (Vincent, Innes, & Vincent, 2007). This suggests that obesity may cause a shift in the cytokine profile toward a pro-oxidant state, while a healthy weight may result in a more balanced oxidative state (Vincent & Taylor,
2006). However, the precise relationship between obesity and the Th1/Th2 axis has not been fully characterized.

3. Effect of Obesity and Depression on Inflammation

Obesity and depression have both demonstrated independent low-grade inflammatory effects. According to a recent study, obese individuals with high depression levels had significantly elevated CRP concentrations in relationship to those without depression or excess body weight (K.-H. Ladwig, Marten-Mittag, Löwel, Döring, & Koenig, 2003). Another study found that adiposity was responsible for elevated levels of inflammatory molecules among depressed individuals, indicating that excessive body fat may affect the relationship between depression and inflammation (Miller, Freedland, Carney, Stetler, & Banks, 2003). However, it is unclear whether obesity and depression have an interactive effect on pro-inflammatory and anti-inflammatory cytokines represented by the Th1 and Th2 pathways, respectively. The association between various inflammatory markers and the risk for CVD, diabetes, and other related health problems has also not been completely determined. The theoretical model for the present study is illustrated in Figure 1 below.
Figure 1. Schematic representation of the interactions between obesity, depression, inflammation, and long term health complications. Obesity and depression are associated with many chronic health problems, and this relationship may be mediated by pro-inflammatory or anti-inflammatory molecules. Stimulatory effects are represented by solid lines and inhibitory effects are represented by dashed lines. Confounding variables include age, gender, ethnicity, education, degree of difficulty meeting family expenses, exercise, diet, and diagnosis of an inflammatory condition.

4. Inflammation and Risk for Chronic Health Problems

Analyzing serum markers of atherosclerosis to determine the likelihood of future cardiovascular problems has received greater attention in recent years. Many factors have been associated with endothelial dysfunction as a result of damage to the cell wall, leading to the development of atherosclerosis (Davignon & Ganz, 2004). For instance, hypercholesterolemia, hypertension, diabetes mellitus, and cigarette smoking are possible causes of endothelial dysfunction which is characterized by reduced vasodilation and a shift toward a pro-inflammatory state to activate an immunological cascade (Vapaatalo & Mervaala, 2001). The higher expression of Th1 cells and pro-inflammatory cytokines (including INF-γ, IL-1, IL-12, IL-15, IL-18, and TNF-α) found in mouse models of atherosclerosis as well as in human plaques differ from the few cells
producing the Th2 cytokines such as IL-4 (Hansson & Libby, 2006). This represents an imbalance in the Th1/Th2 cytokine axis and supports the idea that atherosclerosis is mediated by Th1 cells, which may partially explain the increased risk for coronary heart disease events among individuals who suffer from obesity as well as depression (K. H. Ladwig, Marten-Mittag, Lowel, Doring, & Wichmann, 2006).

When considering the role of cytokines in inflammation related to atherosclerosis and CVD, it is important to distinguish between local inflammation (involved in the activation of immune cells within the plaque microenvironment) and systemic inflammation (involving the presence of acute phase proteins and circulating pro-inflammatory mediators). Locally produced pro-inflammatory mediators with atherogenic activity include IFN-γ, TNF-α, IL-1, IL-8, IL-12, and IL-18. Systemic markers of inflammation include IL-6 and CRP (Greaves & Channon, 2002). Importantly, elevated CRP levels have been found in patients with myocardial infarction and may be an indicator of coronary risk (Libby, 2002), while IL-6 levels have been associated with future cardiovascular events among apparently healthy men (Ridker, Rifai, Stampfer, & Hennekens, 2000). Therefore, analyzing different inflammatory markers will help to determine their overall effects on the body as well as their degree of association with heart disease. Finally, the proper method of treatment for cardiac problems may depend on the degree of local and systemic inflammation based on the extent of atherosclerotic damage and plaque buildup which may be neutralized through anti-inflammatory mechanisms (Galkina & Ley, 2009).

Type 2 diabetes has traditionally been characterized as a metabolic disease. However, inflammatory mediators also play an important role in the development of
insulin resistance and the risk for diabetes. For instance, both TNF-α and IL-6 have been shown to reduce insulin sensitivity by attenuating the insulin signaling cascade which is necessary for proper glucose uptake into insulin sensitive cells (Hotamisligil, Murray, Choy, & Spiegelman, 1994). TNF-α also contributes to insulin resistance by inhibiting the expression of genes which are essential for insulin signaling and adipocyte differentiation (Ruan, Hacohen, Golub, Van Parijs, & Lodish, 2002). The pathogenesis of type 2 diabetes may involve the pro-inflammatory endocrine function of adipose tissue. In addition, hyperactivation of the HPA axis causes an increase in cortisol concentration which can inhibit the effect of insulin and increase blood glucose levels, possibly contributing to insulin resistance and the development of type 2 diabetes. Depression prevalence is almost two fold higher among diabetics compared to individuals without diabetes (Gavard, Lustman, & Clouse, 1993), while many individuals who are obese are also at increased risk for diabetes (Resnick, Valsania, Halter, & Lin, 1998). Therefore, individuals suffering from obesity or depression may be at increased risk for diabetes due to elevated cortisol and chronic inflammation.

In addition to cardiovascular and diabetic complications, autoimmune diseases can also result from cytokine dysregulation. For instance, diseases such as rheumatoid arthritis, multiple sclerosis, type 1 diabetes, Crohn’s disease and autoimmune thyroid disease are associated with a pro-inflammatory response involving an activation of the cellular immune system. On the other hand, allergic diseases such as asthma are characterized by a Th2 response to stimulate the humoral immune response (Elenkov, 2008). Patients with autoimmune diseases normally show a higher incidence of affective disorders and affective symptoms as well compared to patients with non-immune
disorders (Wolfe & Hawley, 1993). For these individuals, an increase in disease activity has been associated with higher rates of depressive symptoms. In addition, obesity may be linked to immune-mediated inflammatory diseases due to the effects of leptin and other pro-inflammatory cytokines that are produced and secreted by adipose tissue (Lago, Gomez, Lago, Gomez-Reino, & Gualillo, 2008). However, the precise impact of depression and obesity on autoimmune diseases based on changes in inflammation requires further analysis.

C. Cytokine Functions

1. Pro-inflammatory Cytokines

   a. TNF-α In order to determine the effects of obesity and depression on overall health problems, it is important to understand the function of some of the inflammatory molecules that can disrupt normal homeostasis in our bodies. For instance, one of the main functions of TNF-α is to promote necrosis in tumors after acute bacterial infection. However, it is also involved in inflammation, metastasis, viral replication, septic shock, fever, and the onset of autoimmune diseases (Locksley, Killeen, & Lenardo, 2001). It has also been determined that TNF-α can upregulate adhesion molecules on the endothelium, stimulate fibroblast proliferation, recruit leukocytes from the circulation into the synovial fluid, and promote angiogenesis and osteoclast differentiation to activate osteoclasts and absorb bone. Many of these effects can lead to joint erosion and inflammatory joint disease (Wong et al., 2008). Finally, other factors such as IFN-α, IFN-β, IL-4, IL-6, and IL-10 have been shown to suppress the production of TNF-α as part of a possible feedback mechanism (Juge-Aubry et al., 2005).
TNF-α has also lead to reduced insulin sensitivity by its ability to down-regulate facilitative glucose transporters in adipose tissue and may therefore play an important role in the onset of obesity-linked insulin resistance (Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995). Hypertriglyceridemia often results from elevated TNF-α levels among animal and human subjects due to increased de novo fatty acid synthesis in the liver and esterification to form triglycerides, induction of lipolysis in adipose tissue, and decreased lipoprotein lipase activity (Warne, 2003). TNF-α is one of the most important cytokines implicated in congestive heart failure as well. Myocardial stress and volume overload can lead to high quantities of TNF-α production in the heart, which have been shown to induce apoptosis in myocytes and endothelial cells. Finally, this cytokine has been linked to vascular injury and may perpetuate atherosclerosis by acting as a pro-inflammatory factor in ruptured plaques (Sarzi-Puttini, Atzeni, Doria, Iaccarino, & Turiel, 2005).

b. **IL-6** IL-6 is a cytokine with multiple effects on the body, such as bone remodeling, neuroendocrine homeostasis, hematopoiesis, and inflammatory response regulation. IL-6 is synthesized by many cell types, including T cells, macrophages, and stromal cells (Heikkila, Ebrahim, & Lawlor, 2008). One of the main functions of this cytokine is to provoke acute phase reactions which can lead to fever as well as production of acute phase proteins (such as CRP) in the liver. Studies have found that IL-6 also has anti-inflammatory functions by decreasing the levels of TNF-α and IFN-γ and increasing the concentration of IL-1 receptor antagonist during the course of an inflammatory response (Mohamed-Ali et al., 1997; Senn et al., 2003). Therefore, IL-6 may play an important role in balancing the pro-inflammatory/anti-inflammatory pathways. Like
TNF-α, IL-6 has been associated with increased lipolysis and decreased lipoprotein lipase activity in human adipose tissue cultures, resulting in impaired lipid metabolism and an increased production of triglycerides (Greenberg et al., 1992). IL-6 also causes partial resistance to insulin dependent glucose uptake through down regulation of the insulin receptor substrate and glucose transporter expression (Kern, Ranganathan, Li, Wood, & Ranganathan, 2001). As one of the main pro-coagulant cytokines, IL-6 functions by enhancing the synthesis of factors such as fibrinogen which is involved in the clotting process as well (Willerson & Ridker, 2004). Finally, circulating IL-6 stimulates the HPA axis which is often associated with central obesity, hypertension, and insulin resistance (Pradhan, Manson, Rifai, Buring, & Ridker, 2001).

c. CRP  CRP is an example of an acute phase protein that is normally elevated when infections are present in the body. However, recent analysis shows that cardiac risk is associated with higher levels of this protein, and that CRP is a strong predictor of cardiac events even after adjusting for traditional risk factors (Yeh, Anderson, Pasceri, & Willerson, 2001). CRP is responsible for inducing the expression of cellular adhesion molecules that mediate adhesion of leukocytes to the vascular endothelium, and CRP also induces monocytes to express specific glycoproteins involved in the coagulation process which is a major factor in causing cardiovascular problems (Nakagomi, Ben Freedman, & Geczy, 2000). CRP mediates LDL cholesterol uptake by macrophages as well, and may be an important marker in the onset of systemic inflammation which is involved in autoimmune diseases as well as atherosclerosis (Zwaka, Hombach, & Torzewski, 2001).
2. Anti-inflammatory Cytokine - IL-10

IL-10 is a cytokine produced by many types of cells such as monocytes, macrophages, and lymphocytes. It has mainly anti-inflammatory properties by suppressing the production of numerous pro-inflammatory cytokines (such as IL-1, IL-6, and TNF-α) and stimulating anti-inflammatory molecules (including IL-1 receptor antagonists and TNF-α receptors) (Esposito et al., 2003). Because of its immunosuppressive properties and its ability to stimulate B cell function which is important in an adequate defense against intestinal parasites and neutralization of bacterial toxins, it is referred to as a Th2 type cytokine (Asadullah, Sterry, & Volk, 2003). However, its biological activity may be more complex than this, and there is growing evidence that IL-10 may have immuno-suppressive and immuno-stimulating effects on adaptive immune cell mediators. IL-10 may be secreted by adipocytes, and show higher levels among obese patients with increased fat tissue (Juge-Aubry et al., 2005). In addition, both over-expression and under-expression of IL-10 have been associated with different types of inflammatory problems, suggesting the need to maintain adequate concentrations of this cytokine in the body. However, many of the overall effects of IL-10 are still unclear. Finally, cytokine molecules can cause significant changes in inflammation and the overall immune response. Therefore, due to the positive association between obesity and depression with multiple chronic diseases, the changes in CRP, IL-6, IL-10, and TNF-α levels as a result of obesity and depression may provide an indication of an individual’s overall health risk.
D. Purpose of the Study

The purpose of this study is to determine the mechanism by which obesity and depression disrupt the normal physiological balance within the body. Therefore, the association between obesity, scores of depression, and markers of inflammation will be studied after controlling for demographic, socioeconomic, health behavioral, and health status variables. The inflammatory analysis will compare systemic and local inflammatory markers within the body and determine whether the balance of the Th1/Th2 axis is altered as a result of obesity and depression. Serum samples of CRP, IL-6, IL-10, and TNF-α will be studied among Seventh-day Adventists (SDAs) who participated in the Biopsychosocial Religion and Health Study (BRHS) to determine the role of these factors on their risk for health problems. It may also be possible to identify segments of the SDA population who are at lower risk for CVD and other inflammatory problems based on their body mass measurements, depression scores, and inflammation level that can be analyzed in future studies to better understand their higher condition of wellbeing.

E. Research Questions

1. Is obesity associated with depression?

2. Are obesity and depression each independently associated with markers of inflammation (i.e., CRP, IL-6, IL-10, TNF-α)?

3. Do obesity and depression have a significant interactive effect on the inflammatory marker concentrations?

4. Are there ethnic and gender specific differences in the inflammatory marker concentrations?
F. Significance to Preventive Care

Preventive care specializes in promoting healthy lifestyles in order to reduce the prevalence of chronic health problems among susceptible individuals. Since excessive body weight and depression are associated with health complications, it is important to determine which molecules become activated in the disease state. Inflammation has been a major contributing factor toward the onset of atherosclerosis, insulin resistance, and other conditions which can lead to greater risk of morbidity and mortality (Mahadik et al., 2008). Therefore, understanding which inflammatory markers are associated with obesity and depression would allow us to better determine the patients’ level of health risk and possibly find suitable methods of prevention. So promoting lifestyle changes such as proper weight maintenance (through a balanced diet and incorporation of adequate exercise), improved psychological well-being, and appropriate stress management may prove to be therapeutic as anti-inflammatory methods of disease prevention.
CHAPTER 2

REVIEW OF LITERATURE

A. Significance of Understanding the Relationship between Obesity, Depression, and Inflammation

It is important to understand the relationship between depression and obesity as well as their mechanism of action that can lead to long term health complications (Markowitz et al., 2008). Studies have shown that systemic inflammation may be the factor leading to cardiovascular disease, diabetes, and other conditions among depressed and obese individuals (Carney et al., 2002; Leonard, 2007; Musaad & Haynes, 2007). Therefore, this literature review will examine the relationship between obesity, depression, and inflammation and their role in chronic health problems. The prevalence and severity of obesity and depression will initially be explained, followed by an analysis of the association between: (a) depression and inflammation, (b) obesity and inflammation, (c) obesity and depression, and (d) obesity, depression, and inflammation. This literature review will analyze the changes in both pro-inflammatory and anti-inflammatory molecules due to increased body weight and depression that can lead to increased morbidity and a lower quality of life. The variable measurements utilized for the present study and the implications for future analysis including the effect of diet and exercise on inflammation will also be discussed.

B. Prevalence and Severity of Obesity

Obesity has become a prevalent condition and is a serious public health problem in the United States (Hedley et al., 2004; Ogden et al., 2006). Data from the National Health and Nutrition Examination Survey (NHANES) as well as the Behavioral Risk
Factor Surveillance System (BRFSS) have shown that the obesity prevalence has continued to increase within the last decade among adults in this country (Yun, Zhu, Black, & Brownson, 2006). A recent analysis has indicated that 23.9% of US adults were obese in 2005, and all states in the US showed an increased prevalence of obesity from 1995-2005 (Blanck et al., 2006). The prevention of obesity is important because significant increases in weight have been associated with increased risk for hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, and some cancers (Haslam & James, 2005; Kopelman, 2000; Must et al., 1999; Pi-Sunyer, 1999). Therefore, one of the objectives of Healthy People 2010 is to reduce the prevalence of obesity in the United States by 15% (Wang, Colditz, & Kuntz, 2007).

C. Prevalence and Severity of Depression

Depression is also a significant public health problem and one of the most serious mental health problems in the United States. Elevated symptoms of depression can often lead to impairments in physical and social functioning, and increase the risk of health complications including coronary heart disease, myocardial infarction, low bone mineral density, insulin resistance and other metabolic problems (J. Abramson et al., 2001; Anderson, Freedland, Clouse, & Lustman, 2001; Pohjasvaara, Vataja, Leppavuori, Kaste, & Erkinjuntti, 2001; Robbins et al., 2001; Schultz et al., 2000). Like obesity, depression contributes substantially to national morbidity and mortality, accounting for 8% of total disability adjusted life years in the United States, and approximately 12% of all years lived with disability worldwide in 2000 (Ustun, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004). Therefore, individuals who suffer from both obesity and depression may be at particularly high risk for health problems and reduced well-being.
D. Association between Depression and Inflammation

Many studies have analyzed the relationship between depression and inflammation and their association with coronary heart disease (CHD) events. For instance, results from the Cardiovascular Health Study (CHS) and the Third National Health and Nutrition Examination Survey (NHANES III) showed that depression symptoms and a history of major depression were associated with elevated CRP levels (Ford & Erlinger, 2004; Kop et al., 2002). Even though both of these studies utilized large samples of healthy individuals, the CHS was based on elderly individuals (65 years or older) and the NHANES III participants were between the ages of 18 to 39. Therefore, these effects may be typical for younger as well as older individuals. Depression was also associated with increased levels of CRP, white blood cells, and fibrinogen based on data from the ATTICA study (Panagiotakos et al., 2004). Therefore, evidence suggests that a correlation exists between depression and inflammation. However, because surveys were utilized to measure the participant’s level of depression in these studies, the results may have been affected by self-report bias. In addition, due to the cross-sectional nature of these studies, the causal nature of association between depression and inflammation is unclear, and the relationship between these risk factors and incident CHD has not been fully characterized.

If depression results from an elevated inflammatory state, then depression treatment would be beneficial in improving one’s quality of life but would not necessarily reduce the risk of cardiovascular disease unless the inflammation is reduced as well. On the other hand, if inflammation mediates the relationship by which depression increases the risk for CHD events, then the benefits of inflammation treatment on CHD would not
be effective over a prolonged period of time unless depression was treated as well
(Shimbo, Chaplin, Crossman, Haas, & Davidson, 2005). According to a prospective
case-control study, depression and various inflammatory markers (including CRP, IL-6,
and fibrinogen) significantly predicted CHD events after adjusting for various
cardiovascular risk factors, including tobacco and alcohol consumption, systolic blood
pressure, and total cholesterol (Empana et al., 2005). In addition, the association between
depression and CHD events was not affected after evaluating depression and
inflammatory markers as covariates in the analysis. This suggests that the causal
pathways by which inflammation and depression increase the risk for CHD may occur
independently. However, the sample participants consisted only of healthy, European,
middle-aged men, making it difficult to generalize the results to other populations. Also,
depression and inflammation were measured at baseline only, so the changes in these
factors over time and the impact on CHD events could not be determined. Therefore,
additional research is needed to determine the causal relationship between depression,
inflammation, and CHD.

Inflammation and the associated secretion of pro-inflammatory cytokines may
also mediate the association of depression and diabetes. Elevations in pro-inflammatory
molecules such as IL-6 and TNF-α have occurred among patients with depression and
with diabetes (Musselman, Betan, Larsen, & Phillips, 2003). In addition, recent
population based prospective studies reported that being depressed at baseline
significantly increases the risk of developing type 2 diabetes, and this relationship
appears to exist independently of other known diabetes risk factors, including obesity,
activity level, smoking, alcohol consumption and family history of diabetes (Everson-
Rose et al., 2004; Kawakami, Takatsuka, Shimizu, & Ishibashi, 1999). However, the study by Kawakami, Takatsuka, Shimizu, & Ishibashi (1999) involved 3,066 male participants from Japan, which limits the external validity of the information obtained due to its gender and ethnic specificity. A study by Everson-Rose et al (2004) involved 2,662 female participants from the Study of Women’s Health Across the Nation (SWAN) who were either Caucasian, African-American, Hispanic, Japanese, or Chinese. This study found that depression was a significant predictor of diabetes among African-American women only, which further illustrates that the effects of depression on diabetes may be specific to gender and ethnic background. Finally, depression may serve as an important risk factor in the development of type 2 diabetes among individuals at risk for this disease, but the exact role of inflammatory cytokines in this process is still unclear.

There is evidence of an association between increased levels of pro-inflammatory cytokines among individuals with major depression (Leonard, 2007). In addition, the number of T helper cells, T memory cells, macrophages, and monocytes that act as a source of cytokines are also increased in depressed individuals (Myint et al., 2005). There appears to be an imbalance between pro-inflammatory and anti-inflammatory molecules within the cellular immune system of depressed individuals, in which the cytokines from the Th1 lymphocytes (pro-inflammatory) are predominant over the cytokines from the Th2 lymphocytes (anti-inflammatory). It is possible that the elevations in inflammatory molecules may result from a reduced threshold to stress. In fact, it has been suggested that cytokine production contributes to a hyperactive hypothalamic pituitary adrenal (HPA) axis which may lead to depression (Lozovaya & Miller, 2003). However, depending on the ability of people to properly manage the stress
in their lives, the effects of inflammation may only be found among certain depressed individuals.

A shift in the pro-inflammatory/anti-inflammatory cytokine balance has been demonstrated among patients suffering from major depression as well. A study by Huang and Lee (2007) showed significantly higher levels of TNF-α in patients with major depressive disorder compared to a control group. This inflammatory molecule may therefore play a very important role in the psychopathology of major depression. TNF-α levels have also been elevated in other clinical conditions such as multiple sclerosis and Alzheimer’s disease, suggesting a possible role in neuronal dysfunction (Sheng, Boop, Mrak, & Griffin, 1994). In addition, a higher pro-inflammatory/anti-inflammatory cytokine ratio (IL-1/IL-10) was found among patients with melancholic depression than in patients with non-melancholic features (T. L. Huang & Lee, 2007). This suggests that melancholic and non-melancholic depression may have different immune patterns, but future studies analyzing the relationship between cytokine levels, depression subtypes, and the interactions between the HPA axis and immune system are still needed.

Similar changes in the cytokine balance has been demonstrated in other studies, including an increase in the IFN-γ/IL-4 ratio among patients with major depression (Myint et al., 2005). The ratio was significantly reduced following antidepressant treatments, which indicates that the balance in the Th1 and Th2 cytokine ratio plays an important role in the pathophysiology of depression. However, different medications were used to treat different groups of patients suffering from depression in this study, so the precise mechanism of action on cytokine levels based on the treatment would be difficult to determine. Another study found that major depression was associated with...
higher levels of IL-4 and IL-13 (Th2 cytokines) and lower levels of IL-2 and IFN-γ (Th1 cytokines) that may have been due to the effects of increased cortisol in the body (Pavon et al., 2006). Yet this study consisted of only a small population of patients exclusively of Mexican ethnicity (n=66), so further studies will be needed in this area. Specifically, the causal relationship between depression and specific inflammatory markers should be examined through longitudinal analysis.

E. Association between Obesity and Inflammation

Studies have found that obesity causes increases in circulating concentrations of many pro-inflammatory, acute phase proteins. For instance, Lemieux et al (2001) determined that CRP concentrations were significantly higher among obese individuals than overweight or normal weight individuals. In addition, the correlation between CRP concentrations and indices of body fatness and abdominal adipose tissue accumulation were analyzed as well. This included BMI, fat mass, waist circumference, and visceral and subcutaneous adipose tissue content. Although all these variables were significantly correlated with CRP concentration, total body fat mass showed the highest correlation. This study was very thorough by using several anthropometric measurements to assess the participant’s fat content. However, because the study consisted of a small sample (n=159) of male subjects from Canada, these effects may be gender or population specific. A more recent study also found a significant correlation between CRP concentration and obesity parameters such as BMI and body fat percentage (Mahadik et al., 2008). This provides further evidence of a link between adipose tissue and inflammation, despite the fact that the study consisted of a small sample (n=134) of Asian Indian subjects which may limit external validity. Nevertheless, the finding of the same
relationship in two very different populations (Asian Indians and male Canadians) lends credence to the idea that the association may exist generally.

Similar results regarding obesity and inflammation were found in a large scale study (n=3042) of participants from Greece, in which there were significantly higher levels of CRP, TNF-α, IL-6, and white blood cells in subjects who were obese with a central body fat distribution (based on waist-to-hip ratio measurements) compared to normal weight participants with a normal body fat distribution. The inflammatory molecules were significantly correlated with BMI and waist-to-hip ratio measurements for male and female subjects as well, even after controlling for variables such as age, smoking, physical activity, dietary habits, blood pressure, and lipid levels (Panagiotakos, Pitsavos, Yannakouli, Chrysohoou, & Stefanadis, 2005). Finally, despite the strong evidence for obesity induced inflammation among various sample populations, the cross sectional design of these studies limit the conclusions that can be drawn regarding the causal relationship between obesity and inflammation.

Obesity is considered to be a state of chronic inflammation, in which adipose tissue is an active endocrine organ as well as a storage depot for lipid energy. Expansion of the adipose tissue depot in obese individuals has been associated with increased levels of pro-inflammatory cytokines (such as TNF-α, IL-6, and CRP) that has been correlated with type 2 diabetes as well as cardiovascular complications such as coronary artery disease, myocardial infarction, stroke, thrombosis, and peripheral arterial disease (Kopp et al., 2003; Saito, Yonemasu, & Inami, 2003; Weyer et al., 2002). There is a great overlap in the prevalence of obesity, type 2 diabetes, and CVD in western society, and it has become apparent that people with obesity are more prone to type 2 diabetes and other
problems based on metabolic abnormalities that serve as major risk factors for CVD. For instance, Saito et al (2003) found a strong association between circulating levels of CRP and CVD risk factors such as smoking, obesity, high blood pressure and dyslipidemia, but the study was a cross-sectional analysis which made it difficult to determine the causal relationship between the variables. In addition, elevated CRP and IL-6 levels in obese patients were found to significantly predict the development of type 2 diabetes in a prospective case-control study by Pradhan et al (2001), but the analysis was based on data from females participants only and was therefore gender specific. Nevertheless, the results of these studies suggest that the pathophysiology of many of the comorbidities associated with obesity may involve pro-inflammatory cytokine production.

In addition to stimulating the production of pro-inflammatory cytokines, obesity may lead to an increased concentration of anti-inflammatory cytokines as well. For instance, it was determined that circulating levels of IL-10 were higher among obese women than non-obese women between the ages of 22 and 44 years old (Esposito et al., 2003). However, the results provided were gender specific since males were not assessed in the study. A more recent study showed that a significantly higher quantity of IL-10 was secreted by white adipose tissue from a small sample of obese patients compared to normal weight patients (Juge-Aubry et al., 2005), although an analysis using a larger sample size may be necessary to validate these results. Manigrasso et al (2005) found that women suffering from android obesity were found to have a significantly lower concentration of IL-10 compared to non-obese women. This suggests that the presence of excess body fat in the abdominal region may explain the lower amount of IL-10 among this study population. However, it is unclear if the same effects are present
among men. It has been well characterized that IL-10 is a major inhibitor of pro-inflammatory cytokines and has a protective role in atherosclerotic lesion formation and stability (Endo et al., 1996; Oslund et al., 1999). Therefore, it is possible that adipose tissue secretes pro-inflammatory as well as anti-inflammatory cytokines into the general circulation that may alter the balance of the Th1/Th2 axis. However, the exact pathophysiological process that contributes to the pro-inflammatory or anti-inflammatory state is currently unknown.

Inflammation does not appear to be an initial cause of obesity. Rather, obesity results from positive energy balance that can lead to inflammation due to effects of cytokines present within fat cells (Musaad & Haynes, 2007). Many of the molecules produced by adipocytes (known as adipocytokines) are associated with increased cardiovascular or diabetes risk by either increasing the expression of inflammatory genes or inducing systemic inflammation (Y. H. Lee et al., 2005; Maachi et al., 2004). In addition to increased CRP concentrations, abnormal fat accumulation has also been associated with other types of inflammatory changes such as the recruitment of macrophages and activation of endothelial cells which can promote vascular disease (Weisberg et al., 2003). These changes in the body can ultimately lead to oxidative stress due to high concentrations of free radicals and reactive oxygen species in the body. Finally, inflammation and oxidative stress due to obesity is a systematic problem that should be corrected by improving antioxidant defenses through exercise, dietary modification, or a combination of both (Vincent & Taylor, 2006).
F. Association between Obesity and Depression

According to current research, an association between obesity and depression does appear to exist (Markowitz et al., 2008). However, it has been difficult to generalize the relationship between these two disorders because of the heterogeneity of the obese population as well as methodological limitations of subject selection and measurement (Friedman & Brownell, 1995). Most of the research in this area has focused on comparing the depression level between obese and non-obese individuals based on measures of psychological functioning, and the investigators have relied predominantly on cross-sectional data to determine the association between obesity and depression among different sample populations (Faith, Matz, & Jorge, 2002).

A study based on 2482 subjects from the 1995 Nova Scotia Health Survey examined the relationship between body mass index and depression assessed with the Center for Epidemiologic Studies Depression scale. The study showed that a higher BMI was significantly associated with an increased risk for depression, with obese individuals having a 41% increased odds of being depressed (Johnston, Johnson, McLeod, & Johnston, 2004). Another study that analyzed a cohort of 1040 Swedish men found that self-reported BMI was associated with increased depression symptoms on a self-report questionnaire. Despite the large sample size, this study was limited by the use of self-report assessments for these two factors which may have biased the data (Rosmond, Lapidus, Marin, & Bjorntorp, 1996).

Based on data from the 2001 Behavioral Risk Factor Surveillance Survey (BRFSS), a sample of 44,800 nationally representative respondents were studied to determine the association between depressed mood and BMI. The results showed that
obesity was associated with an increased likelihood for depressive mood among young women, particularly who were Hispanic. Therefore, the relationship between depressive mood and obesity seemed to be dependent on gender, age and ethnicity. However, because this was a self-report telephone survey, the variables studied may have been susceptible to measurement error. Individuals without telephones would also not be eligible for this study, so the results do not reflect those from a low socioeconomic status.

Finally, these cross-sectional studies suggest a possible relationship between obesity and depression, but the association between these variables is still not conclusive.

Longitudinal studies have found that obesity may predict later onset of depression in adults. For instance, Roberts, Kaplan, Shema, and Strawbridge (2000) analyzed data from a sample of 2730 middle-aged and older adults from Alameda County, and determined that obesity at baseline was found to predict depression one year later (91% increased odds of depression for obese individuals) after controlling for demographic and psychosocial variables. In a 2002 study by the same research group, it was reported that obesity at baseline also predicted depression five years later among the same participant sample when controlling for depression at baseline, but that depression did not predict obesity prospectively when controlling for obesity at baseline (Roberts, Strawbridge, Deleger, & Kaplan, 2002). These longitudinal studies contained large sample sizes, included diagnostic assessment of depression, and sufficiently controlled for baseline variables, indicating that the results are based on strong methodological design.

There is evidence to suggest that some factors may cause certain obese individuals to be at greater likelihood for developing depression. For instance, research has shown that severe obesity puts individuals at greater risk for depression. According
to the Third National Health and Nutrition Examination Survey (NHANES III), the most severely obese individuals (BMI > 40) were at a significantly higher risk for depression than those with a BMI between 30 and 34.9 (Onyike, Crum, Lee, Lyketsos, & Eaton, 2003). In addition, female gender may also be a potential risk factor for depression among obese individuals. Data from NHANES-I showed that there was no significant relationship between obesity status and depression among 1237 men, but obesity was associated with increased depression among 1616 women (Istvan, Zavela, & Weidner, 1992). Low socioeconomic status is another risk factor that has been associated with obesity and depression, suggesting that it may play a role in co-morbid depression and obesity (Everson, Maty, Lynch, & Kaplan, 2002). Therefore, the risk for depression among obese individuals may be increased by severe obesity, female gender, and low socioeconomic status.

Studies have also examined the risk factors and causal mechanisms leading to obesity among depressed individuals. One study compared a sample of children with major depression disorder (MDD) to a control group and found that the group of children with MDD at baseline had significantly higher BMI values compared to the control group after being assessed 10 to 15 years later. This indicates that higher depression levels at baseline may have lead to the elevated BMI in adulthood (Pine, Goldstein, Wolk, & Weissman, 2001). Another longitudinal study involved a large sample of adolescents who completed in home interviews for the National Longitudinal Study of Adolescent Health, and were assessed for depression and BMI at baseline and one year later. It was determined that baseline depressive symptoms independently predicted obesity at follow-up for those participants who were not obese at baseline, even after controlling for
smoking, self-esteem, socioeconomic status, and physical activity (Goodman & Whitaker, 2002). Finally, a study on a cohort of adolescent girls from New Zealand found that depression at baseline lead to more than a twofold increased risk for obesity in adulthood when compared to their non-depressed peers (Richardson et al., 2003). These results suggest that adolescent depression causes an increased risk for obesity later in life. However, a study on the effect of depression on obesity in middle and late adulthood found no significant change (Roberts, Deleger, Strawbridge, & Kaplan, 2003).

A recent cross sectional study analyzed the association between body mass index and depression (based on a Mental Health Inventory) among a large sample of males and females aged 18 to 90 years old from the Netherlands (n=43,534). The results showed that there was a U-shaped association between BMI categories and depression, in which the obese and underweight categories had higher levels of depression compared to the normal and overweight groups (de Wit, van Straten, van Herten, Penninx, & Cuijpers, 2009). This suggests that depression may be associated with a positive as well as a negative calorie balance that lead to changes in body weight. Other factors may therefore affect the relationship between depression and body weight. However, because the BMI values were based on self-reported height and weight measurements, the validity of the results may have been compromised. The causal relationship between BMI and depression could not be determined as well because the study was an observational analysis.

The hypothalamic-pituitary-adrenal (HPA) axis is a biological pathway involving increased stress reactivity with hormonal changes, and could promote weight gain among individuals who are depressed. Activation of the HPA axis can lead to elevated levels of
cortisol which has been found to promote weight gain and contribute to abdominal obesity (Stunkard, Faith, & Allison, 2003). Research has also shown that failing to regulate the HPA axis can elevate the risk for depression and increase susceptibility to atherosclerosis and coronary heart disease (Carney et al., 2002; Holsboer, 2000).

Therefore, this pathway may be an important neuro-endocrine link between depression and the development of obesity, and could affect psychosocial factors such as binge eating, poor adherence to weight loss programs, and reduced self-efficacy and social support which are potential mediators of the obesity-depression relationship (Markowitz et al., 2008).

Obesity may play an important role in causing depression as well as other psychological problems. Depression may also lead to obesity and increased weight gain for many individuals. This bidirectional relationship between depression and obesity may be affected by lifestyle factors that can influence the stress response as well. For instance, physical activity may reduce depression and body weight due to its anti-inflammatory and calorie-burning effects in order to effectively cope with chronic stress (Pereira et al., 2007). However, sedentary behavior may promote the opposite effect, and should be considered when assessing one’s physical and mental health.

G. Association between Obesity, Depression, and Inflammation

Recently, it has been suggested that increased inflammatory responses in depressed individuals may at least partially depend on obesity. For instance, one study compared a group of depressed and non-depressed subjects to determine the differential expression of inflammatory markers implicated in CHD, and found that the former group exhibited significantly higher levels of CRP and IL-6 compared to the latter group. An
analysis of possible mediating factors contributing to this association also showed that the depressed individuals had higher BMI values than the non-depressed subjects, and that adjusting for BMI attenuated the group differences in CRP and IL-6. The results also showed that there was a synergistic association between depression and obesity, in which subjects with depression and high BMI (>30 kg/m²) exhibited significantly higher CRP levels compared to subjects who were either depressed or had a high BMI (Miller, Stetler, Carney, Freedland, & Banks, 2002). Based on this evidence, the researchers argued that depressed individuals may have accumulated excess weight over time as a result of sedentary behavior. As this occurred, increased levels of IL-6 and CRP may have been released by adipose tissue. Unfortunately, the cross-sectional design of this study makes it impossible to determine the direction of causality between the variables.

Other studies using similar methodology have also found a significant association between CRP concentrations and depression, in which the effect disappeared after adjusting for body mass index (Douglas, Taylor, & O'Malley, 2004; Kop et al., 2002). A more recent study also showed that an increase in body weight (based on BMI and waist circumference measurements) mediated the association between depression and CRP levels among a large sample of elderly men and women (n=3609) from the English Longitudinal Study of Aging. However, because inflammatory marker levels were not measured at baseline, the longitudinal relationship between depression and inflammation could not be determined (Hamer, Molloy, de Oliveira, & Demakakos, 2009). Therefore, prospective analysis that measures obesity, depression, and inflammation at multiple time points will be necessary to further understand the relationship between these variables.
In another study, the investigators examined the relationship between depression, obesity, and inflammatory molecules by the use of structural equation modeling (SEM) to examine the validity of competing models specifying different patterns of association between these variables. For the sample population, a group of depressed subjects were compared to a control group with no psychiatric illness who were matched on a case by case basis with respect to age, gender, and ethnicity. Based on SEM analysis, the joint pathway model fit the data significantly better than any other model that was evaluated. This model is consistent with the hypothesis that depression promotes weight accumulation which can stimulate an inflammatory response, by inducing the expression of leptin as well as releasing inflammatory molecules from adipose tissue (Miller et al., 2003). These results identified adiposity and leptin as potential mediating pathways linking depression with inflammation. However, further research will be required to validate this proposed mechanism.

A German study of 3204 male subjects examined whether depressive mood modifies the relationship between obesity and serum concentrations of CRP in a non-clinical population. It was determined that there was a significantly increased concentration of CRP among an obese sample of males with high levels of depression compared to the non-obese group with low levels of depression. More importantly, there was a significant interactive effect (based on the beta coefficient value corresponding to the obesity x depression product) of body mass index and depressive symptoms on CRP concentration among this sample population. Multivariate analysis further demonstrated that depression significantly contributed to elevated levels of CRP in obese men after controlling for confounders such as age, high blood pressure, smoking, physical
inactivity, low social class, and alcohol intake (K. H. Ladwig, Marten-Mittag, Lowel, Doring, & Koenig, 2003). This synergistic effect of obesity and depressive mood on chronic inflammation may play an important role in determining a person’s risk for cardiovascular and metabolic problems. However, further studies are needed to determine if an interactive effect involving obesity and depression affects additional markers of inflammation among other population groups that include males as well as females.

A more recent study analyzed the relationship between symptoms of depression and CRP concentration among a sample of 493 obese patients presenting for bariatric surgery after controlling for identified determinants of CRP levels. Based on linear regression analysis, it was determined that five independent factors were associated with increased CRP. In order of strength of association, the factors were higher BMI, female gender, estrogen therapy, higher depression, and insulin resistance (Dixon et al., 2008). The results also showed that progressively higher depression levels (based on scores from the Beck Depression Inventory) and mean BMI values were correlated with higher concentrations of CRP, indicating that the degree of obesity and the severity of depressive symptoms were independently and incrementally associated with this acute phase protein. These studies provide strong evidence for a positive association between obesity, depression and inflammation, although the cytokines involved in the immune response are still unclear.

**H. Effect of Diet and Exercise on Inflammation**

Lifestyle factors can affect inflammation as well. For instance, many studies have shown that increased levels of physical activity have been associated with lower CRP
concentrations (J. L. Abramson & Vaccarino, 2002; Geffken et al., 2001; Wannamethee et al., 2002), but they are predominantly based on cross-sectional analysis such as the Cardiovascular Health Study which found that CRP levels decreased in a dose-response manner with increasing energy expenditure (Geffken et al., 2001). The results from Health, Aging, and Body Composition Study showed that individuals who accumulated at least 180 minutes of exercise per week had lower CRP levels compared to physically inactive individuals after controlling for age, gender, and ethnicity (Colbert et al., 2004). Analysis based on frequency of activity have reported that physical activity performed only 1 time per week have been associated with lower CRP levels compared with sedentary individuals, and more frequent activity leads to additional reduction in CRP levels (Albert, Glynn, & Ridker, 2004; Colbert et al., 2004). However, the data from these investigations were based on self-reported physical activity and are therefore subject to self-report bias. Some longitudinal studies have been performed as well, including a recent study which found that a 6 month aerobic exercise training program (at a frequency of 4 times per week for 45-60 minutes per session) significantly reduced CRP levels but increased IL-10 levels among 60 overweight individuals with type 2 diabetes (Kadoglou et al., 2007). Similarly, Stewart et al (2007) found that a 12 week training program consisting of aerobic activity (at a frequency of 3 times per week for 20 minutes per session) and resistance exercises (2 sets of 8 repetitions at a frequency of 3 times per week) decreased CRP concentrations but did not affect IL-6 or TNF-α levels among 70 healthy participants. Exercise may therefore reduce CRP levels and provide anti-inflammatory benefits for both healthy and diseased populations, but the overall inflammatory mechanism involved is not fully known.
A number of well-controlled cross sectional studies have examined the relationship between dietary factors and inflammatory markers. A nutritional analysis of 732 healthy women from the Nurses Health Study showed that a prudent dietary pattern consisting of a high intake of fruits, vegetables, legumes, fish, poultry, and whole grains was inversely associated with CRP levels. On the other hand, a western pattern characterized by a higher intake of red and processed meats, sweets, and refined grains was directly associated with increased CRP and IL-6 levels after controlling for potential confounding variables (Lopez-Garcia et al., 2004). Dietary assessments of 5089 men and women from the Multi-Ethnic Study of Atherosclerosis showed that a nutritional pattern consisting of fats and processed meats was positively associated with CRP while a diet made up predominantly of whole grains, fruits, nuts, and green leafy vegetables was inversely associated with CRP and IL-6 levels (Nettleton et al., 2006). Furthermore, 30 long term vegetarians who consumed no meat or fish were found to have significantly lower CRP concentrations when compared with 30 age and gender matched omnivores (Szeto, Kwok, & Benzie, 2004). Dietary components appear to have a direct influence on CRP and IL-6 levels, but their effects on other cytokines have not been determined and require further research.

I. Variable Measurements Utilized for the Present Study

1. Center for Epidemiological Studies Depression (CESD) Scale

The CESD scale is a useful tool for measuring depression among various populations. However, the 11 item short form of the CESD also provides a high degree of reliability in analyzing four symptom dimensions (depressed affect, positive affect, somatic complaints, and interpersonal problems) that are assessed by the original CESD
as well. In addition, very little information and precision is sacrificed when shorter forms are utilized as they have demonstrated high consistency and reliability. The 11 item short form can also be administered in less than half the time needed for the original CESD. This can help to lower costs in studies that utilize large surveys, provide elderly participants with a simpler and more convenient response format, and reduce the emotional stress burden that the original CESD may place on them (Kohout, Berkman, Evans, & Cornoni-Huntley, 1993).

2. **Body Mass Index (BMI) and Waist Circumference (WC)**

Body Mass Index is a standardized method to determine an individual’s height adjusted weight. Due to its ease of measurement and calculation, the BMI is one of the most widely used diagnostic tools to identify weight problems within a population, regardless of whether individuals are underweight (less than 18.5 kg/m²), overweight (25 -29.9 kg/m²), or obese (greater than 30 kg/m²). However, in order to determine the amount of abdominal fat present among the study population, the waist circumference is often measured as well. Waist circumference is highly correlated with visceral fat which is associated with negative health consequences including hypertension, type 2 diabetes, hyperlipidemia, and coronary artery disease (Bergman et al., 2007). Therefore, the waist circumference associated with increased obesity-related health risk is greater than 40 inches for men and 35 inches for women (S. Lemieux, Prudhomme, Bouchard, Tremblay, & Despres, 1996). Therefore, assessments based on BMI and waist circumference can be helpful in determining the risk for chronic health problems due to excessive weight accumulation as measured by two different methods.
J. Implications for Future Analysis

Up to this point, much of the empirical support regarding the causal relationship between obesity, depression, and inflammation has been inconclusive because of the cross-sectional design of the past research. Therefore, it is important to have more comprehensive study designs and analytical methods to better determine the pathways linking these variables. Additional studies on diverse sample populations using structural equation modeling (SEM) and path analysis may be particularly helpful in determining the best statistical fit of various proposed models pertaining to obesity, depression, and inflammation. In addition to evaluating the model that provides the best fit to the data, SEM could also be utilized to specify subgroups for which a particular model is more valid (Hrabosky & Thomas, 2008). Future studies in this area should also include longitudinal, prospective analysis of these variables to determine their relationship over time and better understand the proposed mechanisms and association between these factors. Additional data is also needed to analyze the nature of these variables in the context of lifestyle interventions such as dietary and exercise modifications.
CHAPTER 3

METHODS

A. Study Design

This study was a cross-sectional analysis of the data gathered from a sub-study of the Adventist Health Study-2 (AHS-2) including the Psychosocial Manifestations of Religion Sub-study (PsyMRS) and the Biological Manifestations of Religion Sub-study (BioMRS), collectively referred to as the Biopsychosocial Religion and Health Study (BRHS). The PsyMRS consisted of approximately 20,000 SDAs randomly selected from the AHS-2 sample that were sent a 20 page religion and health questionnaire, in which about 11,000 of those selected returned the questionnaire. The data collection began in September 2006 and was largely completed by August 2007. Their return rate was higher than originally expected, in which 60% of the participants were Caucasians and 31% were African Americans. One of the key categories of variables assessed from the questionnaire was cumulative risk exposure which included physical, socioeconomic, and psychosocial stress. Variables related to religion were also utilized, as well as other mediating variables to help determine the religion/health connection (Lee et al., 2008).

The BioMRS consisted of approximately 508 individuals at least 35 years of age who were selected from the PsyMRS population. Recruitment for the BioMRS involved an initial letter sent to the participants followed by phone calls. In addition to completing the PsyMRS questionnaire, the BioMRS participants also attended clinics in either Loma Linda, Riverside, or Los Angeles to be assessed for biologic indicators and anthropometric measurements (Lee et al., 2008). All participants provided a waking saliva sample, an overnight urine sample, as well as fasting blood and adipose tissue
samples. They also provided physical and cognitive function data, and were assessed for various measures of allostatic load to determine their overall health risk. Anthropometric measurements were done by graduate students who were trained by personnel familiar with the procedures, and the biological samples were taken by certified phlebotomists and nurses. All laboratory experiments were done by experienced laboratory technicians. Finally, data from the BioMRS participants were utilized in the present study to determine the association between obesity, depression, and inflammation after controlling for demographic, socioeconomic, health behavioral, and health status variables.

B. Measurement of Variables

1. Sociodemographic Variables

Sociodemographic variables included age, gender, and ethnicity. Age was measured in years, and gender was categorized as male or female. Ethnicity was coded as Black or White based on self report. Race and ethnicity were divided into Black (Black/African American, West Indian/Caribbean, African or other Black) and White (White non-Hispanic, Hispanic, Middle Eastern, Asian, Native Hawaiian/other Pacific Islander or American Indian) in the current analysis.

2. Socioeconomic Status

SES was assessed based on the participants’ highest level of education as well as the degree of difficulty meeting family expenses for basic needs in the last year. Education was categorized as follows: (a) grade school or some high school, (b) high school or trade school diploma, (c) some college, an Associate’s degree, or a Bachelor’s degree, and (d) a Master’s or Doctoral degree. The response choices for assessing the
degree of difficulty meeting family expenses were not at all, a little, somewhat, fairly, and very.

3. Health Behavior Variables

Behavioral variables included exercise, vegetarian diet consumption, smoking, and alcohol consumption. Exercise was measured by the number of times per week that the participant engaged in regular vigorous activities using previously validated questions (Singh, Fraser, Knutsen, Lindsted, & Bennett, 2001; Singh, Tonstad, Abbey, & Fraser, 1996). A 14-item food frequency questionnaire was used to assess the participants’ dietary intake, and vegetarian status was assessed by whether or not participants consumed (a) red meats, (b) turkey or chicken, or (c) fish over the last 12 months as three separate questionnaire items. A score of 1 indicated that participants never or rarely consumed the food items mentioned above, and a score of 2 indicated that participants consumed any of these foods at least 1 time per month. All values of 1 were coded as vegetarians, and all values of 2 were coded as non-vegetarians. Smoking status and alcohol consumption were assessed based on whether or not the participants currently smoked or consumed alcoholic beverages at least once a week. However, since there was only 1 current smoker and 18 participants who consumed alcohol more than 1 time per week, we did not control for these variables in the analyses. Results did not change substantially when analyses were run with alcohol included as a control variable (data not shown).

4. Health Status Variables

The participants’ health status was assessed based on their risk of obesity, depression, as well as the presence of inflammatory conditions. Obesity levels were
assessed in two ways. First, the subjects’ body mass index (BMI) was determined based on their height and weight as measured by trained personnel. Their weight was measured using the scale function of the Tanita Scale, and their height was measured using a portable stadiometer. Their BMI was then calculated in kilograms per meters squared (reported to the nearest hundredth of a unit) to obtain the height adjusted weight. BMI was used as a continuous variable in this analysis. However, the participants were also classified as normal weight (18.5-24.9 kg/m²), overweight (25-29.9 kg/m²), or obese (>30 kg/m²) to determine the ethnic differences in these body weight categories (as shown in Table 1). In addition, the subject’s waist circumference was assessed to determine the amount of abdominal fat present. The waist circumference was taken midway between the lower rib margin and the iliac crest and measured to the nearest millimeter.

Participant depression levels were based on an 11-item short form of the Center for Epidemiological Studies Depression (CESD) scale which was used in the BRHS (Kohout et al., 1993). Scores from this scale ranged from 0 (not depressed) to 22 (severely depressed). Although each item on the questionnaire was scored on a scale from 0 (rarely or none of the time) to 3 (most or all of the time), options 2 and 3 were combined to make the scoring compatible with the Iowa score form. This questionnaire was then rescaled to match the full 20 item CESD scale based on NHANES data used for validation purposes. Rescaling also allowed comparison against a depression cutoff score of 16 (although the participants’ CESD scores were analyzed as a continuous variable in this study in order to provide more power). Finally, the participants were also assessed for the presence of a disease associated with inflammation based on whether or not they
reported any of the following conditions: type 2 diabetes, stroke, transient ischemic attack, angina pectoris, rheumatoid arthritis, or sleep apnea.

5. **Assessment of Inflammatory Markers**

Serum concentrations of CRP, IL-6, IL-10 and TNF-α were assessed based on fasting blood samples provided by participants. These inflammatory markers were measured by enzyme linked immunosorbent assay (ELISA) kits from Assaypro (CRP), R & D Systems (IL-6), and ThermoScientific (IL-10 and TNF-α). The minimum detectable dose was 100 pg/ml for CRP, 0.039 pg/ml for IL-6, 3 pg/ml for IL-10, and 2 pg/ml for TNF-α. The intra-assay and inter-assay coefficient of variation (CV) were 5.5% and 7.6% for CRP, 6.9% and 7.2% for IL-6, 8.7% and 9.4% for IL-10, and 4.2% and 5.2% for TNF-α. All ELISA kits used were specific for the measurement of natural human CRP, IL-6, IL-10 and TNF-α concentrations in serum, and did not cross-react with other cytokine molecules. Standard ELISA plate readers were used to measure the absorbance of each sample. The assessment protocol for IL-10 and TNF-α are shown in appendix A and B, respectively.

Blood samples were stored in -70°C freezers until they were ready to be tested. In addition, serum sample measurements of inflammatory marker concentrations were tested in duplicate to ensure that the data is accurate and to reduce experimental error. Therefore, the average inflammatory marker concentration values were assessed and compared among the target population. All experimentation involving IL-10 and TNF-α were done by the primary investigator with the assistance of a laboratory technician, while CRP and IL-6 levels were analyzed in a different laboratory by other personnel.
Finally, analyzing these inflammatory markers was important in determining the type of immune response that occurs as a result of changes in the physiological system.

C. Data Management

Double data entry was used for the BioMRS data sheets to ensure that the information was accurate. In addition, the material from the PsyMRS was scanned and checked for inconsistencies such as out-of-range responses.

D. Data Analysis

All statistical analysis was performed using SPSS (version 17). Study population characteristics were stratified by ethnicity. Differences in the study characteristics were determined using independent *t*-tests for continuous variables or chi-square tests for categorical variables (Table 1). In addition, Mann-Whitney tests were also used to determine the differences in inflammation among blacks and whites since the CRP, IL-6, IL-10 and TNF-values were not normally distributed and required log transformation before regression analysis could be performed (Table 2).

Multiple linear regression analysis was utilized to test the association between ethnicity and inflammation among the study population after controlling for demographic, socioeconomic, behavioral and health status variables. Separate models were utilized to test the effects of these variables on inflammation, and each model was analyzed in a stepwise fashion. Ethnicity was included in model 1, while model 2 consisted of age, gender, education, and degree of difficulty meeting family expenses. Model 3 included exercise and vegetarian diet consumption, and BMI was included in model 4 (waist circumference was included in model 4 in a separate regression analysis when BMI was not included). Model 5 additionally assessed whether the participants
were diagnosed with an inflammatory condition. The serum concentrations of log transformed CRP, IL-6, TNF-α, and IL-10 served as continuous outcome variables in separate linear regression equations.

The determinants of depression were assessed by linear regression using predictor variables that were placed into models in a stepwise fashion as illustrated from the regression analysis above. CESD scores served as the continuous outcome variable of the regression equation. The association between depression and inflammatory markers was then assessed through linear regression after controlling for the same predictor variables mentioned above in a stepwise fashion (model 1: depression; model 2: age, gender, ethnicity, education, degree of difficulty meeting family expenses; model 3: exercise, vegetarian diet consumption; model 4: BMI; model 5: diagnosis of an inflammatory condition). Log transformed CRP, IL-6, TNF-α, and IL-10 again served as continuous outcome variables in separate linear regression equations.

It was important to analyze the interactive effect of obesity and depression on inflammation as well, so the product of the centered depression and obesity variables were included in the regression analysis. Centering the depression and obesity variables was necessary to reduce multicollinearity. An interactive effect was determined based on whether the beta coefficient value corresponding to the obesity x depression product differed significantly from zero. Finally, all regression analysis was based on standardized regression coefficient values as well as $R^2$ change values which were used to determine the amount of variability in inflammation and depression explained by the predictor variables in each of the given models.
E. Power Analysis

For the current study, the power estimate was based on the statistical test with the least amount of power to ensure that there was an adequate sample size to detect the effects of the independent variables. Therefore, the association between inflammation and each of the predictors was tested by multiple linear regression analysis based on whether there was an increase in the multiple $R$ when each variable was added. According to G Power 3.0 software based on a small effect size ($f=0.02$), power=80%, and $\alpha=0.05$, the sample size required at least 395 participants. Therefore, a minimum of 395 participants was needed for this study. Finally, because this was a cross-sectional study in which the data from the questionnaires and the blood samples had already been acquired, it was not necessary to compensate for dropout rate.

F. Study Limitations

There were several limitations to this study. First, because of the cross-sectional nature of this study, the causal relationship between the variables could not be determined which reduced the internal validity. In addition, the population of interest included only SDAs consisting mainly of Black and White Adventists, which suggests that the data may not have been representative of the larger population and may compromise external validity. Another limitation of this study is that depression was measured from the CESD scale and subject to self-reporting bias which could influence the reported results and conclusions.

Due to the fact that the participants in this cohort normally maintain healthy lifestyles, they may have been less susceptible to elevated inflammation levels compared to other populations resulting in inflammatory marker concentrations below the minimal
sensitivity values on the ELISA assays. In addition, the median CESD score of this population was 6.45, which is well below the depression cutoff score of 16. The average BMI of the population was 26.8 kg/m², and only 25% of the participants in this study were obese. So there may have been an insufficient number of individuals with high BMI and elevated depression scores for a possible interactive effect on inflammation levels. Therefore, future analysis may require methodological changes in order to account for these factors.

G. Research Ethics

All AHS-2 participants were eligible to participate in the BRHS. However, consent was obtained from each participant before they began the study. All participants were assured that their identities would remain anonymous and that all personal information would be kept confidential. They were informed that no physical harm, danger, or discomfort would be experienced from any of the research procedures, and that they were free to withdraw from the study at any time. Participants were also informed that all anthropometric measurements and biological samples would be taken by adequately trained personnel, and that the samples would be preserved at appropriate storage conditions. If the results showed that the participants were at high risk for health problems, they would be referred to a specialist for the necessary treatment. Finally, the biological and psychosocial manifestations of religion protocol for the BRHS was approved by the Institutional Review Board of Loma Linda University. The form of approval is shown in appendix C.
CHAPTER 4
PUBLISHABLE PAPER

Determinants of Inflammatory Markers in a Bi-ethnic Population

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Referencing is in American Medical Association formatting style and is not in
accordance with the APA specifications of this dissertation as specified by the
journal requirements
Abstract

Background: Inflammation is a common pathophysiological pathway for a number of chronic diseases, and is strongly influenced by sociodemographic factors and lifestyle. Less is known about factors that may influence the inflammatory response in individuals of distinct ethnic backgrounds. Therefore, this study examined the relationship between ethnicity and blood levels of inflammatory markers in a sample of non-smoking church-goers.

Methods: In a cross-sectional investigation, 508 men and women (>35 years old) participated in the Biopsychosocial substudy of the Adventist Health Study 2 (62% White, 38% Black). The contribution of socioeconomic status (education level and difficulty meeting expenses for basic needs) and health covariates (exercise, vegetarian or other type of diet, body mass index, and presence of inflammatory conditions) toward serum levels of c-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) was assessed with linear regression models. Levels of interleukin-10 (IL-10), an anti-inflammatory marker, were also assessed.

Results: Blacks showed higher levels of CRP and IL-6 than Whites. Controlling for sociodemographic and health variables attenuated the ethnic difference in CRP while IL-6 levels remained higher in Blacks than in Whites (β = 0.118; 95% confidence interval = 0.014 – 0.206; p = 0.025). Ethnic differences in IL-10 and TNF-α were not found. Vegetarian diet was associated with lower CRP levels while exercise frequency was associated with higher IL-10 levels.
**Conclusion:** Higher susceptibility of Blacks to inflammatory diseases may reflect higher IL-6, which could be important in assessing health disparities among Blacks and Whites. Vegetarian diet and exercise may counteract effects of disparities.
INTRODUCTION

Ethnic differences in chronic disease incidence have been widely studied, with Blacks having a higher risk of hypertension, stroke and renal failure compared to Whites.\textsuperscript{1} The reason for this disparity has not been completely elucidated but may be due to increased prevalence of disease risk factors among Blacks. Inflammation has been determined to be an important factor associated with chronic diseases, and is often indexed by elevated circulating levels of C-reactive protein (CRP) and pro-inflammatory cytokines.\textsuperscript{2} Behavioral factors have a direct impact on inflammatory processes.\textsuperscript{3}

Large epidemiologic studies have shown that CRP and pro-inflammatory cytokines increase with age in both men and women.\textsuperscript{4,6} In addition, several studies have found that females have higher levels of CRP compared to men, possibly due to higher amounts of fat tissue among women.\textsuperscript{7-8} The association between ethnicity and inflammation has been analyzed as well, showing that Blacks often have higher CRP than Whites.\textsuperscript{9-11} Other inflammatory molecules include interleukin 6 (IL-6) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) which are associated with a pro-inflammatory response while IL-10 has anti-inflammatory effects.\textsuperscript{12} Some studies have shown that Blacks have higher IL-6 levels than Whites,\textsuperscript{11,13} but it is unclear whether IL-10 and TNF-\(\alpha\) levels vary according to ethnic background.\textsuperscript{13-15}

Ethnic specific differences in inflammation may have diverse determinants. For instance, low socioeconomic status (SES) has been associated with increased levels of CRP, IL-6 and TNF-\(\alpha\).\textsuperscript{16-17} It is therefore possible that SES may partially explain why certain ethnicities are at higher risk for inflammatory problems. Health behavior factors also play a role in inflammation. For example, physical activity has been associated with
lower circulating levels of CRP and IL-6 in both healthy and patient populations18 and has been shown to increase concentrations of anti-inflammatory cytokines (such as IL-1 receptor antagonist and IL-10) to lower the inflammatory response.19 In addition, diets rich in fruits, vegetables, whole grains and nuts have been associated with reductions in CRP and IL-6 levels, while diets high in refined grains, red meat, and high fat dairy have been directly correlated with increased inflammation.20-21

Alcohol consumption has also been associated with lower levels of CRP among moderate drinkers compared to non-drinkers and heavy drinkers, a U-shaped pattern.22-23 Cigarette smoking has been found to be a potent risk factor for increased levels of low-grade inflammation based on elevated CRP and IL-6 levels.24-26

One of the populations in which determinants of chronic disease have been studied is Seventh-day Adventists, a conservative religious group that abstain from alcohol and tobacco.27-28 Thus, the population avoids some of the major identified factors involved in increasing inflammation and may provide the opportunity to illuminate the role of other factors, including ethnicity and lifestyle factors. In the present study ethnic differences in inflammation were assessed among a population of church going Adventists. We also determined whether the ethnicity-inflammation relationship is affected by demographic (age and gender), SES (education level and difficulty meeting expenses for basic needs), behavioral (exercise and vegetarian diet) or health status variables (body mass index [BMI] and diagnosis of an inflammatory condition). The inflammatory markers that were analyzed include both pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (IL-10) cytokines as well as an acute phase protein (CRP).
METHODS

A. Study Population

This study was a cross-sectional analysis of the data gathered from a sub-study of the Adventist Health Study-2 (AHS-2) referred to as the Biopsychosocial Religion and Health Study (BRHS).29 Approximately 20,000 subjects randomly selected from the 96,000 case AHS-2 sample were sent a 20 page religion and health questionnaire, and about 11,000 returned the questionnaire. The data collection began in September 2006 and was largely completed by August 2007. The study population consisted mainly of Black and White, Seventh-day Adventist males and females who were at least 35 years of age. In addition to completing the questionnaire, 508 BRHS participants also attended study-specific clinics in Loma Linda, Riverside, or Los Angeles to be assessed for biologic indicators and anthropometric measurements. Cytokine analysis was based on fasting blood samples provided by the participants on the day they attended the clinic. The institutional review board at Loma Linda University approved the study protocol, and informed consent was obtained from all participants.

B. Measurement of Variables

1. Sociodemographic Variables

Sociodemographic variables included age, gender, and ethnicity. Ethnicity was coded as Black or White based on self report. Race and ethnicity were divided into Black (Black/African American, West Indian/Caribbean, African or other Black) and White (White non-Hispanic, Hispanic, Middle Eastern, Asian, Native Hawaiian/other Pacific Islander or American Indian) in the current analysis.
2. Socioeconomic Status

SES was assessed based on the participants' highest level of education as well as the degree of difficulty meeting family expenses for basic needs in the last year. Education was categorized as follows: (a) grade school or some high school, (b) high school or trade school diploma, (c) some college, an Associate's degree, or a Bachelor's degree, and (d) a Master's or Doctoral degree. The response choices for assessing the degree of difficulty meeting family expenses were not at all, a little, somewhat, fairly, and very.

3. Health Behavior Variables

Behavioral variables included exercise, vegetarian diet, smoking, and alcohol consumption. Exercise was measured by the number of times per week that the participant engaged in regular vigorous activities using previously validated questions.30, 31 A 14-item food frequency questionnaire was used to assess the participants' dietary intake, and vegetarian status was assessed by whether or not participants consumed (a) red meats, (b) turkey or chicken, or (c) fish over the last 12 months as three separate questionnaire items. A score of 1 indicated that participants never or rarely consumed the food items mentioned above, and a score of 2 indicated that participants consumed any of these foods at least 1 time per month. All values of 1 were coded as vegetarians, and all values of 2 were coded as non-vegetarians. Smoking status and alcohol consumption were assessed based on whether or not the participants currently smoked or consumed alcoholic beverages at least once a week. However, since there was only 1 current smoker and 18 participants who consumed alcohol more than 1 time per week, we did not
control for these variables in the analyses. Results did not change substantially when analyses were run with alcohol included as a control variable (data not shown).

4. Health Status Variables

The participants’ health status was assessed based on their height, weight, and calculated BMI as well as the presence of inflammatory conditions. The participants were assessed for the presence of a disease associated with inflammation based on whether or not they reported any of the following conditions: type 2 diabetes, stroke, transient ischemic attack, angina pectoris, rheumatoid arthritis, or sleep apnea.

5. Inflammatory Markers

Serum concentrations of CRP, IL-6, IL-10 and TNF-α were assessed based on fasting blood samples provided by participants that were processed and stored in -70°C freezers until they were ready to be tested. These inflammatory markers were measured in duplicate by enzyme linked immunosorbent assay (ELISA) kits from Assaypro (CRP), R & D Systems (IL-6), and ThermoScientific (IL-10 and TNF-α). The minimum detectable dose was 100 pg/ml for CRP, 0.039 pg/ml for IL-6, 3 pg/ml for IL-10, and 2 pg/ml for TNF-α. The intra-assay and inter-assay coefficient of variation (CV) were 5.5% and 7.6% for CRP, 6.9% and 7.2% for IL-6, 8.7% and 9.4% for IL-10, and 4.2% and 5.2% for TNF-α. All ELISA kits used were specific for the measurement of natural human CRP, IL-6, IL-10 and TNF-α concentrations in serum, and did not cross-react with other cytokine molecules. Standard ELISA plate readers were used to measure the absorbance of each sample.
C. Statistical Analysis

All statistical analysis was performed using SPSS (version 17). Ethnic differences in the study characteristics were determined using independent $t$ tests for continuous variables or chi-square tests for categorical variables (Table 1). In addition, Mann-Whitney tests were also used to determine the differences in inflammation between Blacks and Whites since the CRP, IL-6, IL-10 and TNF-values were not normally distributed and required log transformation before regression analysis could be performed (Table 2).

Multiple linear regression analysis was utilized to test the association between ethnicity and inflammation among the study population after controlling for demographic, socioeconomic, behavioral and health status variables. Separate models were utilized to test the effects of these variables on inflammation, and each model was analyzed in a stepwise fashion. Ethnicity was included in model 1, while model 2 added age, gender, education, and degree of difficulty meeting family expenses. Model 3 additionally added exercise and vegetarian diet consumption, and BMI was included in model 4. Model 5 added whether the participants were diagnosed with a medical condition. The serum concentrations of log transformed CRP, IL-6, TNF-$\alpha$, and IL-10 served as continuous outcome variables in separate linear regression equations. Cytokine concentrations below the limit of detection of were given a value of 0.001 representing a near zero value as demonstrated in a previous study.\textsuperscript{32} The regression analysis was based on standardized regression coefficient values as well as $R^2$ change values which were used to determine the amount of variability in inflammation explained by the predictor variables in each of the given models.
RESULTS

The study population characteristics and differences between Blacks and Whites are shown in Table 1. The mean age of the population was 68.8 years (range: 36 – 102); 63% of the participants were women and 37% were Black. Black participants were younger, achieved a lower level of education, and had more difficulty meeting family expenses for basic needs in the last year. Blacks were also less likely to consume a vegetarian diet, and had a higher mean BMI and waist diameter value. Table 2 shows that higher levels of CRP and IL-6 were present among Black participants compared to the White participants while IL-10 and TNF-α levels did not differ between the two groups.

Regression coefficients for the association of each predictor variable with serum concentrations of CRP, IL-6, IL-10 and TNF-α are illustrated in Table 3, 4, 5 and 6, respectively. CRP levels were found to be significantly higher among Blacks than Whites, but this relationship was no longer significant after controlling for the demographic and SES variables in model 2. After controlling for all covariates, CRP levels were positively associated with age, female gender, BMI, and non-vegetarianism (Table 3). IL-6 was positively associated with age, BMI, and Black ethnicity after controlling for all other factors. Increased exercise frequency was associated with a decrease in IL-6 levels, while consumption of a vegetarian diet was associated with a decrease in IL-10 levels. However, these relationships were no longer significant after controlling for BMI (Table 4 and 5). Nevertheless, IL-10 was positively associated with exercise frequency after controlling for all other covariates.
The $R^2$ change values were also provided in Tables 3-6. A significant proportion of variability in CRP was explained by ethnicity (F (1, 432) = 3.979, p = 0.047), age, gender, education, and difficulty meeting family expenses for basic needs (F (4, 428) = 5.409, p = 0.000), health behavior variables (F (2, 426) = 9.298, p = 0.000), and BMI (F (1, 425) = 53.844, p = 0.000). Likewise, ethnicity (F (1, 432) = 6.511, p = 0.011), age, gender, education, and difficulty meeting family expenses for basic needs (F (4, 428) = 4.042, p = 0.003), health behavior variables (F (2, 426) = 3.090, p = 0.047) and BMI (F (1, 425) = 8.644, p = 0.003) also explained significant amounts of variability in IL-6 levels. Finally, health behavior variables (mainly exercise frequency) explained a significant proportion of IL-10 variability based on the regression analysis provided (F (2, 397) = 5.137, p = 0.006).

DISCUSSION

This study has shown that IL-6 may serve as an important ethnic-specific cytokine that is higher among Blacks than Whites, while the other inflammatory markers or anti-inflammatory marker (IL-10) did not differ between Blacks and Whites. Healthy lifestyle behaviors were associated with a lower inflammatory status, after controlling for ethnic differences, as increased exercise frequency was associated with increased IL-10 levels, and consumption of a vegetarian diet was associated with lower CRP levels.

The present study provides further evidence of an association between ethnicity and inflammation, in line with other studies that have found higher IL-6 levels among Blacks compared to Whites. However, these studies did not control for variables related to lifestyle practice (such as diet or exercise) when assessing the ethnic differences in IL-6, and may be confounded by differences between Blacks and Whites in
regard to these factors. Earlier study designs were based on a much smaller sample size than was used for the current investigation.

Adipose tissue synthesizes inflammatory markers such as IL-6, and increased body mass has been associated with higher inflammation levels among Blacks.\(^{35-36}\) However, the ethnic specific difference in IL-6 was significant even after controlling for BMI in the current study, so other factors may be responsible for this relationship. One possible reason for the ethnic variations in the level of this cytokine is due to differences in cytokine gene polymorphisms. It has been suggested that specific allelic variations in the regulatory regions of inflammatory cytokine genes may alter the expression of some cytokines.\(^{37}\) One of the genotypes that results in high IL-6 production (G/G IL-6 genotype) has been predominantly found in Blacks, and may explain higher levels of this cytokine among this ethnic group.\(^{38}\)

Although there were higher CRP levels among Blacks in the present study, this association was no longer significant after controlling for the other covariates. Studies among the US population have shown that there are higher CRP levels among Blacks in relation to Whites.\(^{7,39-41}\) However, like the results from the present study, the ethnicity-CRP association found in some of the investigations ultimately disappeared after controlling for various health-related factors.\(^{42-43}\) In the current study, the ethnicity-CRP relationship was largely attenuated after controlling for vegetarian diet consumption as well as exercise frequency, and even more attenuated after controlling for BMI. Therefore, future studies should investigate lifestyle components as well as other variables associated with obesity that may serve as important mediating factors between ethnicity and CRP.
No significant differences in IL-10 and TNF-α levels were found between Black and White subjects in the present study. Although lower levels of IL-10 among Blacks compared to Whites have been reported previously, the analysis was based on stimulated peripheral blood samples rather than basal cytokine levels in the blood. Therefore, no direct evidence of ethnic differences in circulating IL-10 levels was shown. As far as ethnic differences in TNF-α are concerned, the results are mixed. A study by Kalra et al found that TNF-α levels were higher among Blacks in relation to Whites from the UK, while Elkind et al found no significant difference in TNF-α levels among Blacks and Whites from the US. Therefore, geographic location and environmental factors may play an important role in the onset of inflammation among different ethnic groups.

Based on the results provided, engaging in exercise and consuming a vegetarian diet did appear to provide health benefits. We found that a vegetarian diet was associated with a reduction in CRP which is in accordance with previous research showing that long-term vegetarians have a lower risk of coronary heart disease and an improved antioxidant and inflammatory status compared to non-vegetarians. In addition, greater amounts of regular physical activity have been associated with elevated IL-10 levels in healthy older males, and aerobic exercise training has been reported to exert anti-inflammatory effects in type 2 diabetics due to increases in IL-10 concentrations. Specifically, high intensity exercise has been more strongly associated with increased IL-10 levels when compared to moderate and light intensity exercises among healthy, well-trained participants, suggesting that more vigorous exercise may provide stronger anti-inflammatory effects.
Strengths and Limitations

This study assessed pro-inflammatory and anti-inflammatory cytokines that were not examined together in previous studies. The absence of cigarette smokers and low amount of alcohol consumption allowed the confounding effects of smoking and excess alcohol on inflammatory markers to be avoided. Finally, the current study sample size provided sufficient power for detecting associations between the study variables.

This was a cross-sectional study so causality between ethnicity and other variables and levels of inflammatory markers cannot be assumed. There were differences in baseline factors between Blacks and Whites. We adjusted for these factors, but residual confounding may remain. The study population consisted of only Adventist church goers and generalizability to the larger population may be limited. Genetic markers of the inflammatory cytokines were not examined, limiting our analysis to cytokine concentrations rather than specific gene activity. More importantly, this population of church goers may have been less susceptible to increased inflammation based on their overall health and lifestyle practices, making it difficult to detect some of the inflammatory markers. Therefore, since the TNF-α concentration of many of the study participants was below the limit of detection, future analysis of inflammation using TNF-receptor 1 as a surrogate marker for TNF-α may be preferable since TNF-α has a short half-life (~15 minutes) and can be difficult to detect among certain populations using standard ELISA kits.48

Conclusion

Inflammatory based risk for health problems may vary according to ethnicity and other demographic factors. Yet inflammation levels also seem to be influenced by other
variables such as diet, exercise, and overall body mass. Interventions that focus on improving these health behaviors may therefore be the key to reducing the risk for chronic diseases.

ACKNOWLEDGEMENTS

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References


Table 1. Main characteristics of the study population according to ethnicity

<table>
<thead>
<tr>
<th>Factor</th>
<th>Whites (n =314)</th>
<th>Blacks (n =191)</th>
<th>P-value (Whites vs Blacks)</th>
<th>Total Population (n=505)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Mean (SD))</td>
<td>71.4 (11.6)</td>
<td>64.4 (10.1)</td>
<td>0.000</td>
<td>68.8 (11.6)</td>
</tr>
<tr>
<td>Gender (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>128 (41.0)</td>
<td>58 (30.5)</td>
<td></td>
<td>186 (37.1)</td>
</tr>
<tr>
<td>Female</td>
<td>184 (59.0)</td>
<td>132 (69.5)</td>
<td>0.018</td>
<td>316 (62.9)</td>
</tr>
<tr>
<td>Education (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade school or some high school</td>
<td>5 (1.6)</td>
<td>5 (2.7)</td>
<td></td>
<td>10 (2.0)</td>
</tr>
<tr>
<td>High school or Trade school diploma</td>
<td>11 (3.5)</td>
<td>19 (10.1)</td>
<td></td>
<td>30 (6.0)</td>
</tr>
<tr>
<td>Some college, Associate’s degree, or Bachelor’s degree</td>
<td>146 (47.1)</td>
<td>124 (66.0)</td>
<td></td>
<td>270 (54.2)</td>
</tr>
<tr>
<td>Master’s or Doctoral degree</td>
<td>148 (47.7)</td>
<td>40 (21.3)</td>
<td>0.000</td>
<td>188 (37.8)</td>
</tr>
<tr>
<td>Difficulty meeting family expenses for basic needs in last year (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td>264 (85.2)</td>
<td>138 (74.6)</td>
<td></td>
<td>402 (81.2)</td>
</tr>
<tr>
<td>A little</td>
<td>26 (8.4)</td>
<td>25 (13.5)</td>
<td></td>
<td>51 (10.3)</td>
</tr>
<tr>
<td>Somewhat</td>
<td>8 (2.6)</td>
<td>6 (3.2)</td>
<td></td>
<td>14 (2.8)</td>
</tr>
<tr>
<td>Fairly</td>
<td>6 (1.9)</td>
<td>10 (5.4)</td>
<td></td>
<td>16 (3.2)</td>
</tr>
<tr>
<td>Very</td>
<td>6 (1.9)</td>
<td>6 (3.2)</td>
<td>0.043</td>
<td>12 (2.4)</td>
</tr>
<tr>
<td>Alcohol consumption within the last 12 months (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumed 3 drinks or less per month</td>
<td>299 (95.5)</td>
<td>187 (97.9)</td>
<td></td>
<td>486 (96.4)</td>
</tr>
<tr>
<td>Consumed 4 drinks per month or more</td>
<td>14 (4.5)</td>
<td>4 (2.1)</td>
<td>0.163</td>
<td>18 (3.6)</td>
</tr>
<tr>
<td>Frequency of Vigorous Activities per Week (n (%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never or less than once per week</td>
<td>108 (36.4)</td>
<td>63 (35.4)</td>
<td></td>
<td>171 (36.0)</td>
</tr>
<tr>
<td>1-2 times per week</td>
<td>51 (17.2)</td>
<td>29 (16.3)</td>
<td></td>
<td>80 (16.8)</td>
</tr>
<tr>
<td>3-4 times per week</td>
<td>81 (27.3)</td>
<td>66 (37.1)</td>
<td></td>
<td>147 (30.9)</td>
</tr>
<tr>
<td>5 or more times per week</td>
<td>57 (19.2)</td>
<td>20 (11.2)</td>
<td>0.048</td>
<td>77 (16.2)</td>
</tr>
<tr>
<td>Vegetarian Diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>152 (48.4)</td>
<td>137 (71.7)</td>
<td></td>
<td>289 (57.2)</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>162 (51.6)</td>
<td>54 (28.3)</td>
<td>0.000</td>
<td>216 (42.8)</td>
</tr>
<tr>
<td>BMI (Mean (SD))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Weight (n (%))</td>
<td>151 (48.1)</td>
<td>61 (31.9)</td>
<td></td>
<td>212 (42.0)</td>
</tr>
<tr>
<td>Overweight (n (%))</td>
<td>107 (34.1)</td>
<td>62 (32.5)</td>
<td></td>
<td>169 (33.5)</td>
</tr>
<tr>
<td>Obese (n (%))</td>
<td>56 (17.8)</td>
<td>68 (35.6)</td>
<td>0.000</td>
<td>124 (24.6)</td>
</tr>
<tr>
<td>Waist Diameter (Mean (SD))</td>
<td>35.9 (5.5)</td>
<td>37.6 (6.3)</td>
<td>0.004</td>
<td>36.6 (5.9)</td>
</tr>
<tr>
<td>Diagnosed with any of the following conditions (n (%)):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 Diabetes, Stroke, TIA, Angina Pectoris, Rheumatoid Arthritis, Sleep Apnea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Disease Diagnosis</td>
<td>236 (75.2)</td>
<td>121 (65.4)</td>
<td></td>
<td>357 (71.5)</td>
</tr>
<tr>
<td>Diagnosed with Disease</td>
<td>78 (24.8)</td>
<td>64 (34.6)</td>
<td>0.020</td>
<td>142 (28.5)</td>
</tr>
</tbody>
</table>

SD = standard deviation
### Table 2. Ethnic differences in inflammatory marker concentrations

<table>
<thead>
<tr>
<th>Inflammatory Marker Concentrations (Median (IQR))</th>
<th>Total Population (n=493)</th>
<th>Whites (n=306)</th>
<th>Blacks (n=187)</th>
<th>P-value (Whites vs Blacks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/ml)</td>
<td>0.10 (0.04-0.24)</td>
<td>0.09 (0.04-0.21)</td>
<td>0.12 (0.05-0.29)</td>
<td>0.010</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>2.72 (1.59-5.62)</td>
<td>2.34 (1.53-4.52)</td>
<td>3.37 (1.95-7.40)</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>5.38 (3.24-8.80)</td>
<td>5.11 (2.96-8.80)</td>
<td>5.83 (3.74-9.03)</td>
<td>0.082</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.01 (0.01-7.49)</td>
<td>0.001 (0.001-8.07)</td>
<td>0.001 (0.001-7.38)</td>
<td>0.668</td>
</tr>
</tbody>
</table>

IQR = interquartile range, CRP = c-reactive protein, IL-6 = interleukin-6, IL-10 = interleukin 10, TNF-α = tumor necrosis factor alpha
Table 3. Regression analysis models illustrating the association between various predictor variables and CRP

<table>
<thead>
<tr>
<th>Factor</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>B (SE)</td>
<td>P-value</td>
<td>β</td>
<td>B (SE)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blacks vs Whites</td>
<td>0.096</td>
<td>0.108</td>
<td>0.047</td>
<td>0.093</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>(0.054)</td>
<td>(0.057)</td>
<td></td>
<td></td>
<td>(0.057)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>0.108</td>
<td>0.005</td>
<td>0.029</td>
<td>0.093</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>(0.002)</td>
<td>(0.002)</td>
<td></td>
<td></td>
<td>(0.002)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>0.175</td>
<td>0.193</td>
<td>0.000</td>
<td>0.093</td>
<td>0.105</td>
</tr>
<tr>
<td>Females vs Males</td>
<td>(0.054)</td>
<td>(0.057)</td>
<td></td>
<td></td>
<td>(0.057)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td>-0.059</td>
<td>-0.049</td>
<td>0.247</td>
<td>-0.008</td>
<td>-0.007</td>
</tr>
<tr>
<td></td>
<td>(0.042)</td>
<td>(0.043)</td>
<td></td>
<td>(0.043)</td>
<td></td>
</tr>
<tr>
<td><strong>Difficulty meeting family expenses in the</strong></td>
<td>0.009</td>
<td>0.006</td>
<td>0.845</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>last year**</td>
<td>(0.029)</td>
<td>(0.028)</td>
<td></td>
<td>(0.028)</td>
<td></td>
</tr>
<tr>
<td><strong>Frequency of vigorous activities per week</strong></td>
<td>-0.110</td>
<td>-0.026</td>
<td>0.022</td>
<td>-0.059</td>
<td>-0.014</td>
</tr>
<tr>
<td></td>
<td>(0.011)</td>
<td>(0.011)</td>
<td></td>
<td>(0.011)</td>
<td></td>
</tr>
<tr>
<td><strong>Vegetarian diet</strong></td>
<td>-0.170</td>
<td>-0.185</td>
<td>0.000</td>
<td>-0.108</td>
<td>-0.118</td>
</tr>
<tr>
<td>Vegetarian vs Non-Vegetarian</td>
<td>(0.053)</td>
<td>(0.051)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.344</td>
<td>0.336</td>
<td>0.000</td>
<td>0.341</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>(0.005)</td>
<td>(0.005)</td>
<td></td>
<td>(0.005)</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory Condition Diagnosis</strong></td>
<td>0.012</td>
<td>0.014</td>
<td>0.803</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease vs No Disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R² Change</strong></td>
<td>0.009</td>
<td>0.048</td>
<td>0.039</td>
<td>0.102</td>
<td>0.000</td>
</tr>
</tbody>
</table>

β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
<table>
<thead>
<tr>
<th>Factor</th>
<th>Model 1 β (SE)</th>
<th>P-value</th>
<th>Model 2 β (SE)</th>
<th>P-value</th>
<th>Model 3 β (SE)</th>
<th>P-value</th>
<th>Model 4 β (SE)</th>
<th>P-value</th>
<th>Model 5 β (SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity Blacks vs Whites</td>
<td>0.122 (0.044)</td>
<td>0.011</td>
<td>0.165 (0.047)</td>
<td>0.001</td>
<td>0.154 (0.047)</td>
<td>0.003</td>
<td>0.124 (0.048)</td>
<td>0.015</td>
<td>0.113 (0.048)</td>
<td>0.016</td>
</tr>
<tr>
<td>Age</td>
<td>0.180 (0.002)</td>
<td>0.007</td>
<td>0.000 (0.002)</td>
<td>0.163</td>
<td>0.006 (0.002)</td>
<td>0.001</td>
<td>0.175 (0.002)</td>
<td>0.007</td>
<td>0.000 (0.002)</td>
<td>0.167</td>
</tr>
<tr>
<td>Gender Females vs Males</td>
<td>-0.005 (0.045)</td>
<td>0.924</td>
<td>-0.017 (0.045)</td>
<td>0.924</td>
<td>-0.015 (0.044)</td>
<td>0.732</td>
<td>-0.012 (0.044)</td>
<td>0.805</td>
<td>-0.010 (0.045)</td>
<td>0.843</td>
</tr>
<tr>
<td>Education</td>
<td>-0.072 (0.035)</td>
<td>-0.049</td>
<td>0.157 (0.036)</td>
<td>-0.050</td>
<td>-0.034 (0.036)</td>
<td>0.338</td>
<td>-0.047 (0.035)</td>
<td>0.363</td>
<td>-0.045 (0.035)</td>
<td>0.318</td>
</tr>
<tr>
<td>Difficulty meeting family expenses in the last year</td>
<td>-0.042 (0.024)</td>
<td>-0.021</td>
<td>0.388 (0.024)</td>
<td>-0.044</td>
<td>-0.022 (0.024)</td>
<td>0.356</td>
<td>-0.049 (0.024)</td>
<td>0.303</td>
<td>-0.051 (0.024)</td>
<td>0.290</td>
</tr>
<tr>
<td>Frequency of vigorous activities per week</td>
<td>-0.116 (0.009)</td>
<td>-0.022</td>
<td>0.018 (0.009)</td>
<td>-0.094</td>
<td>-0.018 (0.009)</td>
<td>0.055</td>
<td>-0.091 (0.009)</td>
<td>0.017</td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td>Vegetarian diet Vegetarian vs Non-Vegetarian</td>
<td>-0.029 (0.044)</td>
<td>-0.026</td>
<td>0.556 (0.044)</td>
<td>-0.003</td>
<td>-0.002 (0.044)</td>
<td>0.957</td>
<td>0.001 (0.045)</td>
<td>0.988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.147 (0.004)</td>
<td>0.013</td>
<td>0.003 (0.004)</td>
<td>0.141</td>
<td>0.003 (0.004)</td>
<td>0.012</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory Condition Diagnosis Disease vs No Disease</td>
<td>0.030 (0.050)</td>
<td>0.030</td>
<td>0.554</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2$ Change: 0.015, 0.036, 0.014, 0.019, 0.001

β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
Table 5. Regression analysis models illustrating the association between various predictor variables and IL-10

<table>
<thead>
<tr>
<th>Factor</th>
<th>Model 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>B (SE)</td>
<td>P-value</td>
<td>$\beta$</td>
<td>B (SE)</td>
<td>P-value</td>
<td>$\beta$</td>
<td>B (SE)</td>
<td>P-value</td>
</tr>
<tr>
<td>Ethnicity Blacks vs Whites</td>
<td>0.054</td>
<td>0.080</td>
<td>0.275</td>
<td>0.051</td>
<td>0.074</td>
<td>0.344</td>
<td>0.064</td>
<td>0.16</td>
<td>0.064</td>
</tr>
<tr>
<td>Age</td>
<td>-0.009</td>
<td>-0.01</td>
<td>0.858</td>
<td>0.024</td>
<td>0.001</td>
<td>0.646</td>
<td>0.031</td>
<td>0.002</td>
<td>0.558</td>
</tr>
<tr>
<td>Gender Females vs Males</td>
<td>0.006</td>
<td>0.008</td>
<td>0.911</td>
<td>0.033</td>
<td>0.047</td>
<td>0.532</td>
<td>0.037</td>
<td>0.054</td>
<td>0.473</td>
</tr>
<tr>
<td>Education</td>
<td>-0.026</td>
<td>-0.028</td>
<td>0.629</td>
<td>-0.022</td>
<td>-0.024</td>
<td>0.685</td>
<td>-0.019</td>
<td>-0.021</td>
<td>0.723</td>
</tr>
<tr>
<td>Frequency of vigorous activities per week</td>
<td>0.137</td>
<td>0.041</td>
<td>0.008</td>
<td>0.148</td>
<td>0.045</td>
<td>0.005</td>
<td>0.147</td>
<td>0.044</td>
<td>0.005</td>
</tr>
<tr>
<td>Vegetarian diet Vegetarian vs Non-Vegetarian</td>
<td>-0.102</td>
<td>-0.146</td>
<td>0.049</td>
<td>-0.091</td>
<td>-0.130</td>
<td>0.084</td>
<td>-0.092</td>
<td>-0.132</td>
<td>0.082</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.073</td>
<td>0.010</td>
<td>0.171</td>
<td>0.076</td>
<td>0.010</td>
<td>0.168</td>
<td>0.082</td>
<td>0.085</td>
<td>0.168</td>
</tr>
<tr>
<td>Inflammatory Condition Disease vs No Disease</td>
<td>-0.012</td>
<td>-0.018</td>
<td>0.832</td>
<td>0.005</td>
<td>0.005</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

$\beta = $ standardized regression coefficient, $B =$ unstandardized regression coefficient, $SE =$ standard error
Table 6. Regression analysis models illustrating the association between various predictor variables and TNF-α

<table>
<thead>
<tr>
<th>Factor</th>
<th>Model 1</th>
<th></th>
<th>P-value</th>
<th>Model 2</th>
<th></th>
<th>P-value</th>
<th>Model 3</th>
<th></th>
<th>P-value</th>
<th>Model 4</th>
<th></th>
<th>P-value</th>
<th>Model 5</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity Blacks vs Whites</td>
<td>β -0.019</td>
<td>B (SE) -0.081</td>
<td>0.699</td>
<td>β -0.003</td>
<td>B (SE) -0.011</td>
<td>0.962</td>
<td>β -0.004</td>
<td>B (SE) -0.017</td>
<td>0.939</td>
<td>β -0.048</td>
<td>B (SE) -0.011</td>
<td>0.837</td>
<td>β -0.047</td>
<td>B (SE) -0.011</td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>β -0.012</td>
<td>0.009</td>
<td>0.820</td>
<td>β -0.011</td>
<td>0.002</td>
<td>0.820</td>
<td>β -0.008</td>
<td>0.001</td>
<td>0.820</td>
<td>β -0.011</td>
<td>0.009</td>
<td>0.820</td>
<td>β -0.011</td>
<td>0.009</td>
<td>0.820</td>
</tr>
<tr>
<td>Gender Females vs Males</td>
<td>β -0.101</td>
<td>0.210</td>
<td>0.050</td>
<td>β -0.010</td>
<td>0.010</td>
<td>0.210</td>
<td>β -0.099</td>
<td>0.010</td>
<td>0.210</td>
<td>β -0.099</td>
<td>0.010</td>
<td>0.210</td>
<td>β -0.099</td>
<td>0.010</td>
<td>0.210</td>
</tr>
<tr>
<td>Education</td>
<td>β 0.037</td>
<td>0.165</td>
<td>0.490</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.490</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.490</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.490</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.490</td>
</tr>
<tr>
<td>Difficulty meeting family expenses in the last year</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.868</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.868</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.868</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.868</td>
<td>β -0.008</td>
<td>0.115</td>
<td>0.868</td>
</tr>
<tr>
<td>Frequency of vigorous activities per week</td>
<td>β -0.003</td>
<td>0.045</td>
<td>0.957</td>
<td>β 0.002</td>
<td>0.045</td>
<td>0.957</td>
<td>β 0.002</td>
<td>0.045</td>
<td>0.957</td>
<td>β 0.002</td>
<td>0.045</td>
<td>0.957</td>
<td>β 0.002</td>
<td>0.045</td>
<td>0.957</td>
</tr>
<tr>
<td>Vegetarian diet Vegetarian vs Non-Vegetarian</td>
<td>β -0.010</td>
<td>0.214</td>
<td>0.847</td>
<td>β -0.005</td>
<td>0.214</td>
<td>0.847</td>
<td>β -0.005</td>
<td>0.214</td>
<td>0.847</td>
<td>β -0.005</td>
<td>0.214</td>
<td>0.847</td>
<td>β -0.005</td>
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<td>0.847</td>
</tr>
<tr>
<td>Body Mass Index</td>
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<td>0.021</td>
<td>0.535</td>
<td>β 0.034</td>
<td>0.022</td>
<td>0.535</td>
<td>β 0.034</td>
<td>0.022</td>
<td>0.535</td>
<td>β -0.001</td>
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<td>0.535</td>
<td>β -0.001</td>
<td>0.021</td>
<td>0.535</td>
</tr>
<tr>
<td>Inflammatory Condition Diagnosis Disease vs No Disease</td>
<td>β -0.001</td>
<td>0.246</td>
<td>0.981</td>
<td>β -0.001</td>
<td>0.246</td>
<td>0.981</td>
<td>β -0.001</td>
<td>0.246</td>
<td>0.981</td>
<td>β -0.001</td>
<td>0.246</td>
<td>0.981</td>
<td>β -0.001</td>
<td>0.246</td>
<td>0.981</td>
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<th>R^2 Change</th>
<th>Model 1</th>
<th></th>
<th>P-value</th>
<th>Model 2</th>
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<th>P-value</th>
<th>Model 3</th>
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<th>P-value</th>
<th>Model 4</th>
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<th>Model 5</th>
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<tbody>
<tr>
<td></td>
<td>β 0.000</td>
<td>0.013</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
CHAPERN 5
ADDITIONAL FINDINGS

Inflammation is an important part of any healthy biological system, but can be affected by many different factors. Additional findings from the present study may be useful in assessing the subjects’ overall health status and risk for inflammatory problems. Topics to be discussed include the effects of age, gender, waist circumference, and depression on the other study variables including inflammation.

A. Effects of Age and Gender

In addition to the ethnic differences in inflammation mentioned in chapter 4, evidence of gender and age-related changes in inflammation were also found and commented on here due to insufficient space in Chapter 4. Gender specific differences in CRP have been illustrated in many studies with similar outcomes to the current study results in which females had consistently higher CRP levels than males (Khera et al., 2005; Nazmi & Victoria, 2007; Valentine, Vieira, Woods, & Evans, 2009; Zhang et al., 2008). Large scale studies conducted in the US reported similar findings, including the Multiethnic Study of Atherosclerosis which found higher CRP levels in females than males among a population of Caucasian, African American, Hispanic, and Chinese participants. The gender differences in CRP remained significant after accounting for potential confounding variables as well (Lakoski et al., 2006). Since SES, health behavioral, and health status variables did not affect the gender-CRP relationship in the present study, it is possible that gender may be an independent determinant of changes in CRP levels based on physiological or genetic differences between males and females.

Prior studies that examined large populations of adults have also found age related
increases in IL-6 and CRP (Ballou et al., 1996; Ershler et al., 1993; Stowe et al., 2009; Wei, Xu, Davies, & Hemmings, 1992). Elderly individuals may therefore be affected by a low grade pro-inflammatory state characterized by increased levels of cytokines and acute phase proteins. However, it is unclear whether the inflammatory state associated with aging results from an impaired mechanism that prevents the inflammatory response from shutting off, or is due to an accumulation of unhealthy lifestyle practices (Ferrucci et al., 2005). The age-inflammation association in the present study remained significant after controlling for SES and health behavior factors, so it is more likely that an age associated dysregulation of IL-6 and CRP production may have affected this population of SDAs. Accumulated stress may lead to a reduced compensatory effect resulting in loss of equilibrium between cytokines and stress hormones, which could be responsible for the age associated increase in inflammation (Butcher & Lord, 2004). Reduced concentrations of sex hormones, growth hormones, and Vitamin D levels that accompany the aging process have also been associated with increases in inflammatory protein concentrations (Hunt, Walsh, Voegeli, & Roberts, 2010a), and should be investigated through further study.

B. Effects of Waist Circumference

Waist circumference was assessed as an indicator of obesity in the present study, and showed the same associations with the markers of inflammation as BMI. In addition, there was a high correlation between BMI and waist circumference so their associations with CRP, IL-6, IL-10, and TNF-α were assessed through separate regression analysis. Because these two obesity assessment parameters were significantly correlated with CRP
and IL-6 levels, BMI and waist circumference may both be reliable tools for determining inflammatory health risk.

**C. Effects of Depression**

Evidence has shown that depression is often associated with increased levels of inflammation (Howren, Lamkin, & Suls, 2009; J. C. Stewart, Rand, Muldoon, & Kamarck, 2009). The depression-inflammation relationship was therefore an important area to investigate among the current population of SDAs. Knowing which factors are associated with depression may be important in identifying subjects at high risk for depression related health problems. Therefore, regression analysis was utilized to determine the contribution of demographic (age, gender and ethnicity), socioeconomic (education level and difficulty meeting family expenses for basic needs), health behavioral (exercise and vegetarian diet) and health status variables (body mass index and diagnosis of an inflammatory condition) toward depression scores based on the 11 item short form of the Center for Epidemiological Studies Depression (CESD) scale. The predictor variables were placed into separate models and analyzed in a stepwise fashion as described in chapter 3. Regression models were also used to determine the association between depression and each of the markers of inflammation (CRP, IL-6, IL-10, and TNF-α) using the same control variables mentioned above.

Regression coefficients for the association between each predictor variable and depression are illustrated in table 7. Higher depression scores were found among females compared to males, and depression was positively correlated with BMI, increased difficulty meeting family expenses in the last year, and the diagnosis of an inflammatory condition. These associations were found after controlling for all other covariates.
Interestingly, consumption of a vegetarian diet was associated with decreased depression, but this relationship was no longer significant after controlling for the diagnosis of an inflammatory condition. As shown in tables 8-11, depression scores were not significantly correlated with any of the inflammatory markers in the regression models provided. This may have occurred since many SDAs from the current study reported low CESD scores, in which 82% of the depression scores were below the depression cutoff value of 16. In addition, a recent meta-analysis by Howren et al (2009) showed that stronger associations exist between depression and inflammatory cytokines among clinically depressed populations that were assessed using clinical interviews compared to community based samples that were assessed using self-report measures of depression. Therefore, the method used to measure depression levels in the present study may have contributed to the results found. Finally, incorporating depression scores into the regression analysis did not significantly change the correlation coefficient values between the other predictor variables and each of the inflammatory markers when compared to the previous regression models in chapter 4 when depression was not analyzed.

$R^2$ change values were also provided in tables 7-11. Factors such as age, gender, education, and degree of difficulty meeting family expenses for basic needs ($F (4, 429) = 9.676, p = 0.000$), as well as health behavior variables ($F (2, 427) = 4.936, p = 0.008$), BMI ($F (1, 426) = 7.999, p = 0.005$), and inflammatory conditions ($F (1, 425) = 9.309, p = 0.002$) were responsible for significant variability in depression (table 7). As illustrated by the $R^2$ change values in tables 8-11, CRP, IL-6, IL-10, and TNF-α were not significantly associated with depression among the target population.
The results from the regression analysis showed that depression did not differ according to ethnicity but did vary according to gender. Many studies have shown that females exhibit higher depression levels than men (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Lucht et al., 2003; Piccinelli & Wilkinson, 2000; Weissman & Klerman, 1977), but the results are mixed regarding differences in depression between Blacks and Whites. Although there is evidence that Black people experience higher rates of depressive symptoms than White people (Dunlop, Song, Lyons, Manheim, & Chang, 2003; Plant & Sachs-Ericsson, 2004; Tweed et al., 1990), some studies that used diagnostic criteria for major depression typically found no ethnic differences in depression (F. Y. Huang, Chung, Kroenke, & Spitzer, 2006; Somervell, Leaf, Weissman, Blazer, & Bruce, 1989). One of the reasons for the discrepancy may be that Blacks often have lower SES than Whites but still maintain high levels of social support (Plant & Sachs-Ericsson, 2004). In the present study, Black SDAs were less educated and had more difficulty meeting family expenses for basic needs in the last year compared to White SDAs. Previous studies have shown that chronic resource deficit can have a negative impact on people’s psychological well-being due to stress which can be a strong predictor of depression (Ennis, Hobfoll, & Schroder, 2000; Hobfoll, Johnson, Ennis, & Jackson, 2003). However, increased social support has been associated with reduced levels of depression (Plant & Sachs-Ericsson, 2004). No ethnic differences in social support were found in the present study, so it is possible that social support may be a stronger determinant of ethnic related depression levels among SDAs than SES. Future studies among different ethnic populations should therefore analyze the effects of SES and social support on depression.
Depression has been shown to vary according to gender. Estimates have shown that nearly twice as many women are depressed as men in adulthood (Lucht et al., 2003), and the World Health Organization has indicated that major depression is the leading cause of disease related disability worldwide among females (Kessler, 2003). Research also indicates that the gender difference in depression emerges during adolescence and may result from a variety of factors, including women’s higher level of affiliative needs, self-esteem fluctuation, body dissatisfaction, and exposure to negative events throughout life (Hyde, Mezulis, & Abramson, 2008). Fluctuations in hormone levels that occur during puberty (such as DHEA, estrogen, and testosterone) may also be responsible for higher depression among females and should be analyzed in further studies (Remer, Boye, Hartmann, & Wudy, 2005; van Broekhoven & Verkes, 2003). Another important area of future research will be to identify the genes that contribute to the gender difference in depression and determine whether gene interactions occur.

Body mass index (BMI) was also positively associated with depression after controlling for the other covariates in the present study. Indeed, cross-sectional studies have shown that there is a positive association between depression and increased body weight (McElroy et al., 2004; Rosmond, 2004), and longitudinal studies have found that obesity may predict later onset of depression in adults (Roberts et al., 2002). Some discrepancies exist in the literature which may be a result of variations in demographic factors such as age, gender, and ethnicity (Greenman & Stern, 2007). Therefore, the association between body mass index and depression may vary based on the population studied. The type of depression experienced by an individual can also affect his or her weight differently. Typical or melancholic depression is often associated with anorexia
and decreased food intake, while atypical depression is primarily characterized by increased appetite and weight gain (Dipietro et al., 1992). Nevertheless, obesity and depression may co-exist due to the presence of common emotional factors. The negative thoughts and attitudes characteristic of depression may contribute to weight management problems due to low levels of self-efficacy (Markowitz et al., 2008), and obesity may contribute to a poor self-image and, hence, increased risk of depression.

Depression was positively associated with the diagnosis of inflammatory conditions such as type 2 diabetes, stroke, transient ischemic attack, angina pectoris, rheumatoid arthritis, or sleep apnea. Previous research has shown that depression is often associated with inflammatory problems, but the directional relationship involved is still unclear. A recent meta-analysis of prospective studies analyzing the depression-diabetes relationship determined that depression was associated with a 60% increased risk of type 2 diabetes (Mezuk, Eaton, Albrecht, & Golden, 2008), suggesting that there is a strong and robust relationship between these two conditions. High rates of depression have also been found among people with obstructive sleep apnea (Harris, Glozier, Ratnavadivel, & Grunstein, 2009), and a recent longitudinal study determined that major depression at baseline was associated with several long-term medical conditions, including heart disease, arthritis, asthma, bronchitis, hypertension and migraines (Patten et al., 2008). Changes in cytokine levels may be responsible for depression related diseases that are associated with an elevated inflammatory response (Howren et al., 2009). However, further analysis is still needed before depression can be considered a primary risk factor for many inflammatory conditions.
In conclusion, BMI was associated with increased concentrations of CRP, IL-6 and elevated depression scores while females demonstrated higher CRP levels and depression scores compared to males among this SDA population. Therefore, gender as well as weight status may serve as important risk factors for health problems associated with depression and elevated inflammation. However, despite the fact that depression has been directly associated with inflammation based on previous research, no relationship was found in the current study. This null finding may be due to the use of self-reported depression measurement based on CESD scores. Nevertheless, future studies should consider the psychological as well as physical health status of research participants when assessing their level of inflammation since they both have been shown to effect cytokine levels.
Table 7. Regression analysis models illustrating the association between various predictor variables and Depression

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE)</td>
<td>B (SE)</td>
<td>B (SE)</td>
<td>B (SE)</td>
<td>B (SE)</td>
</tr>
<tr>
<td>Ethnicity Blacks vs Whites</td>
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<td>-0.035</td>
<td>-0.057</td>
<td>-0.085</td>
<td>-0.099</td>
</tr>
<tr>
<td></td>
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<td>(0.888)</td>
<td>(0.890)</td>
<td>(0.900)</td>
<td>(0.895)</td>
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<td>-0.028</td>
<td>-0.038</td>
<td>-0.027</td>
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<tr>
<td></td>
<td>(0.036)</td>
<td>(0.036)</td>
<td>(0.036)</td>
<td>(0.036)</td>
<td>(0.036)</td>
</tr>
<tr>
<td>Gender Females vs Males</td>
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<td>2.660</td>
<td>0.154</td>
<td>0.159</td>
<td>0.170</td>
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<td>(0.835)</td>
<td>(0.836)</td>
<td>(0.829)</td>
<td>(0.824)</td>
</tr>
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<td>-0.866</td>
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<td>(0.655)</td>
<td>(0.650)</td>
<td>(0.650)</td>
<td>(0.645)</td>
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<td>0.192</td>
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<td>(0.448)</td>
<td>(0.445)</td>
<td>(0.445)</td>
<td>(0.441)</td>
</tr>
<tr>
<td>Frequency of vigorous activities per week</td>
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<td>-0.217</td>
<td>-0.039</td>
<td>-0.141</td>
<td>-0.027</td>
</tr>
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<td>(0.173)</td>
<td>(0.173)</td>
<td>(0.173)</td>
<td>(0.172)</td>
</tr>
<tr>
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<td>0.006</td>
<td>-1.080</td>
<td>-0.089</td>
</tr>
<tr>
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<td>(0.832)</td>
</tr>
<tr>
<td>Body Mass Index</td>
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<td>(0.080)</td>
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<td>(0.082)</td>
<td>(0.082)</td>
<td>(0.082)</td>
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<td>Inflammatory Condition Diagnosis</td>
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<td>0.017</td>
<td>0.150</td>
</tr>
<tr>
<td>Diagnosis vs No Diagnosis</td>
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<td></td>
<td>(0.944)</td>
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R² Change 0.001 0.083 0.021 0.017 0.019

β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
Table 8. Regression analysis models illustrating the association between depression and CRP after controlling for various predictor variables

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
<th>Model 3</th>
<th></th>
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<th></th>
<th>Model 5</th>
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</thead>
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<tr>
<td></td>
<td>β</td>
<td>B (SE)</td>
<td>P-value</td>
<td>β</td>
<td>B (SE)</td>
<td>P-value</td>
<td>β</td>
<td>B (SE)</td>
<td>P-value</td>
<td>β</td>
<td>B (SE)</td>
<td>P-value</td>
<td>β</td>
</tr>
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<td>0.005</td>
<td>0.131</td>
<td>0.037</td>
<td>0.002</td>
<td>0.457</td>
<td>0.003</td>
<td>0.000</td>
<td>0.947</td>
<td>-0.043</td>
<td>-0.003</td>
<td>0.363</td>
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<td>(0.003)</td>
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<tr>
<td>Ethnicity</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Blacks vs Whites</td>
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<td>0.111</td>
<td>0.056</td>
<td>0.064</td>
<td>0.072</td>
<td>0.211</td>
<td>-0.005</td>
<td>-0.006</td>
<td>0.914</td>
<td>-0.008</td>
<td>-0.009</td>
<td>0.879</td>
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<tr>
<td></td>
<td></td>
<td>(0.058)</td>
<td></td>
<td></td>
<td>(0.057)</td>
<td></td>
<td></td>
<td>(0.055)</td>
<td></td>
<td></td>
<td>(0.056)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.113</td>
<td>0.005</td>
<td>0.025</td>
<td>0.107</td>
<td>0.005</td>
<td>0.034</td>
<td>0.130</td>
<td>0.006</td>
<td>0.007</td>
<td>0.124</td>
<td>0.006</td>
<td>0.012</td>
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<tr>
<td></td>
<td></td>
<td>(0.002)</td>
<td></td>
<td></td>
<td>(0.002)</td>
<td></td>
<td></td>
<td>(0.002)</td>
<td></td>
<td></td>
<td>(0.002)</td>
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<td></td>
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<tr>
<td>Gender</td>
<td>0.172</td>
<td>0.189</td>
<td>0.001</td>
<td>0.175</td>
<td>0.192</td>
<td>0.000</td>
<td>0.194</td>
<td>0.213</td>
<td>0.000</td>
<td>0.196</td>
<td>0.216</td>
<td>0.000</td>
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</tr>
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<td>Females vs Males</td>
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<td>(0.054)</td>
<td></td>
<td></td>
<td>(0.051)</td>
<td></td>
<td></td>
<td>(0.052)</td>
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<tr>
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<td>0.009</td>
<td>0.823</td>
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<tr>
<td></td>
<td></td>
<td>(0.042)</td>
<td></td>
<td></td>
<td>(0.043)</td>
<td></td>
<td></td>
<td>(0.040)</td>
<td></td>
<td></td>
<td>(0.040)</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.008</td>
<td>0.788</td>
<td>0.017</td>
<td>0.010</td>
<td>0.812</td>
<td>0.015</td>
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<td>0.014</td>
<td>0.009</td>
<td>0.757</td>
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<tr>
<td></td>
<td></td>
<td>(0.030)</td>
<td></td>
<td></td>
<td>(0.029)</td>
<td></td>
<td></td>
<td>(0.028)</td>
<td></td>
<td></td>
<td>(0.028)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of vigorous activities per week</td>
<td>-0.104</td>
<td>-0.024</td>
<td>0.033</td>
<td>-0.058</td>
<td>-0.013</td>
<td>0.211</td>
<td>-0.056</td>
<td>-0.013</td>
<td>0.230</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(0.011)</td>
<td></td>
<td></td>
<td>(0.011)</td>
<td></td>
<td></td>
<td>(0.011)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vegetarian diet</td>
<td>-0.188</td>
<td>-0.204</td>
<td>0.000</td>
<td>-0.133</td>
<td>-0.145</td>
<td>0.005</td>
<td>-0.131</td>
<td>-0.142</td>
<td>0.006</td>
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<tr>
<td>Vegetarian vs Non-Vegetarian</td>
<td></td>
<td>(0.054)</td>
<td></td>
<td></td>
<td>(0.051)</td>
<td></td>
<td></td>
<td>(0.052)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
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<td>0.036</td>
<td>0.000</td>
<td>0.337</td>
<td>0.035</td>
<td>0.000</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.005)</td>
<td></td>
<td></td>
<td>(0.005)</td>
<td></td>
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</tr>
<tr>
<td>Inflammatory Condition Diagnosis</td>
<td>0.005</td>
<td>0.053</td>
<td>0.043</td>
<td>0.099</td>
<td>0.000</td>
<td></td>
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$R^2$ Change: 0.005 0.053 0.043 0.099 0.000

$\beta =$ standardized regression coefficient, $B =$ unstandardized regression coefficient, $SE =$ standard error
Table 9. Regression analysis models illustrating the association between depression and IL-6 after controlling for various predictor variables

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R^2 Change | 0.003 | 0.048 | 0.013 | 0.018 | 0.000

β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
Table 10. Regression analysis models illustrating the association between depression and IL-10 after controlling for various predictor variables

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<td>B (SE)</td>
<td>P-value</td>
<td>( \beta )</td>
<td>B (SE)</td>
<td>P-value</td>
<td>( \beta )</td>
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<td>0.052</td>
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<td>0.019</td>
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<td>Frequency of vigorous activities per week</td>
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\( \beta \) = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
Table 11. Regression analysis models illustrating the association between depression and TNF-α after controlling for various predictor variables

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β = standardized regression coefficient, B = unstandardized regression coefficient, SE = standard error
CHAPTER 6

CONCLUSIONS

A. Summary and Findings

The results of the present study have shown that a number of factors can affect inflammation which may be associated with chronic health problems. For instance, inflammation was found to vary according to age, gender, and ethnicity. In particular, higher levels of pro-inflammatory cytokines were exhibited among Blacks and female participants compared to Whites and male participants. The Blacks and the female SDAs may therefore be at an increased risk for future cardiovascular disease and other inflammatory health problems. Increased age was also associated with an elevated pro-inflammatory response, which has been associated with many detrimental effects that contribute to frailty and disability among elderly populations (Hunt, Walsh, Voegeli, & Roberts, 2010b). However, healthy lifestyle practices may counteract these effects, and function as important determinants of quality of life as individuals get older.

Lifestyle factors played an important role in the level of inflammation among the population of SDAs from the current study, as increased exercise frequency and the consumption of a vegetarian diet were found to have anti-inflammatory effects. Practicing these behaviors may prove to have other important health benefits as well, which should be further assessed through interventional studies to determine if they have a dose-response effect on inflammation and other physiological factors. The positive association between increased body mass and elevated levels of CRP and IL-6 has been illustrated in previous studies (Berg & Scherer, 2005; Nicklas et al., 2004; Panagiotakos et al., 2005), and was further verified in the present investigation. On the other hand, the
association between BMI and anti-inflammatory cytokines has not been fully determined. The present study showed no significant association between BMI and IL-10, so it is possible that this cytokine is not directly stimulated by elevated body mass. However, further analysis on this particular relationship is still needed. Finally, factors related to SES were not significantly associated with inflammation levels, but low SES has been associated with increased inflammation based on previous research (Gruenewald, Cohen, Matthews, Tracy, & Seeman, 2009; Koster, Bosma, Penninx, Newman, Harris, van Eijk, Kempen, Simonsick, Johnson, Rooks, Ayonayon, Rubin, & Kritchevsky, 2006; K. L. Petersen et al., 2008). Many of the SDAs in the current population achieved high levels of education and reported no significant difficulties meeting family expenses for basic needs in the last year, so they may not have been prone to elevated inflammation based on their socioeconomic background.

B. Limitations

The original objective of this study was to assess the effects of obesity and depression on both pro-inflammatory and anti-inflammatory markers as well as determine whether there were ethnic and gender specific differences in inflammation. Yet the results showed that this population of SDAs did not have high scores of depression, and there were no significant associations between depression and the markers of inflammation. It was therefore necessary to examine other possible determinants of inflammation among SDAs to assess their overall risk for inflammatory based health problems. SDAs have lower mortality rates and report better physical and mental health than the general population (J. W. Lee et al., 2009). However, factors related to SES and overall lifestyle practice may play an important role in determining the quality of life of
current SDAs. Therefore, education level, degree of difficulty meeting family expenses for basic needs, exercise frequency, and vegetarian diet consumption were examined through regression analysis to determine if these factors were associated with inflammation in this population.

There was no significant interactive effect of obesity (based on BMI) and depression on the inflammatory marker concentrations from the regression analysis (data not shown). Although a significant interactive effect of BMI and depressive symptoms on CRP concentrations has been reported previously, the results were based on a large population of German males which suggests that the effect may have been gender and population specific (K. H. Ladwig et al., 2003). The inflammatory effects of obesity and depression may therefore function independently among SDAs, possibly involving distinct cytokine pathways.

C. Areas for Future Research

The current investigation may serve as an important pilot study for future analysis of inflammatory determinants among distinct populations. Because this was a cross-sectional study, the directional relationship between the predictor variables and the inflammatory markers could not be determined. However, the effect of healthy lifestyle practices such as a vegetarian or high fiber diet as well as increased physical activity on inflammation levels should be investigated. Study participants should be randomly assigned to a control and treatment group, and matched according to demographic (age, gender, and ethnicity) and socioeconomic status. Because there are a number of factors that can lead to elevated inflammation, it will be important to control for certain variables such as smoking status, alcohol consumption, and previous history of inflammatory
conditions. The goal of this type of intervention would be to determine the optimal method for reducing inflammation and chronic disease risk among healthy individuals.

In addition to pro-and anti-inflammatory cytokines, future studies should also analyze the ratio of pro-inflammatory to anti-inflammatory cytokines among participants. Many diseases are associated with an imbalance of inflammatory cytokines, so this analysis would help to determine people’s risk for different inflammatory conditions in a more specific way. In the present study, we did analyze the ratio of TNF-\(\alpha\) to IL-10 among the SDA population, but the TNF-\(\alpha\) concentration was below the detection limit for many of the participants which prevented the ratio values from being accurately determined. Genetic markers of inflammation should also be examined to check for possible hereditary differences in pro-inflammatory and anti-inflammatory cytokine levels. Finally, since ethnic differences in inflammation were found in the present investigation, a cohort of participants from different racial backgrounds should be assessed in future studies to determine the effectiveness of lifestyle interventions on reducing inflammation among these ethnic groups.

D. Implications for Preventive Care

The more data that can be obtained about inflammation and its effects on the body, the better equipped we will be in dealing with many health problems. Although there is much about the immune system that is still unknown, new information is constantly being discovered that may be useful in trying to maintain a healthy inflammatory balance for many individuals. The key is to better understand the signaling pathways by which circulating levels of inflammatory markers impact the pathobiology
of common diseases. This level of information may help in finding effective techniques and interventions for improving the quality of life as people age.
References


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Esposito, K., Pontillo, A., Giugliano, F., Giugliano, G., Marfella, R., Nicoletti, G., et al. (2003). Association of low interleukin-10 levels with the metabolic syndrome in


in-vivo and in 3T3-L1 adipocytes – A possible role for interleukin-6 in cancer cachexia. *Cancer Research, 52*(15), 4113-4116.


Appendix A
IL-10 ELISA Protocol

1. Prepare 6 standards, one for each standard curve point: 600, 240, 96, 38.4, 15.36, and 0 pg/ml. Use a 1:2.5 serial dilution for the standard curve as follows:
   a. Pipette 240 µl of appropriate diluent into each tube.
   b. Pipette 160 µl of the reconstituted standard into the first tube (600 pg/ml) and mix
   c. Pipette 160 µl of this dilution into the second tube labeled (240 pg/ml) and mix
   d. Repeat the serial dilutions (using 160 µl) three more times to complete the standard curve points
2. Add 50 µl of reconstituted standards or test samples in duplicate to each well.
3. Add 50 µl of Biotinylated Antibody Reagent to all wells containing standards or samples.
4. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate for two hours at room temperature, 20-25°C.
5. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of three washes. Blot plate onto paper towels or other absorbent material.
6. For one complete 96-well plate, add 30 µl of Streptavidin-HRP Concentrate to 12 ml of Streptavidin-HRP Dilution Buffer and mix gently.
7. Add 100 µl of prepared Streptavidin-HRP Solution to each well.
8. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
9. Carefully remove the plate cover and discard plate contents. Wash plate as described in step 5.
10. Pipette 100 µl of TMB Substrate Solution into each well.
11. Allow enzymatic color reaction to develop at room temperature in the dark for 45 minutes. Do not cover plate with aluminum foil or a plate sealer.
12. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well.
13. Measure absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate.
14. Use the standard curve to determine IL-10 amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding IL-10 concentration (pg/ml) on the horizontal (X) axis.
15. The amount of IL-10 in each sample is determined by interpolating from the absorbance value (Y axis) to the IL-10 concentration (X axis) using the standard curve.
Appendix B
TNF-α ELISA Protocol

1. Prepare 7 standards, one for each standard curve point: 1000, 500, 250, 125, 62.5, 31.2, 15.6 and 0 pg/ml. Use a 1:2 serial dilution to generate the standard curve points as follows:
   a. Pipette 200 µl of Sample Diluent into each tube.
   b. Pipette 200 µl of the reconstituted Standard into the first tube (1,000 pg/ml) and mix.
   c. Pipette 200 µl of this dilution into the next tube (500 pg/ml) and mix.
   d. Repeat serial dilutions (using 200 µl) five more times to complete the standard curve points.

2. Add 50 µl Sample Diluent to each well.
3. Add 50 µl standard or sample to each well in duplicate.
4. Carefully cover plate with an adhesive plate cover. Ensure that all edges and strips are sealed tightly by running your thumb over the edges and down each strip. Incubate for one hour at room temperature, 20-25°C.
5. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of three washes. Blot plate onto paper towels or other absorbent material.
6. Add 100 µl of the Biotinylated Antibody Reagent to each well.
7. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over the edges and down each strip. Incubate for one hour at room temperature, 20-25°C.
8. Carefully remove the adhesive plate cover. Wash plate as described in step 5.
9. Add 100 µl of Streptavidin-HRP Reagent to each well.
10. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
11. Carefully remove the adhesive cover and discard plate contents. Wash plate as described in step 5.
12. Pipette 100 µl of TMB Substrate Solution into each well.
13. Allow enzymatic reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer.
14. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well.
15. Measure absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate.
16. Generate the standard curve by plotting the average absorbance (450 nm minus 550 nm) obtained for each standard concentration on the vertical (Y) axis vs. the corresponding TNFα concentration on the horizontal (X) axis.
17. The amount of TNFα in each sample is determined by interpolating from the absorbance value (Y axis) to the TNFα concentration (X axis) using the standard curve.
Appendix C
Documentation of IRB approval for BioMRS

INSTITUTIONAL REVIEW BOARD
Extension Requested - Approval Notice (Expeditied)
OFFICE OF SPONSORED RESEARCH • 1188 Anderson Street • Loma Linda, CA 92350
(909) 558-4531 (voice) • (909) 558-0131 (fax)

To: Fraser, Gary E.
Department: Epidemiology & Biostatistics
Protocol: Biological and psychosocial manifestations of religion

Your request to extend the protocol indicated above has been reviewed administratively.

Extension Request: Approved
Risk to research subjects: Minimal
Approval period begins 01-Jun-2009 and ends 31-May-2010
Stipulations of approval are: Waiver of documentation of informed consent for Adventist Religion & Health Survey, per 46.117(c)(1)

Consent Form
If this study was approved on the condition that a consent form is required AND subjects are still being enrolled, only the consent form bearing the IRB authorization stamp can be used. This will become your OFFICIAL consent form for the dates specified and should be used as the new master for making copies to give prospective subjects.

☐ Master consent form with up-dated authorized stamp is enclosed.
☐ Updated consent form not required. Approval limited to data analysis or follow-up of currently enrolled subjects only.
☐ Not applicable; IRB approved a waiver of informed consent, as noted above.

IRB Communications
Please continue to notify the IRB in writing of any modifications or adverse events relating to the approved research protocol. Your assistance in providing the PI's name and the protocol's IRB # on all communications with the IRB about this project will expedite necessary communications.

Thank you for your cooperation in LLU's shared responsibility for the ethical use of human subjects in research.

Signature of IRB Chair/Designee: [Signature]

Loma Linda University Adventist Health Sciences Center holds Federalwide Assurance (FWA) No. 6447 with the U.S. Office for Human Research Protections, and the IRB registration no is IORG0003841. This Assurance applies to the following institutions: Loma Linda University, Loma Linda University Medical Center (including Loma Linda University Children's Hospital, LLU Community Medical Center), Loma Linda University Behavioral Medicine, and affiliated medical practices groups.

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