Inflammatory Pathways Linking Type 2 Diabetes Mellitus and Depression

A dissertation presented to

the faculty of

the College of Arts and Sciences of Ohio University

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy

Todd A. Doyle

August 2012

This dissertation titled

Inflammatory Pathways Linking Type 2 Diabetes Mellitus and Depression

by

TODD A. DOYLE

has been approved for

the Department of Psychology

and the College of Arts and Sciences by

Mary de Groot

Associate Professor of Psychology

Howard Dewald

Interim Dean, College of Arts and Sciences

Abstract

DOYLE, TODD A., Ph.D., August 2012, Psychology

Inflammatory Pathways Linking Type 2 Diabetes Mellitus and Depression (75 pp.)

Director of Dissertation: Mary de Groot

Up-regulated levels of interleukin-6 (IL6), TNF-α (TNF), and C-reactive protein (CRP) are common to both type 2 diabetes (T2DM) and depression, yet inflammation as a possible biological link between these disorders has gone unexamined. This study examined the role of inflammation in the relationship between T2DM and depression. Baseline and longitudinal data were analyzed from 3,014 adults, 70-79 years, participating in the Health, Aging and Body Composition Study. Presence of T2DM was assessed per self-report, medication use, fasting glucose and/or glucose tolerance test results. Depressed mood was categorized using the Center for Epidemiologic Studies Depression (CES-D) scale using a cut-score of ≥20. Participants who had both T2DM and depressed mood demonstrated significantly higher levels of IL-6 ($F[1, 2761]=5.3$, $p<.05$, $\eta^2=<.001$) compared to those with T2DM alone, depressed mood alone, and healthy controls. Similarly, participants with T2DM and depressed mood also had significantly higher levels of CRP ($F[1, 2875]=5.5$, $p<.05$, $\eta^2=.002$) compared to those with depressed mood alone and health controls. Among T2DM participants, those with higher levels of IL-6 had significantly increased risk of depressed mood compared to T2DM participants with lower levels of IL-6 (OR = 10.43, 95% CI = 1.65 – 66.09, Max-rescaled $R^2 = 0.13$). Further, up-regulated levels of TNF-α at baseline predicted depressed mood status at year 2 among T2DM patients (OR=7.02, 95% CI 1.34-36.71, Max-

rescaled R^2=.28). These findings support an additive model of inflammation linking T2DM and depressed mood, using self-reported depressive symptoms. Further investigation into these relationships could aid in understanding the biological pathways underlying the relationship between T2DM and depression.

Approved: _____

Mary de Groot

Associate Professor of Psychology

Table of Contents

Page

List of Tables

Page

List of Figures

Page

Introduction

Depression is a significant co-morbid condition in type 2 diabetes mellitus (T2DM; Anderson, Freedland, Clouse, & Lustman, 2001). Patients with T2DM are two times more likely to experience depression than individuals without T2DM (Anderson et al., 2001). While the causal mechanisms linking T2DM and depression are multifaceted and represent a complex interaction between genetic, biological, and psychosocial factors, one mechanism common to both disorders is systemic, low-grade inflammation, characterized by up-regulated levels of pro-inflammatory cytokines (e.g., interleukin-6 [IL-6] & tumor necrosis factor-alpha [TNF-α]) and acute-phase reactants such as C-reactive protein (CRP; Kiecolt-Glaser & Glaser, 2002; Pickup, 2004). However, there is no research that has examined inflammation as a possible biological link between T2DM and depression. This problem persists despite a growing numbers of studies that suggest chronic, low-grade inflammation is closely related to the underlying disease processes associated with T2DM (e.g., insulin resistance; Coppola et al., 2006; Corrado, Rizzo, Muratori, Coppola, & Novo, 2006; Hogue, Lamarche, Tremblay, Bergeron, & Gagne, 2008; Leinonen, Hurt-Camejo, Wiklund, Hulten, & Hiukka, 2003; Muhlestein, May, Jensen, Horne, & Lanman, 2006; Temelkova-Kurktschiev, Siegert, Bergmann, Henkel, & Koehler, 2002). In addition, up-regulated levels of inflammation have been shown to be associated with depressive symptoms in patients who are otherwise medically healthy as well as those with established inflammatory diseases, such as cardiovascular disease (Empana et al., 2005; Irwin & Miller, 2007; Ladwig, Marten-Mittag, Lowel, Doring, &

Koenig, 2005; Pickup, 2004; Raison, Capuron, & Miller, 2006). To demonstrate that chronic, low-grade inflammation may be a common link between these disorders, a review of the literature will be provided on inflammation as it relates to T2DM and depression, respectively. This review will provide the foundation for a conceptual framework that illustrates the overlapping inflammatory processes involved in co-morbid T2DM and depressed mood.

Inflammation and Type 2 Diabetes Mellitus

Among individuals diagnosed with T2DM, numerous cross-sectional and longitudinal studies have confirmed that IL-6, TNF-α, and CRP are positively correlated with measures of insulin resistance, BMI/waist circumference, circulating triglycerides, and the progression of atherosclerosis (Coppola et al., 2006; Corrado et al., 2006; Hogue et al., 2008; Leinonen et al., 2003; Mishima et al., 2001; Muhlestein et al., 2006; Nilsson et al., 1998; Temelkova-Kurktschiev et al., 2002). Elevated levels of IL-6 and CRP have been found to be related to insulin resistance states in both controlled and uncontrolled samples of T2DM patients (Leinonen et al., 2003; Temelkova-Kurktschiev et al., 2002). In addition, numerous studies have confirmed that serum levels of TNF-α are consistently elevated among obese T2DM patients compared to non-obese T2DM patients and healthy controls (Katsuki et al., 1998; Mishima et al., 2001; Nilsson et al., 1998; Winkler et al., 1998). Two studies have shown a graded relationship between TNF-α and insulin resistance, with TNF-α levels being highest among those with severe insulin resistance (Mishima et al., 2001; Nilsson et al., 1998).

Several randomized and non-randomized clinical drug trials examining the anti-inflammatory and cholesterol-lowering effects of hydroxymethylglutaryl-CoA reductase inhibitors (i.e., statins) on reducing inflammatory markers have shown that at baseline, T2DM patients diagnosed with dyslipidemia exhibit elevated levels of TNF-α, IL-6, CRP, and monocyte chemoattractant protein-1 (Hogue et al., 2008; Muhlestein et al., 2006; Tan, Tan, & Berk, 2007). Further, cross-sectional studies among T2DM patients have provided correlational evidence to support a relationship between elevated inflammatory markers and dyslipidemia (Bullo, Garcia-Lorda, Megias, & Salas-Salbado, 2003; Frohlich et al., 2000; Pereira, Frode, & Medeiros, 2006). Markers of inflammation also play a prominent role in the accelerated progression of atherosclerosis that is often associated with T2DM. At least four studies have shown that atherosclerosis, assessed through ultrasound or the cardio-ankle vascular index, is positively associated with CRP (Coppola et al., 2006; Corrado et al., 2006; Wakabayashi & Masuda, 2006; Yu, Sheu, Song, Liu, & Lee, 2004). Similarly, endothelial dysfunction, an underlying process associated with atherosclerosis, has also been linked to inflammatory markers in T2DM. Increased levels of both CRP and IL-6 have been positively associated with one or more plasma markers of endothelial dysfunction including von Willebrand factor, tissue-type plasminogen activator, soluble E-selectin, and soluble vascular cell adhesion molecule-1 (de Jager et al., 2006; Natali et al., 2006; Stehouwer et al., 2002).

It is important to recognize that pro-inflammatory cytokines are released as a function of these separate, but often overlapping T2DM-related disease processes (i.e., insulin resistance, dyslipidemia, & atherosclerosis). The presence of one or more of these

disease processes can contribute to the up-regulation of immune markers, which could potentially fuel the development of additional inflammatory-related T2DM disease processes and/or further the progression of those already in existence. This up-regulation of inflammatory markers in T2DM is similar to elevated levels of inflammation associated with depressive symptoms observed in patients who are otherwise medically healthy as well as those with established inflammatory diseases, such as cardiovascular disease (Pizzi et al., 2009; Irwin & Miller, 2007; Ladwig, Marten-Mittag, Lowel, Doring, & Koenig, 2005; Pickup, 2004; Raison, Capuron, & Miller, 2006). To understand the role of inflammation in the link between T2DM and depression, it is also necessary to review the literature on the association between inflammation and depressive symptoms.

Inflammation and Depression

Patients with major depression who are otherwise medically healthy have been repeatedly observed to have increased pro-inflammatory cytokines and acute-phase reactants (Irwin & Miller, 2007; Raison et al., 2006). These markers of inflammation are likely related to such underlying mechanisms as increased hypothalamic-pituitary-adrenal (HPA) axis activity, impaired glucocorticoid receptor expression and function, reduced synthesis of serotonin through a peripheral depletion of tryptophan, and noradrenaline dysregulation (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Jansen, Schmidt, Voorn, & Tilders, 2003; Pace, Hu, & Miller, 2007; Ruhe, Mason, & Schene, 2007; Wichers & Maes, 2002). Elevated levels of serum or plasma concentrations of IL-6, Interleukin-1, TNF-α, and CRP have most frequently been observed in cross-sectional studies among depressed versus non-depressed controls (Irwin & Miller, 2007; Raison et

al., 2006; Ford & Erlinger, 2004; Danner, Kasl, Abramson, & Vaccarino, 2003; Glaser, Robles, Sheridan, Malarkey, & Kiecolt-Glaser, 2003; Penninx, Kritchevsky, Yaffe, Newman, & Simonsick et al., 2003; Suarez, Krishman, & Lewis, 2003).

In addition to these findings, depression or depressive symptoms are associated with pro-inflammatory cytokines and acute-phase reactants in several medical illnesses, including CVD, certain types of cancer, & acute myelogenous leukemia (Pizzi, Mancini, Angeloni, Fontana, & Mansoli et al., 2009; Empana, Luc, Juhan-Vague, Arveiler, & Ferrieres et al., 2005; Ferketich, Ferguson, & Binkley, 2005; Ladwig, Marten-Mittag, Lowel, Doring, & Koeing, 2005; Lesperance, Frasure-Smith, Theroux, & Irwin, 2004; Meyers, Albitar, & Estey 2005; Musselman, Miller, Porter, Manatunga, & Gao et al., 2001; Appels, Frits, Bar, Bar, & Bruggeman et al., 2000). These relationships have been reported most commonly within the CVD literature, with a variety of studies demonstrating that, compared to controls, depressive symptoms are associated with the inflammatory response within not only those at risk for CVD, but also among patients who have experienced some CVD-related event (Pizzi et al., 2009; Ferketich, Ferguson, & Binkley, 2005; Ladwig et al., 2005; Miller, Freedland, Duntley, & Carney, 2005). Ladwig and colleagues (2005) found a significant interaction between CRP and depressive symptoms in predicting cardiac-related events among N=3,021 men participating in the MONICA-KORA Ausburg Cohort study. Levels of CRP predicted cardiac-related events to a much greater extent among depressed men, compared to non-depressed men, suggesting that CRP and depressive symptoms had an additive effect in the development of CVD in men (Ladwig et al., 2005). In addition, Pizzi and colleagues

(2009) examined levels of inflammation among N=100 patients diagnosed with coronary heart disease (CHD) who were undergoing treatment for depression. Data from this randomized, double-blind, prospective study demonstrated that after 20 weeks of treatment, CHD patients treated with sertraline showed significantly ($p<.01$) lower levels of depressive symptoms, IL-6, and CRP compared to CHD patients who received placebo (Pizzi et al., 2009). Further cross-sectional evidence shows that CRP (Lesperance, Frasure-Smith, Theroux, & Irwin, 2004; Miller et al., 2005) and TNF-α (Ferketich et al., 2005) are significantly increased among depressed (versus non-depressed) patients with established CVD. Together findings from these studies suggest that depressive symptoms are associated with the inflammatory response among patients with established inflammatory diseases such as CVD. Given that many of the disease-processes associated with T2DM are related to inflammation, it is reasonable to assume that depressive symptoms, in the presence of T2DM, are associated with heightened levels of inflammatory markers.

Conceptualization of Type 2 Diabetes Mellitus, Depression, and Inflammation

Evidence for the up-regulation of IL-6, TNF-α, and CRP in studies of T2DM and depression, respectively, in conjunction with the high rates of observed co-morbidity, suggests that chronic, low-grade inflammation may be a common link between these disorders. Figure 1 provides a conceptual framework that illustrates the overlapping inflammatory processes involved in co-morbid T2DM and depressed mood. Currently, the literature on inflammation in T2DM and depression, respectively, has not been integrated. Figure 1 presents a theorized representation of how the inflammatory response

may be linked to T2DM and depression, in part, through its association with increased HPA axis hyperactivity, which is known to be independently related to pro-inflammatory cytokines in both disorders. However, this relationship is bi-directional since 1) pro-inflammatory cytokines can directly influence activation of the HPA axis and the release of glucocorticoids and 2) continued neuroendocrine dysfunction (resulting from impaired negative feedback mechanisms), through its effects on adipose and hepatic tissues, likely contributes to the pathological and clinical markers of T2DM through the indirect up-regulation of pro-inflammatory cytokines and acute-phase reactants (Figure 1).

A brief overview of Figure 1 is outlined below. This overview will first summarize the link between pro-inflammatory markers and T2DM, and then the link between pro-inflammatory markers and depression will be discussed. In obese states, it is hypothesized that the metabolic overload of adipocytes triggers localized inflammatory responses within adipose tissue, which includes the up-regulation of pro-inflammatory cytokines and chemokines, resulting in the infiltration of macrophages into adipose tissue (Figure 1; Alexandraki, Piperi, Kalofoutis, Singh, & Alaveras, et al., 2006; O'Connor, 2006; Shoelson et al., 2006; Wellen & Hotamisligil, 2005). The co-localization of macrophages and visceral adipocytes would then produce an increased amount of pro-inflammatory cytokines and free fatty acids which circulate via the portal and systemic vascular systems and directly contribute to the release of CRP from the liver and the development of insulin resistance in peripheral tissues (Figure 1; Alexandraki et al., 2006; Pickup, 2000). This process likely occurs through the inhibition of subsequent downstream intracellular insulin signaling mechanisms such as phosphoinositide 3-

kinase, Akt kinase, and translocation of glucose transporter 4 to the plasma membrane (Figure 1; Alexandraki et al., 2006; Kirwan & del Aguila, 2003; Pickup, 2000; Schmidt, Duncan, Sharrett, Lindberg, & Savage, et al., 1999; Cong, Chen, Li, Zhou, & McGibbon et al., 1997). The increased hepatic triglyceride-rich VLDL production, as seen in dyslipidemia, would likely result from the large of amounts of pro-inflammatory cytokines released from adipose tissue as well as an increase in the re-esterification of fatty acids that occurs from both enhanced lipolysis and increased hepatic fatty acid synthesis (Figure 1; Mazzone, Chait, & Plutzky, 2008; Esteve, Ricart, & Fernandez-Real, 2005). The presence of metabolic abnormalities such as dyslipidemia may also accelerate atherosclerotic processes since excess circulating lipid particles leads to increased lipid deposition and retention within the arterial wall, contributing to macrophage infiltration and pro-inflammatory cytokine release (Figure 1; Hansson, 2005; Leitinger, 2003; Skalen, Gastafsson, Rydberg, Hulten, & Wiklund et al, 2002). Further, as levels of pro-inflammatory cytokines rise, they can stimulate atherosclerosis through the induction of protein-kinase C, NF-κB activation, and increased release of superoxide from phagocytes, all of which contribute to cellular functioning within the arterial wall (Venugopal, Devaraj, Yang, & Jialal, 2002).

Up-regulated levels of pro-inflammatory cytokines stemming from any one of these underlying T2DM disease processes could circulate through the vascular system and stimulate the mechanisms commonly observed in patients with depressed mood, such as increased HPA axis activity and alterations to both serotonergic (5-HT) and noradrenergic activities (Figure 1; Ruhe, Mason, & Schene, 2007; Dantzer et al., 2008;

Wichers & Maes, 2002; Heyes, Saito, Crowley, Davis, & Demitrack et al, 1992; Jansen et al., 2003; Zhu, Shamburger, Li, & Ordway, 2000). Pro-inflammatory cytokines are believed to alter HPA axis activity through the impairment of glucocorticoid receptor expression and functioning, which is commonly referred to as glucocorticoid resistance (Figure 1; Pace et al., 2007; Raision et al., 2006; Miller et al., 1999). A variety of pro-inflammatory cytokine signaling pathways are involved in glucocorticoid resistance including up-regulation of NF-κB, protein kinases, signal transducers, gene transcription factors, and protein isoforms that increase the expression of various genes that encode inflammatory markers (Pace et al., 2007; Webster, Oakley, Jewell, & Cidlowski, 2001).

Evidence also exists to suggest that disturbances in 5-HT functioning may play a causal role in the pathophysiology of depression. It is speculated that pro-inflammatory cytokines such as TNF-α affect 5-HT metabolism indirectly, by stimulating major enzymes that metabolize tryptophan (i.e., indoleamine 2,3 dioxygenase [IDO]; Dantzer et al., 2008), which is actively transported into the brain for the purpose of synthesizing serotonin. Thus, IDO leads to a peripheral depletion of tryptophan since it degrades tryptophan, and as a result reduces synthesis of 5-HT in the brain since the latter depends on the plasma availability of tryptophan (Dantzer et al., 2008; Wichers & Maes, 2002; Heyes, Saito, Crowley, Davis, & Demitrack et al, 1992). In addition to the degradation of tryptophan, pro-inflammatory cytokines have also been shown to affect 5-HT levels by up-regulating the 5-HTT transporter, causing a depletion of extracellular 5-HT (Mossner, Heils, Stober, Okladnova, & Daniel et al., 1998; Ramamoorthy, Ramamoorthy, Prasad, Bhat, & Mahesh et al., 1995).

Finally, it may be that pro-inflammatory cytokines influence the release of noradrenaline through reduced expression of pre-synaptic α2-adrenoceptors in the paraventricular nucleus (PVN) and reduced re-uptake of noradrenaline from axon terminals (Jansen et al., 2003; Zhu, Shamburger, Li, & Ordway, 2000). A2-adrenoceptors are an important receptor in the negative feedback control of noradrenaline release, and decreases to the pre-synaptic expression of these receptors would lead to an exaggerated norepinephrine release within the brain (Maes, van Gastel, Delmeire, & Meltzer, 1999; Dunn, 1988; Kabiersch, del Rey, Honegger, & Besedovsky, 1988). Thus, because pro-inflammatory cytokines promote increased levels of noradrenaline, they indirectly up-regulate the HPA axis through the influence of noradrenaline on increasing corticotrophin-releasing hormone (CRH) transcription factors and activating CRH neurons and secretion of CRH into the portal blood (Sawchenko, Brown, Chan, Ericsson, & Li et al., 1996). Together the evidence presented here suggests that inflammatory markers play a role in the pathogenesis of depression through their relationship with one or all of the underlying mechanisms commonly observed in depressed mood.

It has now been shown that many of the underlying disease processes associated with T2DM, including insulin resistance, dyslipidemia, and atherosclerosis are individual manifestations of a common inflammatory pathway that is believed to occur in T2DM (Figure 1). The presence of one or more of these disease processes can contribute to the up-regulation of inflammatory markers, which could potentially fuel the development and/or progression of mechanisms related to depressive symptoms (e.g., dysregulation of monoamine neurotransmission and HPA-axis hyperactivity; Figure 1). In turn, elevated

glucocorticoid concentrations that result from increased HPA-axis activation can further the progression of T2DM-related disease processes (Figure 1). Although these reciprocal processes have yet to be empirically tested, an initial step towards demonstrating that they might exist is to show that individuals with both T2DM and depressed mood have significantly higher levels of inflammation than individuals with either disorder alone or healthy controls. If T2DM and depressed mood are related through inflammatory processes, then one disorder may help to stimulate, increase, or sustain the inflammatory response in the other (i.e., an additive effect).

The primary aim (1) of this study was to examine the additive effects of T2DM and depression symptom status on levels of IL-6, TNF-α, and CRP in a sample of men and women participating in the Health, Aging, and Body Composition study. It was hypothesized that inflammation would be greatest among those with T2DM and elevated depressive symptoms (T2DM + DEP), followed by (T2DM Only) or elevated depressive symptoms alone (DEP Only), followed by healthy controls (Healthy).

The secondary aims of this study were (2) to assess the association between depressive symptoms and continuous levels of IL-6, TNF-α, and CRP in a sub-sample (n=707) of adults with T2DM to determine whether an increased likelihood of clinically depressive symptoms was associated with elevated inflammatory markers; and (3) to investigate the role of baseline levels of IL-6, TNF-α, and CRP as potential predictors of depression symptom status at year 2 among adults with T2DM.

Methods

This study used 2 (T2DM present/absent) X 2 (depression symptoms increased/minimal) between-subjects cross-sectional and longitudinal designs among a sample of 3,075 men and women participating in the Health, Aging, and Body Composition (Health ABC) study (National Institute on Aging, 2009). Year 2 longitudinal data on depression symptom status were also used during analysis of the secondary aims. The Health ABC study was a 11-year prospective cohort study designed to investigate the extent of changes in body composition and the impact of these changes on strength, endurance, disability, and weight-related health conditions associated with aging (National Institute on Aging, 2009). Well-functioning men and women, aged 70-79 years at baseline, of whom approximately 41.6% were Black, were included in the study (National Institute on Aging, 2009; Penninx et al., 2003). Participants were recruited from April 1997 to June 1998 and drawn from a sample of Medicare beneficiaries residing in Pittsburgh, Pennsylvania and Memphis, Tennessee (Penninx et al., 2003). To be eligible, participants had to be free of disability and functional limitations (e.g., no difficulty walking one quarter mile, climbing 10 steps, or performing basic activities of daily living; National Institute on Aging, 2009; Penninx et al., 2003). Participants also had to be free of life-threatening illnesses (e.g., cancers under active treatment) and willing to remain in the geographic area for at least 3 years (National Institute on Aging, 2009; Penninx et al., 2003).

During baseline, detailed questionnaires on social demographics, health behaviors, indicators of socioeconomic status, health service utilization, and depression

symptom status were administered within the home. Participants also underwent a clinical examination immediately following their baseline interview that included biological and body composition measures as well as indicators of weight-related health conditions and physical performance measures. Annually, participants took part in follow-up interviews and clinical examination during which time the HABC study variables were re-evaluated. However, some study variables were only assessed every two years during the annual follow-up periods (e.g., depressive symptoms). In order to examine the association between inflammation and depressive symptoms over time, depression screenings from the year 2 follow-up period were used as part of the current investigation.

The Health ABC Study was reviewed and approved by the institutional review boards of the clinical sites. All participants provided informed consent before participating in the study.

Measurements

Inflammatory Markers. Measures for the pro-inflammatory cytokines IL-6 and TNF-α, along with the acute-phase protein, CRP, were obtained from frozen stored plasma taken at baseline (Penninx et al., 2003). All blood samples were obtained in the morning (Penninx et al., 2003). After processing, the specimens were aliquoted into cryovials, frozen at -70°C, and shipped to the Health ABC Core Laboratory at the University of Vermont (Penninx et al., 2003). Plasma IL-6 and TNF-α levels were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN). The detectable limit for IL-6 (by HS600 Quantikine kit) was .10

pg/mL, and for TNF-α (by HSTA50 kit) .18 pg/mL. Plasma levels of CRP were also measured in duplicate by ELISA based on purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, CA). C-reactive protein assays were standardized according to the World Health Organization First International Reference Standard (i.e., a sensitivity of 0.08 μg/mL). Circulating IL-6 and CRP levels have been shown to be reproducible and representative over extended periods (Penninx et al., 2003; Macy, Hayes, & Tracy, 1997; Rao, Pieper, Currie, & Cohen, 1994).

Diabetes Status Measurements. At baseline, participants were asked whether a doctor had ever diagnosed them with type 2 diabetes (yes/no; Figaro, Kritchevsky, Resnick, Shorr, & Butler et al., 2006; de Rekeneire, Peila, Ding, Colbert, & Visser et al., 2006). In addition, each participant had their HbA1c measured (Bio-Rad, Hercules, CA; Figaro et al., 2006). To assess for the use of diabetes medications, participants were asked to bring prescribed and over-the-counter medications used in the preceding 2 weeks (de Rekeneire et al., 2006). Participants also had a fasting glucose measured and received a 75-g oral glucose tolerance test if they were not currently taking diabetes medications at baseline (Figaro et al., 2006; de Rekeneire et al., 2006). The oral glucose tolerance test was performed after at least an 8-hour overnight fast (de Rekeneire et al., 2006). Glucose parameters were measured on a Johnson & Johnson Vitros 950 analyzer (de Rekeneire et al., 2006). Biological specimens were processed according to standardized protocols by the Laboratory of Clinical Biochemistry at the University of Vermont (de Rekeneire et al., 2006).

Diabetes status was determined using standardized algorithms developed by the National Institute on Aging for use in the Health ABC study (2004). These algorithms were based on criteria from the American Diabetes Association (Figaro et al., 2006; de Rekeneire et al., 2006). A diagnosis of diabetes was based on 1) self-reported diagnosis from a medical doctor and/or use of diabetic medications; 2) measured fasting glucose results ≥ 126 mg/dl; or 3) oral glucose tolerance test results ≥ 200 mg/dl.

*Center for Epidemiologic Studies Depression (CES-D) Scale***.** Two versions of the CES-D scale were used during this investigation (i.e., the full-scale CES-D and a brief 10-item version, the CES-D 10). At baseline, depressive symptoms were measured with both the CES-D and the CES-D 10. At year 2, depressive symptoms were measured using only the CES-D 10. Because the performance characteristics of the full-scale CES-D have been well documented in comparison to the CES-D 10 (Wancata, Alexandrowicz, Marquart, Weiss, & Friedrich, 2006; Haringsma, Engels, Beekman, & Spinhoven, 2004; Beekman et al., 1997; Oxman, Berkman, Kasl, Freeman, & Barrett, 1992), the full-scale version was used during all cross-sectional analyses (i.e., study aims 1 & 2). The CES-D 10 was used only during longitudinal analyses (i.e., study aim 3).

The full-scale version of the CES-D is a 20-item self-report questionnaire designed to measure depressive symptoms experienced during the previous week (Radloff, 1977). The scale, ranging from 0 to 60, has been shown to be a valid and reliable instrument in older populations (Beekman et al., 1997). The internal consistency has been demonstrated to be high: Cronbach's $\alpha = .82$. When compared to other measures

for detecting depressive symptoms among older adults (e.g., Geriatric Depression Scale), the CES-D has demonstrated comparable criterion validity (Wancata et al., 2006).

Given that many of the physical symptoms of depressed mood (e.g., fatigue, weight/appetite fluctuations, & sleep disturbances) often overlap with symptoms of chronic illness, the use of higher cut-scores on the CES-D scale have been proposed in order to reduce the potential for increased false-positive rates of clinically meaningful depressive symptoms (Haringsma et al., 2004) . To help reduce false-positive rates of clinically meaningful depressive symptoms, this study employed a CES-D cut-score of ≥ 20. Haringsma and colleagues (2004) have reported the sensitivity (93.2%) and specificity (43.2%) of the CES-D (cut-score ≥ 20) in detecting clinically relevant depressive symptoms among older adults (ages 55-85).

The brief 10-item version of the CES-D scale (i.e., the CES-D 10; Andersen, Malmgran, Carter, & Patrick, 1994), ranges from 0 to 30, and has been shown to be a valid and reliable instrument for screening depression in older adults and among adults with diabetes (Irwin, Artin, & Oxman, 1999; Zauszniewski & Graham, 2009). Items from the 10-item version of the CES-D scale have been found to be highly correlated with items from the full, 20-item CES-D scale ($r = 0.96$; Zauszniewski & Graham, 2009). Consistent with findings by Zauszniewski & Graham (2009), the correlation coefficient between the 20-item CES-D scale and the 10-item CES-D scale for this study was ($r = 0.95$, $p<.0001$). To identify respondents with a level of depressive symptomatology that is clinically meaningful, the cut-off score of ≥ 10, which has good criterion validity for major depression was employed (Andersen et al., 1994).

*Covariates***.** Demographic variables including age, race (White/Black), study site (Pittsburgh/Memphis), smoking status (current/former/never smoker), and average alcohol use during the past year (none/< 1 ounce per week/1-7 drinks per week/> 1 drink per day) were assessed during the baseline interview (National Institute on Aging, 1997). A person's total kilograms of body fat mass was determined by dual-energy x-ray absorptiometry (QDR 4500A, software version 8.21; Hologic, Waltham, MA). The baseline presence of lung disease (including asthma/chronic bronchitis/emphysema/chronic obtrusive pulmonary disorder), cardiovascular disease (including stroke/TIA/coronary heart disease/abnormal electrocardiogram/angioplasty/coronary artery bypass surgery/myocardial infarction/angina pectoris/congestive heart failure/peripheral arterial disease), renal insufficiency, and rheumatoid arthritis was coded using standardized algorithms that incorporated various sources of information (e.g., self-report, medication use, medical examination, and data from physicians; Healthy Aging and Body Composition Study, 2004). All medications taken regularly in the past 2 weeks were recorded and coded according to the Iowa Drug Information System code (Pahor et al 1994). Using this drug inventory, the daily use of anti-inflammatory drugs (including Cox-2 Inhibitors/Oral Steroids/Salicylates/NSAIDs) and anti-depressants (including MAO Inhibitors/Tricyclics/SSRIs) were assessed.

Analyses

Statistical analyses were conducted using SAS 9.1 (SAS Institute, 2003). Data were examined for normality, homoscedasticity, skewedness, kurtosis, and

multicollinearity prior to analysis. Because plasma levels of inflammatory markers and triglycerides were found to be non-normally distributed, continuous measurements of these variables were log-transformed to produce normally distributed data. Statistical analyses were performed for each inflammatory marker separately.

Analysis of covariance was used to examine the mean differences in levels of log-transformed IL-6, TNF-α, and CRP by T2DM status, depression symptom status, and the interaction term (T2DM X depression symptom status). This produced four groups of participants for comparison during post-hoc analyses: T2DM and elevated depressive symptoms (T2DM+DEP), (T2DM Only), elevated depressive symptoms only (DEP Only), and healthy controls (Healthy). Scheffé tests were used in all post-hoc comparisons in order to reduce the probability of type 1 error. Covariates assessed for inclusion into the models included site, age, gender, total fat mass, smoking status, alcohol use, lung disease, heart disease, arthritis, use of anti-inflammatory drugs and use of anti-depressant medications. The presence of multicollinearity among covariates was assessed through use of the variance inflation factor (VIF; the inverse of the proportion of variance not accounted for by other independent variables/covariates). Commonly referenced VIF guidelines for multicollinearity suggest that no VIF should be ≥ 10 and a mean VIF for each regression model be < 2 (Strotmeyer, Cauley, Orchard, Steenkiste, & Dorman, 2006; Menard, 1995; Chatterjee & Price, 1991; Mason, Gunst, & Hess, 1989). Further, the utility of covariates was examined through use of pooled within-cell correlations (Tabachnick & Fidell, 2001). These correlations helped to determine if a covariate was related to the dependent variable (i.e., inflammation) once adjustment was

made for other covariates. Covariates that were not significantly related to inflammation following this procedure were not included in the final ANCOVA models. Effect sizes were calculated using eta squared.

Logistic regression analyses were used to determine the relationship between continuous plasma levels of log-transformed inflammatory markers (baseline assessment) and elevated depressive symptoms (baseline assessment) after adjustment for significant covariates. Overall model fit was evaluated using Hosmer-Lemeshow goodness-of-fit statistic (Tabachnick & Fidell, 2001). Test of discrimination of depression status was evaluated by examining the area under the curve (Tabachnick & Fidell, 2001). The predictive power of the independent variables on determining depressed mood status was measured using the Max-rescaled R^2 value. These values can range from 0 to 1, with smaller values representing lower predictive power of the model (Allison, 1999).

Finally, logistic regression analyses were used to determine the extent to which log-transformed markers of inflammation (baseline assessment) predicted elevated depressive symptom status (at year 2) after controlling for significant covariates. Overall model fit was evaluated using Hosmer-Lemeshow goodness-of-fit statistic (Tabachnick & Fidell, 2001). Test of discrimination of depression status was evaluated by examining the area under the curve (Tabachnick & Fidell, 2001). The predictive power of the independent variables on determining depressed mood status was measured using the Max-rescaled R^2 value. These values can range from 0 to 1, with smaller values representing lower predictive power of the model (Allison, 1999).

Results

Of the 3,075 participants in the Health ABC study, a total of n = 30 were missing all three markers of inflammation (i.e., IL-6, TNF-α, & CRP) and were subsequently excluded from the sample. Of the remaining 3,045 participants, n = 7 were excluded because they had inadequate data for determining T2DM status. An additional n = 24 participants were excluded because they did not have complete CES-D scores. Thus, a sample of N = 3,014 participants was used in all further analyses.

Demographics

Demographic characteristics are presented in Table 1 by T2DM and elevated depressed mood status. Approximately 23.5% of the sample had T2DM while 2.3% of participants had elevated levels of depressive symptoms according to the CES-D (cut-score ≥ 20). In both the DEP only and T2DM + DEP groups, women reported more elevated depression scores than men. The T2DM only and T2DM + DEP groups contained higher proportions of participants who were Black, had less than a high school education, or were diagnosed with cardiovascular disease. Total percent body fat did not differ across the study groups.

Table 1. Baseline Demographic and Medical Record Characteristics by T2DM and Depressed Mood Status

	Healthy (n = 2256)	DEP Only (n = 51)	T2DM Only (n = 690)	T2DM + DEP (n = 17)	Total Sample (n = 3014)	p[a]
Age (years)	73.6 (2.9)	73.4 (2.9)	73.6 (2.8)	74.0 (3.3)	73.6 (2.9)	.21[†]
Gender						<.0001
Men	46.9	33.3	55.2	41.2	48.5	
Women	53.1	66.7	44.8	58.8	51.5	
Ethnicity						<.0001
White	61.8	74.5	48.1	47.1	58.8	
Black	38.3	25.5	51.9	52.9	41.2	
Site						.51
Memphis	49.9	49.0	50.7	23.5	49.9	
Pittsburgh	50.1	51.0	49.3	76.5	50.1	
Marital Status						.28
Married	55.0	51.1	54.8	42.9	54.8	
Widowed	30.7	40.0	30.5	50.0	30.9	
Divorced/Separated	9.3	4.4	7.2	7.1	9.4	
Never Married	5.1	4.4	4.8	------	5.0	
Education						<.0001
Less than H.S.	21.5	17.7	30.6	35.3	23.6	
H.S.	27.5	41.2	27.1	47.1	27.7	
Trade/Vocational	6.3	9.8	7.0	11.8	6.5	
Part College	18.8	15.7	15.9	5.9	18.0	
College	13.6	13.7	9.8	------	12.7	
Post-College	12.3	2.0	9.8	------	11.5	
Income						<.05
< 10,000	12.0	17.8	16.2	29.4	13.2	
10,000-25,000	38.1	37.8	41.0	47.1	38.8	
25,001-49,999	32.6	33.3	29.8	17.7	31.9	
≥ 50,000	17.3	11.1	13.0	5.9	16.1	
Current Smoker	10.7	13.7	8.1	23.5	10.3	.07
Total Body Fat (Kg)	35.0 (7.9)	37.5 (7.1)	35.0 (7.6)	34.2 (8.7)	35.0 (7.8)	.08[†]

Table 1 (Continued). Baseline Demographic and Medical Record Characteristics by T2DM and Depressed Mood Status

	Healthy (n = 2256)	DEP Only (n = 51)	T2DM Only (n = 690)	T2DM + DEP (n = 17)	Total Sample (n = 3014)	p[a]
Lung Disease	10.8	21.6	13.2	------	11.5	.42
Heart Disease	24.8	29.4	37.0	35.3	27.7	<.0001
Rheumatoid Arthritis	20.7	26.3	27.5	16.7	22.2	.22
Anti-inflammatory drugs	53.0	62.8	52.4	58.8	53.1	.72
Antidepressant drugs	2.7	15.7	0.7	17.7	2.5	<.0001
MAOI	0.3	------	0.2	------	0.2	
Tricyc	2.4	13.7	2.6	5.9	2.6	
SSRI	3.0	5.9	1.5	17.7	2.8	
Other	1.0	7.8	0.2	------	0.9	
Statins	12.4	11.8	15.2	5.9	13.0	.04
Diabetes Duration	------	------	13.6 (12.0)	14.1 (12.7)	13.7 (12.0)	.63
A1c	6.0 (0.6)	5.9 (0.5)	7.6 (1.6)	7.5 (1.5)	6.4 (1.1)	<.0001[†b,c,d,e]
Insulin Use	------	------	26.8	36.4	27.0	.87
OHAs	------	------	59.5	63.6	59.5	.52
Cholesterol	203.6 (38.1)	202.3 (27.2)	200.1 (40.4)	191.4 (31.8)	202.7 (38.6)	.08[†]
HDL	55.2 (17.0)	54.9 (15.2)	50.2 (16.4)	50.1 (20.1)	54.0 (17.0)	<.0001[†b]
LDL	122.4 (34.1)	121.1 (28.8)	118.4 (36.4)	109.6 (35.8)	121.4 (34.6)	<.05[†b]
Triglycerides	131.5 (75.4)	140.7 (72.8)	161.6 (100.1)	158.5 (96.2)	138.7 (82.7)	<.0001[†b, c]

Data presented as mean (SD) or %.

[a] p -values are based on χ^2 tests for categorical variables and [†]ANOVA for continuous variables.

[b]significant comparison between Healthy and T2DM Only groups.

[c]significant comparison between DEP Only and T2DM Only groups.

[d]significant comparison between Healthy and T2DM + DEP groups.

[e]significant comparison between DEP Only and T2DM + DEP groups.

Zero-Order Correlations between Predictor and Outcome Variables and Covariates

Plasma levels of IL-6, TNF-α, and CRP were moderately intercorrelated (Table 2). The strongest correlation was observed for IL-6 and CRP ($r = 0.47$, $p<.0001$). The correlation between IL-6 and TNF-α was $r = 0.28$ ($p<.0001$), and between CRP and TNF-α was $r = 0.14$ ($p<.0001$). Depressive symptoms measured by the CES-D (baseline) and CES-D10 (both at baseline and at year 2) were moderate to strongly intercorrelated. The strongest correlation was observed for the CES-D and the CES-D10 (baseline; $r = 0.95$, $p<.0001$). The correlation between the CES-D and the CES-D10 (year 2) was $r = 0.49$ ($p<.0001$), and between the CES-D10 (baseline) and CES-D10 (year 2) was $r = 0.50$ ($p<.0001$). Small correlations were observed between IL-6 and the CES-D ($r = 0.04$, $p<.05$) and the CES-D10 (year 2; $r = 0.05$, $p<.05$) as well as CRP and the CES-D10 (year 2; $r = 0.07$, $p<.0001$). Table 3 shows the zero-order correlations between predictor and outcome variables and covariates.

Table 2. Zero-Order Correlations between Predictor and Outcome Variables

Baseline Variables	IL-6	TNF-α	CRP	CES-D	CES-D 10	Year 2 CES-D 10
IL-6	1.0	0.28**	0.47**	0.04*	0.05*	0.05*
TNF-α		1.0	0.14**	0.03	0.03	0.03
CRP			1.0	0.03	0.07**	0.07**
CES-D				1.0	0.49**	0.49**
CES-D 10					1.0	0.50**
Year 2 CES-D 10						1.0

*p<.05; **p<.0001

Table 3. Zero-Order Correlations between Predictor and Outcome Variables and Covariates

Baseline Variables	IL-6	TNF-α	CRP	CES-D	CES-D 10	Year 2 CES-D 10
Age	0.04*	0.08**	-0.06*	0.01	0.01	0.04*
Total % Body Fat	0.04*	0.02	0.25**	0.07*	0.08**	0.10**
Cholesterol	-0.10**	-0.02	0.03	0.02	0.01	0.02
HDL	-0.16**	-0.29**	0.01	0.03	0.03	0.01
LDL	-0.06*	-0.01	0	0	0	0.01
Triglycerides	0.05*	0.30**	0.05*	0.02	0.01	0.02
Ethnicity[a]	0.10**	-0.12**	0.16**	0.01	0.02	0.08**
Income[a]	-0.09**	0.01	-0.13**	-0.16**	-0.15**	-0.18**
Marital Status[a]	0.03	-0.04*	0.10**	0.08**	0.08**	0.09**
Education[a]	-0.09**	-0.02	-0.10**	-0.13**	-0.12**	-0.16**
Study Site[a]	-0.06*	0.04	-0.01	-0.02	-0.04*	0.01
Gender[a]	-0.07*	-0.07*	0.14**	0.10**	0.11**	0.12**
Smoking Status[a]	0.08**	0.06*	0.05*	-0.01	0	-0.01
# Alcohol Drinks	-0.04*	-0.01	-0.04*	-0.03	-0.02	-0.05*
Lung Disease[a]	0.10**	0.01	0.09**	0.09**	0.06*	0.07*
Heart Disease[a]	0.14**	0.14**	0.02	0.02	0.03	0.06*
Rheumatoid Arthritis[a]	0.05	0.08*	0.02	0.02	-0.01	0.06
Kidney Disease[a]	0.04*	0.07*	0.01	0.01	0.01	0.03
Anti-Depressants[a]	-0.02	0.02	0.01	0.01	0.09**	0.09**
Anti-Inflammatory Medications[a]	0.02	0.04*	0.03	0.03	0	0.04
Statins[a]	0.03	0.05*	-0.01	-0.01	-0.02	0.01

*p<.05; **p<.0001
a=Spearman correlation coefficients

Unadjusted Mean Inflammatory Marker Levels According to T2DM and Depressed Mood Status

Table 4 shows the mean plasma concentrations of inflammatory markers by T2DM and depressed mood status. Mean levels of IL-6 and CRP were significantly higher ($p<.0001$) in persons with T2DM+DEP compared to those in the T2DM or DEP Only groups, followed by Healthy Controls. Individuals with T2DM Only had significantly ($p<.0001$) elevated mean levels of TNF-α compared to Healthy Controls. However, a relationship in mean TNF-α levels was not observed across the groups.

Table 4. Unadjusted Mean Inflammatory Marker Levels According to Diabetes and Depressed Mood Status

	Healthy (n = 2153) Mean (SD) (95%CI)	DEP Only (n =50) Mean (SD) (95%CI)	T2DM Only (n = 659) Mean (SD) (95%CI)	T2DM + DEP (n =14) Mean (SD) (95%CI)	p†
IL6 (pg/mL)	2.3 (1.9) (2.2-2.4)	2.6 (2.2) (2.0-3.2)	2.7 (2.0) (2.6-2.9)	4.2 (2.8) (2.7-5.7)	<.0001[a,b,c,d]
TNF-α (pg/mL)	3.4 (1.7) (3.3-3.5)	3.7 (1.5) (3.3-4.1)	3.7 (1.8) (3.6-3.9)	4.2 (1.6) (3.4-5.1)	<.0001[b]
CRP (mg/L)	2.8 (4.5) (2.6-3.0)	3.0 (4.2) (1.8-4.2)	3.6 (5.4) (3.2-4.0)	5.7 (4.9) (3.2-8.2)	<.0001[a,b,c,d]

† p -values are based on ANOVA for continuous variables with pair-wise comparisons.
[a]significant comparison between Healthy and T2DM + DEP groups.
[b]significant comparison between Healthy and T2DM Only groups.
[c]significant comparison between DEP Only and T2DM + DEP groups.
[d]significant comparison between T2DM Only and T2DM + DEP groups.

Note. Cell sizes differ from the overall sample size due to variation in the availability of inflammatory markers for each participant.

Primary Aim (1): Differences in Inflammatory Markers by T2DM and Depressed Mood Status (CES-D ≥ 20)

The interaction between T2DM and depressed mood (CES-D ≥ 20) status on levels of IL-6 (pg/mL) was significant, $F(1, 2761)=5.3$, $p<.05$, $\eta^2=<.001$, after controlling for race, gender, study site, percent body fat, smoking status, and heart and lung disease. A Scheffé test was performed to test for significant pair-wise differences among the four group means. Figure 2 shows that the adjusted marginal means for IL-6 were significantly higher ($p<.05$) among those with T2DM+DEP compared to all other groups (4.4±2.9 versus 2.7±2.0 [T2DM Only], 2.6±2.2 [DEP Only], & 2.3±1.9 [HC]).

Similarly, after adjustment for race, gender, percent body fat, triglycerides, smoking status, and lung disease, the interaction between T2DM and depressed mood status on levels of CRP (mg/L) was significant, $F(1, 2875)=5.5$, $p<.05$, $\eta^2=.002$. A Scheffé test was performed to test for significant differences among the four group means. Figure 3 shows that the adjusted marginal means for CRP were significantly higher ($p<.05$) among those with T2DM+DEP (5.3±4.8) compared to DEP Only (2.9±4.2) and Healthy Controls (2.8±4.5). The difference in levels of CRP between those with T2DM+DEP (5.3±4.8) and T2DM (3.6±5.5) approached significance ($p=.11$). The interaction between T2DM and depressed mood status on levels of TNF-α (pg/mL) was not significant, $F(1, 2736)=0.00$, $p=0.96$, after adjustment for gender, race, percent body fat, and heart disease.

Secondary Aim (2): Risk of Depressed Mood at Baseline Associated with High Inflammation among those with T2DM

Logistic regression analyses were used to calculate the odds ratios and confidence intervals of depressed mood for continuous plasma levels of log-transformed inflammatory markers in a sub-sample of n=707 T2DM patients. Analyses were conducted for depressed mood using a cut-score of CES-D ≥ 20. Results shown in Table 5 demonstrate that T2DM participants with higher levels of IL-6 had a significantly increased likelihood of experiencing depressed mood compared to individuals with lower levels of IL-6 (OR = 10.43, 95% CI = 1.65 – 66.09, Max-rescaled $R^2 = 0.13$) after controlling for gender, site, and antidepressant usage. The likelihood of experiencing depressed mood was 10.4 times greater among T2DM participants with elevated levels of IL-6 than T2DM participants with lower levels of IL-6.

Table 5. Adjusted Risk of Depressed Mood (CES-D ≥ 20) Associated with High Levels of IL-6 in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	0.25	0.54	0.21	1.28	0.45-3.67
Study Site	1.11	0.59	3.50	3.03	0.95-9.67
Depression Medications	2.70	0.98	7.66*	14.95	2.20-101.55
IL-6	2.34	0.94	6.19*	10.43	1.65-66.09
Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 654) = 5.46$; p = 0.71		ROC = 0.77		Max-rescaled $R^2 = 0.13$

*p<.05

However, results in Table 6 suggest that individuals with higher levels of TNF-α were not significantly more likely to have depressed mood than those with lower levels of TNF-α after adjustment for gender, site, and antidepressant usage.

Table 6. Adjusted Risk of Depressed Mood (CES-D ≥ 20) Associated with High Levels of TNF-α in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	0.09	0.57	0.03	1.09	0.36-3.33
Study Site	1.40	0.67	4.39*	4.05	1.10-15.0
Depression Medications	3.31	0.86	14.74*	27.30	5.05-147.69
TNF-α	1.61	1.60	1.01	5.02	0.22-116.50
Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 654) = 16.83$; p = 0.03		ROC = 0.75		Max-rescaled $R^2 = 0.15$

*p<.05

Further, results in Table 7 suggest that individuals with higher levels of CRP were not significantly more likely to have depressed mood than those with lower levels of CRP after adjustment for gender, site, and antidepressant usage.

Table 7. Adjusted Risk of Depressed Mood (CES-D ≥ 20) Associated with High Levels of CRP in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	0.21	0.53	0.16	1.24	0.44-3.51
Study Site	1.26	0.60	4.44*	3.53	1.09-11.4
Depression Medications	2.74	0.86	10.21*	15.43	2.88-82.68
CRP	1.15	0.62	3.42	3.17	0.93-10.74
Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 578) = 5.01$; p = 0.76		ROC = 0.74		Max-rescaled $R^2 = 0.14$

*p<.05

Secondary Aim (3): Predicting Year 2 Depressed Mood from Inflammation at Baseline among those with T2DM

Logistic regression analyses were conducted to determine if continuous levels of IL-6, TNF-α, and CRP (at baseline) were predictive of depressed mood status in year 2

using scores from the CES-D 10 (i.e. depressed mood ≥ 10 vs. no depressed mood < 10) in a sub-sample of n=707 T2DM patients. Preliminary analyses were conducted to identify significant predictors of depressed mood at year 2 among demographic and diabetes status variables. Significant predictors of depressed mood at year 2 included: baseline CES-D 10 scores, total percent body fat, gender, race, family income, education level, and marital status. In the final logistic regression models, all variables were entered simultaneously. It is worth noting that comorbid conditions such as cardiovascular and lung disease, renal insufficiency, and arthritis were not included in the final logistic regression models because they were not significantly related to depressed mood at year 2.

Results shown in Table 8 demonstrate that elevated levels of TNF-α at baseline significantly ($p<.05$) predicted depressed mood status at year 2 (OR = 12.30, 95% CI 2.24-67.59), in the presence of baseline depressed mood status, total percent body fat, gender, race, family income, and education and marital status. A total of 4% of the variance in depressed mood status at year 2 was accounted for by TNF-α. Although the estimated logistic regression coefficients and odds ratios are high for both TNF-α and year 1 depressed mood status (Table 8), examination of the variance inflation factor for the model did not indicate the presence of severe multicollinearity. This suggests that the risk of experiencing depressed mood at year 2 was 12.3 times greater among T2DM participants with elevated levels of TNF-α at baseline than T2DM participants with lower levels of TNF-α at baseline.

Numerous studies have demonstrated that TNF-α is over-expressed within the adipose tissue of obese T2DM patients (Miyazaki et al., 2003; Mishima et al., 2001; Katsuki et al., 1998; Nilsson et al., 1998; Winkler et al., 1998). To better understand the present findings, ad-hoc analyses were conducted to determine if there were significant differences in the total percent body fat (baseline assessment) between T2DM participants with and without depressed mood symptoms at year 2. It was determined that T2DM participants with depressed mood at year 2 had significantly more total percent body fat (M = 36.8 ± 7.8) at baseline than T2DM participants without depressed mood (M = 35.0 ± 7.4; t[621] = -2.02, p<.05, *d* = 0.24).

Table 8. Logistic Regression Model to Assess Baseline Predictors of Year 2 Depressed Mood Status

Predictors	B	S.E.	Wald	OR	95% CI
Y1 Depressed Mood Status	3.13	0.47	43.61**	23.00	9.06-58.19
Total Percent Body Fat	0.09	0.03	8.01*	1.10	1.03-1.17
Gender	-1.01	0.49	4.24*	0.37	0.14-0.95
Race	0.28	0.35	0.66	1.33	0.67-2.65
Family Income	-0.31	0.23	1.77	0.74	0.47-1.16
Education	-0.08	0.12	0.43	0.93	0.74-1.16
Marital Status	0.05	0.18	0.07	1.05	0.73-1.50
TNF-α	2.51	0.87	8.33*	12.30	2.24-67.59
Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 486) = 10.75$; p = 0.22		ROC = 0.82		Max-rescaled $R^2 = 0.28$

*p<.05 **p<.0001

Results shown in Table 9 demonstrate that elevated levels of IL-6 at baseline did not significantly predict depressed mood status at year 2, in the presence of baseline depressed mood status, total percent body fat, gender, race, family income, and education and marital status.

Table 9. Logistic Regression Model to Assess Baseline Predictors of Year 2 Depressed Mood Status

Predictors	B	S.E.	Wald	OR	95% CI
Y1 Depressed Mood Status	2.66	0.44	37.17**	14.35	6.10-33.79
Total Percent Body Fat	0.08	0.03	6.57*	1.09	1.02-1.16
Gender	-1.19	0.49	5.92*	0.31	0.12-0.79
Race	0.13	0.34	0.15	1.14	0.59-2.22
Family Income	-0.42	0.23	3.51	0.65	0.42-1.02
Education	-0.07	0.12	0.34	0.94	0.75-1.17
Marital Status	0.01	0.18	0.00	1.01	0.71-1.43
IL-6	0.81	0.58	1.93	2.24	0.72-7.03

Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 495) = 8.73$; $p = 0.36$	ROC = 0.81	Max-rescaled $R^2 = 0.25$

*p<.05 **p<.0001

Results shown in Table 10 demonstrate that elevated levels of CRP at baseline did not significantly predict depressed mood status at year 2, in the presence of baseline depressed mood status, total percent body fat, gender, race, family income, and education and marital status.

Table 10. Logistic Regression Model to Assess Baseline Predictors of Year 2 Depressed Mood Status

Predictors	B	S.E.	Wald	OR	95% CI
Y1 Depressed Mood Status	2.64	0.42	38.70**	13.95	6.08-31.99
Total Percent Body Fat	0.08	0.03	5.83*	1.08	1.02-1.15
Gender	-1.19	0.47	6.38*	0.30	0.12-0.77
Race	0.15	0.33	0.22	1.17	0.61-2.23
Family Income	-0.44	0.22	4.04*	0.65	0.42-0.99
Education	-0.03	0.11	0.08	0.99	0.78-1.20
Marital Status	0.07	0.17	0.15	1.07	0.76-1.50
CRP	0.19	0.41	0.22	1.21	0.54-2.70

Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 514) = 10.81$; $p = 0.21$	ROC = 0.79	Max-rescaled $R^2 = 0.24$

*p<.05 **p<.0001

Discussion

This work represented the first investigation to demonstrate that the presence of T2DM and elevated depressive symptoms were associated with a graded relationship in levels of specific inflammatory markers. Results showed that those with T2DM and elevated depressive symptoms had significantly higher levels of IL-6 compared to those with T2DM or elevated depressive symptoms alone, compared to healthy controls even after adjustment for potential confounders. The significance of this finding is that it reveals the presence of an additive effect for IL-6, which is important because it supports the conceptualization of inflammation as an underlying biological mechanism linking T2DM and depressed mood (Figure 1). Because such a link has not been established previously, the current finding for IL-6 suggests that interrelationships between the two disorders are at least plausible (Figure 1), and that the presence of inflammation in one disorder could conceivably help to stimulate, increase, or sustain the inflammatory response in the other disorder.

Findings from this study also demonstrate that the presence of T2DM, but not elevated depressive symptoms, is associated with increased levels of CRP. In both adjusted (Figure 3) and unadjusted (Table 4) analyses, higher circulating levels of CRP were observed in the T2DM groups compared to elevated depressive symptoms alone and healthy control groups. This suggests that the presence of T2DM is important to the up-regulation of CRP. It may be that the underlying disease processes related to T2DM (e.g., insulin resistance, dyslipidemia, &/or atherosclerosis) contributed to greater circulating

levels of CRP in the T2DM groups. In addition, the inability to detect an additive effect for CRP versus IL-6 may suggest that differential effects of T2DM and elevated depressive symptoms exist for various markers of inflammation.

When considering the above patterns of results for both IL-6 and CRP, it is important to note that these findings are not likely due to type 1 error. It was recognized that conducting numerous post-hoc comparisons between the study groups for each of these inflammatory markers added to the risk of making a type 1 error. However, to address this issue, Scheffé tests were used to help protect against the increased type 1 error generated from making multiple pair-wise comparisons among unequal cell sizes.

It should also be highlighted that the study population consisted of relatively healthy older adults, and extrapolating the current findings to younger adults or frail older adults may not be appropriate. Despite this point, there is no evidence to suggest that the current findings for IL-6 and CRP were the result of using study groups that were irregular in some systematic way due to demographic or medical characteristics at baseline. Significant variables that emerged from these characteristics (see Table 1) were examined for inclusion as covariates in later analyses. Thus, it can be concluded with greater confidence that variables such as poor health status among those with T2DM cannot explain, for example, the up-regulation of CRP associated with diabetes, but not depressed mood, since all analyses were adjusted for confounders (e.g., CVD and/or rheumatoid arthritis). The possibility that age-related changes to innate immunity could have accounted for the observed results was also considered. However, this explanation is also unlikely since the average age of participants across the study groups was

approximately equivalent and non-significant. The inability of both statistical and methodological considerations to explain the observed differential effects for IL-6 and CRP provides support for the notion that the present findings are not simply due to chance or the result of some observable systematic difference(s). Further investigation is warranted to better understand these complex relationships and to explore many of the individual pathways illustrated in Figure 1.

The presence of an additive effect of T2DM and elevated depressive symptoms on TNF-α was not observed in this study. One reason for this finding may have been the lack of variability in total percent body fat across the study participants (see Table 1). Obesity is a state of chronic inflammation, often characterized by the over-expression of TNF-α within adipose tissue, which is important because visceral obesity plays a pivotal role in the development of T2DM (Dandona, Aljada, & Bandyopadhyay, 2004). Given there were no significant differences in the mean total percent body fat reported across the study groups, the ability to detect an interaction between T2DM and elevated depressive symptoms may have been obscured by the fact that TNF-α was already up-regulated among participants relative to IL-6 and CRP. In this sense, these findings at least support prior research demonstrating that TNF-α is strongly correlated with obesity in both T2DM and non-T2DM samples. While this does not exclude TNF-α as an important inflammatory marker related to T2DM and elevated depressive symptoms it does demonstrate the importance of assessing the association between obesity and TNF-α during future investigations.

When the association between elevated depressive symptoms and each of the inflammatory markers was examined independently, the odds of depressed mood for T2DM participants with elevated continuous levels of IL-6 was approximately 10 times higher than the odds of elevated depressive symptoms for T2DM participants with lower levels of IL-6. Similar patterns were observed for continuous levels of TNF-α and CRP, although non-significant. This may suggest that of the three inflammatory markers, IL-6 was the one most closely associated with elevated depressive symptoms in those with T2DM. However, it could also be that IL-6 was the least variable of the three inflammatory markers, and as a result of being measured continuously, was a stronger correlate of elevated depressive symptoms. This highlights the prognostic value of using IL-6 as an indicator of depressed mood status in T2DM.

Finally, it was demonstrated that longitudinally, elevated levels of inflammation at baseline significantly predicted elevated depressive symptoms at year 2. Participants with T2DM who had higher levels of TNF-α at baseline had a 12-fold increased risk of depressed mood at year 2 compared to T2DM participants with lower levels of TNF-α. This finding suggests that elevated depressive symptoms among individuals with T2DM may be a function of elevated levels of TNF-α, not IL-6 and CRP, which could thus influence the development or exacerbation of depressive symptoms over time. What remains unclear is why TNF-α would be unrelated to T2DM and elevated depressive symptoms during cross-sectional analysis, yet emerge as a significant predictor of increased depressive symptoms longitudinally.

Currently, it can only be speculated as to what factors would contribute to this pattern of results. For example, one factor may have been greater total adiposity at baseline. Given that obesity is often strongly correlated with TNF-α (Miyazaki et al., 2003; Mishima et al., 2001; Katsuki et al., 1998; Nilsson et al., 1998; Winkler et al., 1998), up-regulated levels of this inflammatory marker might be explained by the presence of greater total body fat at baseline among T2DM participants with elevated depressive symptoms at year 2. Ad-hoc t-test analyses demonstrated that T2DM participants with elevated depressive symptoms at year 2 had significantly more total percent body fat (~ 1.8%) at baseline than T2DM participants with minimal depressive symptoms. This finding supports the possibility that more body fat among T2DM participants with elevated depressive symptoms contributed to higher levels of TNF-α. However, while this group difference was statistically significant, it is unclear whether a relatively small difference in total percent body fat between these groups would allow for disproportionately higher levels of TNF-α. It could also be that factors such as gender differences and adipose tissue location (e.g., abdominal visceral fat versus subcutaneous fat) played a role in the up-regulation of TNF-α at baseline (Cartier, Cote, Lemieux, Perusse, & Tremblay et al., 2009; Beasley, Koster, Newman, Javaid, & Ferrucci et al., 2009). However, no such gender differences were found during follow-up analyses for this study and adipose depot location was not assessed as part of this investigation.

Other design/methodological considerations could have contributed to the association between up-regulated TNF-α and elevated depressive symptoms at year 2. For example, among the overall n=629 T2DM participants available for longitudinal data

analysis, n=143 participants were excluded due to missing covariate data. The resulting sub-sample of T2DM participants (i.e., n=486) may have produced a higher average level of TNF-α than what would have been observed if the whole sample of T2DM participants had been used. However, this explanation is unlikely given that the mean levels of TNF-α for the overall sample and sub-sample of T2DM participants were equivalent (~3.0 pg/mL). It could also be that TNF-α was related to elevated depressive symptoms longitudinally due to some systematic difference between baseline and year 2 data on demographic and/or medical record variables not previously examined. Regrettably, investigating these systematic differences was not possible because many of the demographic and medical variables reported at baseline were not available at year 2 for this secondary data analysis. This is recognized by the author as a limitation of the current investigation. However, it does make the argument that more research is needed to better understand the mechanism(s) responsible for the longitudinal relationship between up-regulated TNF-α and increased risk of depressive symptoms among T2DM participants. It may be that this relationship is the result of some complex interaction between fatty acid mobilization, oxidation, and storage (Blaak, 2001).

This study has several limitations. The study design does not allow for causal inferences to be made regarding the directionality of IL-6 and CRP in the association between T2DM and depression. In addition, the study sample was comprised of well-functioning older adults and as a result had a relatively low prevalence of depressive symptoms in comparison with other community-based samples of adults. However, the low prevalence of depressive symptoms did not interfere with the ability to detect

significant differences in inflammation between the relatively small number of individuals within the T2DM+DEP and DEP Only groups. Replication of these findings within a study population demonstrating a higher prevalence of depressive symptoms is warranted. Finally, this study used a self-report scale to measure depressive symptomatology and did not include a clinical and/or diagnostic interview for determining depression. It should be noted though that when working with medical populations, the use of a more stringent cut-score for depressive symptoms on the CES-D scale may help to reduce the potential for high false-positive rates for depressed mood (Haringsma et al., 2004).

The significance of this investigation is that it demonstrates the presence of heightened inflammation (i.e., IL-6) among individuals with both T2DM and elevated depressive symptoms compared to individuals with either disorder alone and healthy controls. This finding provides proof of concept that T2DM and elevated depressive symptoms are likely related through inflammatory processes. Given that one disorder may help to stimulate, increase, or sustain the inflammatory response in the other disorder, more research is needed to improve our understanding of the pathophysiology of depression in T2DM. Conversely, an additive effect of T2DM and elevated depressive symptoms did not emerge for CRP. Instead, significantly increased levels of CRP were observed in the presence of T2DM, but not elevated depressive symptoms, which may suggest that differential effects for T2DM and depressive symptoms appear to exist for various markers of inflammation.

In addition to these findings, results also showed that the presence of inflammation increases the risk of elevated depressive symptoms among T2DM participants compared to T2DM participants without elevated inflammatory markers. This suggests that chronic low-grade inflammation as a biological link between these two conditions may provide a mechanistic explanation for the increased prevalence and persistence of elevated depressive symptoms in T2DM and warrants further investigation. Further, results from this study provide new insight into the predictors of elevated depressive symptoms in T2DM by showing that higher levels of inflammation are associated over time (2 years) with an increased risk of depressive symptoms among participants with T2DM. Broadening our understanding of the predictors of depression in T2DM may elucidate potential ways to improve depression treatment (e.g., translational research strategies that focus on the management of depressive symptoms using novel anti-inflammatory therapies to target the up-regulation of inflammation, in conjunction with anti-depressants and/or psychotherapy). By expanding our knowledge on inflammation and the link between T2DM and depression, it should be possible to improve our understanding of the development, progression, and treatment of these co-morbid conditions.

References

Allison, P. D. (1999). *Logistic regression using the SAS system: Theory and application.* Cary, NC: SAS Institute Inc.

American Diabetes Association: Clinical practice recommendations (2010). *Diabetes Care*, 33, S1.

American Heart Association (2010). Inflammation, heart disease and stroke: The role of c-reactive protein. Retrieved from http://www.americanheart.org/presenter.jhtml?identifier=4648.

Anderson, R. J., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2001). The prevalence of comorbid depression in adults with diabetes: A meta-analysis. *Diabetes Care,* 24 (6),1069-78. DOI:10.2337/diacare.24.6.1069

Beasley, L. E., Koster, A., Newman, A. B., Javaid, M. K., Ferrucci, L., & Kritchevsky, S. B. et al. (2009). Inflammation and race and gender differences in computerized tomography-measured adipose depots. *Obesity*, 17, 1062-1069. DOI:10.1038/oby.2008.627

Beekman, A. T. F., Deeg, D. J. H., van Limbeek, J., Braam, A. W., & de Vries, M. Z. et al. (1997). Criterion validity of the center for epidemiologic studies depression scale (CES-D): Results form a community-based sample of older subjects in the Netherlands. *Psychological Medicine*, 27 (1), 231-235. DOI:10.1017/S0033291796003510

Blaak, E. (2001). Gender differences in fat metabolism. *Current Opinions in Clinical Nutrition and Metabolic Care,* 4, 499-502. DOI:10.1097/00075197-200111000-00006

Bullo, M., Garcia-Lorda, P., Megias, I., & Salas-Salbado, J. (2003). Systemic inflammation, adipose tissue tymor necrosis factor, and leptin expression. *Obesity Research*, 11 (4), 525-531. DOI:10.1038/oby.2003.74

Cartier, A., Cote, M., Lemieux, I., Perusse, L., & Tremblay, A. et al. (2009). Sex differences in inflammatory markers: what is the contribution of visceral adiposity? *American Journal of Clinical Nutrition*, 89, 1307-1314. DOI:10.3945/ajcn.2008.27030

Chatterjee, S. & Price, B. (1991). *Regression Analysis by Example*. 2nd Ed. New York, John Wiley and Sons, p. 191-192.

Cohen, J. (1992). A power primer. *Psychological Bulletin*, 112, 155-159. DOI:10.1037/0033-2909.112.1.155

Coppola, G., Corrado, E., Muratori, I., Tantillo, R., & Vitale, G. et al. (2006). Increased levels of c-reactive protein and fibrinogen influence the risk of vascular events in patients with NIDDM. *International Journal of Cardiology*, 106, 16-20. DOI:10.1016/j.ijcard.2004.12.051

Corrado, E., Rizzo, M., Muratori, I., Coppola, G., & Novo, S. (2006). Association of elevated fibrinogen and c-reactive protein levels with carotid lesions in patients with newly diagnosed hypertension or type II diabetes. *Archives of Medical Research*, 37, 1004-1009. DOI:10.1016/j.arcmed.2006.06.005

Dandona, P., Aljada, A., & Bandyopadhyay, A. (2004). Inflammation: The link between insulin resistance, obesity and diabetes. TRENDS in Immunology, 25 (1), 4-7. DOI:10.1016/j.it.2003.10.013

Danner, M., Kals, S.V., Abramson, J.L., & Vaccarino,V. (2003). Association between depression and elevated c-reactive protein. *Psychosomatic Medicine*, 65, 347-356. DOI:10.1097/01.PSY.0000041542.29808.01

Dantzer, R., O'Connor, J. C., Freund, G. C., Johnson, R. W., & Kelley K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature*, 9, 46-57.

Empana, J. P., Sykes, D. H., Luc, G., Juhan-Vague, I., & Arveiler, D. et al. (2005). Contributions of depressive mood and circulating inflammatory markers to coronary heart disease in healthy european men: The prospective epidemiological study of myocardial infarction (PRIME). *Circulation*, 111, 2299-2305. DOI:10.1161/01.CIR.0000164203.54111.AE

Ferketich, A.K., Ferguson, J.P., & Binkley, P.F. (2005).Depressive Symptoms and inflammation among heart failure patients. *American Heart Journal*, 150 (2), 132-136. DOI:10.1016/j.ahj.2004.08.029

Ford, D.E. & Erlinger, T.P. (2004).Depression and C-reactive protein in Us Adults: Data from the third national health and nutrition examination survey. *Archives of Internal Medicine*, 164, 1010-1014. DOI:10.1001/archinte.164.9.1010

Frohlich, M., Imhof, A., Berg, G., Hutchinson, W. L., & Pepys et al. (2000). Association between c-reactive protein and features of the metabolic syndrome. *Diabetes Care*, 23, 1835-1839. DOI:10.2337/diacare.23.12.1835

Haringsma, R., Engels, G. I., Beekman, A. T. F., & Spinhoven, P. (2004). The criterion validity of the Center for Epidemiological Studies Depression Scale (CES-D) in a sample of self-referred elders with depressive symptomatology. *International Journal of Geriatric Psychiatry*, 19, 558-563. DOI: 10.1002/gps.1130

Harris, T. B., Ferrucci, L., Tracy, R. P., Corti, M. C., & Wacholder, S. et al. (1999). Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *American Journal of Medicine*, 106, 506-512. DOI:10.1016/S0002-9343(99)00066-2

Hogue, J., Lamarche, B., Tremblay, A. J., Bergeron, J., & Gagne, C. (2008). Differential effect of atorbastatin and fenofibrate on plasma oxidized low-density lipoprotein, inflammation markers, and cell adhesion molecules in patients with type 2 diabetes mellitus. *Metabolism Clinical and Experimental*, 57, 380-386. DOI:10.1016/j.metabol.2007.10.014

Irwin, M. R. & Miller, A. H. (2007). Depressive disorders and immunity: 20 years of progress and discovery. *Brain, Behavior and immunity*, 25, 374-383. DOI:10.1016/j.bbi.2007.01.010

de Jager, J., Dekker, J. M., Kooy, A., Kostense, P. J., & Nijpels, G. et al. (2006). Endothelial dysfunction and low-grade inflammation explain much of the excess

cardiovascular mortality in individuals with type 2 diabetes: The hoorn study. *Arteriosclerosis, Thrombosis& Vascular Disease,* 26, 1086-1093.

Jansen,A.S.P., Schmidt,E.D., Voorn,P., & Tilders, F.J.H. (2003). Substance induced plasticity in noradrenergic innervation of paraventricular hypothalamic nucleus. *European Journal of Neuroscience*, 17, 298-306. DOI:10.1046/j.1460-9568.2003.02453.x

Katsuki, A., Sumida, Y., Murashima, S., Murata, K. & Takarada, Y. et al. (1998). Serum levels of tumor necrosis factor-α are increased in obese patients with noninsulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, 83, 859-862. DOI:10.1210/jc.83.3.859

Kiecolt-Glaser, J. K. & Glaser, R. (2002). Depression and immune function: Central pathways to morbidity and mortality. *Journal of Psychosomatic Research*, 53, 873-876. DOI:10.1016/S0022-3999(02)00309-4

Ladwig, K., Marten-Mittag, B., Lowel, H., Doring, A., & Koenig, W. (2005). C-reactive protein, depressed mood, and the prediction of coronary heart disease in initially healthy men: Results from the MONICA-KORA Ausburg cohort study 1984-1998. *European Heart Journal*, 26, 2537-2542. DOI:10.1093/eurheartj/ehi456

Leinonen, E., Hurt-Camejo, E., Wiklund, O., Hulten, L. M, & Hiukka, A. (2003). Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclersosis,* 166, 387-394. DOI:10.1016/S0021-9150(02)00371-4

Lesperance, F., Frasure-Smith, N., Theroux, P., & Irwin, M. (2004). The association between major depression and levels of soluble intercellular adhesion molecule 1, interleukin-6, and c-reactive protein in patients with recent acute coronary syndromes. *American Journal of Psychiatry*, 161, 271-277. DOI:10.1176/appi.ajp.161.2.271

Macy, E. M., Hayes, T. E., & Tracy, R. P. (1997). Variability in the measurement of C-reactive protein in healthy subjects: Implications for reference intervals and epidemiological applications. *Clinical Chemistry*, 43, 52-58.

Mason, R. L., Gunst, R. F., & Hess, J. L. (1989). *Statistical Design and Analysis of Experiments: Applications to Engineering and Science*. New York: Wiley.

Menard, S. (1995). *Applied Logistic Regression Analysis: Sage University Series on Quantitative Applications in the Social Sciences.* Thousand Oaks, CA: Sage.

Miller, G. E., Freedland, K. E., Duntley, S., Carney, R. M. (2005). Relation of depressive symptoms to c-reactive protein and pathogen burden (cytomegalovirus, herpes simplex virus, Epstein-barr virus).in patients with earlier acute coronary syndromes. *American Journal of Cardiology*, 95, 317-321. DOI:10.1016/j.amjcard.2004.09.026

Mishima, Y., Kuyama, A., Tada, A., Takahashi, K., & Ishioka, T. et al. (2001). Relationship between serum tumor necrosis factor-α and insulin resistance in obese men with type 2 diabetes mellitus. *Diabetes Research and Clinical Practice*, 52, 119-123. DOI:10.1016/S0168-8227(00)00247-3

Muhlestein, J. B., May, H. T., Jensen, J. R., Horne, B. D., & Lanman, R. B. (2006). The reduction of inflammatory biomarkers by statin, fibrate, and combination therapy among diabetic patients with mixed dyslipidemia. The DIACOR Study. *Journal of the American College of Cardiology*, 48 (2), 396-401. DOI:10.1016/j.jacc.2006.05.009

Natali, A., Toschi, E., Baldeweg, S., Ciociaro, D. & Favilla, S. et al. (2006). Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. *Diabetes*, 55, 1133-1140. DOI:10.2337/diabetes.55.04.06.db05-1076

National Institute on Aging. (1997). *Health ABC telephone screening interview* (Version 1.0b) [Annotated Form]. Retrieved from https://rds185.epi-ucsf.org/ucsf_cc/version3.1/habc/docs/Y1_Annotated_Forms.pdf.

National Institute on Aging. Healthy Aging and Body Composition Study. (2004). *Prevalent disease variables included in dataset* [Standardized Algorithms]. Retrieved from https://rds185.epi-ucsf.org/ucsf_cc/version3.1/habc/sasfiles.asp.

National Institute on Aging, National Institutes of Health (2009). *Health ABC Description*. Retrieved from: http://www.nia.nih.gov/ResearchInformation/ScientificResources/HealthABCDescription.htm

Nilsson, J., Jovinge, S., Niemann, A., Reneland, R., & Lithell, H. (1998). Relation between plasma tumor necrosis factor-α and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arteriosclerosis, Thrombosis& Vascular Biology*, 18, 1199-1202. DOI:10.1161/01.ATV.18.8.1199

Oxman, T. E., Berkman, L. F., Kasl, S., Freeman, D. H., & Barrett, J. (1992). Social support and depressive symptoms in the elderly. *American Journal of Epidemiology*, 135, 356-368.

Pace, T. W. W., Hu, F., & Miller, A. H. (2007). Cytokine-effects on glucocorticoid receptor function: Relevance to glucocorticoid resistance and pathophysiology and treatment of major depression. *Brain, Behavior and Immunology*, 21 (1) 9-19. DOI:10.1016/j.bbi.2006.08.009

Pahor, M., Chrischilles, E. A., Guralnik, J. M., Brown, S. L., & Wallace, R. B. et al., (1994). Drug data coding and analysis in epidemiologic studies. *European Journal of Epidemiology,* 10, 405-411. DOI:10.1007/BF01719664

Pereira, F. O., Frode, T. A., & Medeiros, Y. S. (2006). Evaluation of tumour necrosis factor alpha, interleukin-2 soluble receptor, nitric oxide metabolites, and lipids as inflammatory markers in type 2 diabetes mellitus. *Mediators of Inflammation*, 2006, 1-7. DOI:10.1155/MI/2006/39062

Pickup, J. C. (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care*, 27, 813-823. DOI:10.2337/diacare.27.3.813

Pizzi, C., Mancini, S., Angeloni, L., Fontana, F., & Manzoli, L. et al. (2009). Effects of selective serotonin reuptake inhibitor therapy on endothelial function and inflammatory markers in patients with coronary heart disease. *Clinical Pharmacology and Therapeutics*, 86 (5), 527-532. DOI:10.1038/clpt.2009.121

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*, 1, 385-401. DOI:10.1177/014662167700100306

Raison, C.L., Capuron, L., & Miller, A.H. (2006). Cytokines sing the blues: Inflammation and the pathogenesis of depression. *Trends in Immunology*, 27 (1), 24-31. DOI:10.1016/j.it.2005.11.006

Rao, K. M., Pieper, C. S., Currie, M. S., & Cohen, H. J. (1994). Variability of plasma IL-6 and crosslinked fibrin dimers over time in community dwelling elderly subjects. *American Journal of Clinical Pathology*, 102, 802-805.

de Rekeneire, N., Peila, R., Ding, J., Colbert, L. H., & Shorr, R. I. et al. (2006). Diabetes, hyperglycemia, and inflammation in older individuals. *Diabetes Care*, 29, 1902-1908. DOI:10.2337/dc05-2327

Ruhé,H.G., Mason, N.S., & Schene, A.H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels on human: A meta-analysis of monoamine depletion studies. *Molecular Psychiatry*, 12, 331-359. DOI:10.1038/sj.mp.4001949

SAS Institute, *version 9.03e*. Cary, NC. 2003.

Stehouwer, C. D. A., Gall, M., Twisk, J. W. R., Knudsen, E., & Emeis, J. J. et al. (2002). Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes. *Diabetes*, 51, 1157-1165. DOI:10.2337/diabetes.51.4.1157

Strotmeyer, S. E., Cauley, J. A., Orchard, T. J., Steenkiste, A. R., & Dorman, J. S. (2006). Middle-aged premenopausal women with type 1 diabetes have lower bone mineral density and calcaneal quantitative ultrasound than nondiabetic women. *Diabetes Care*, 29, 306-311. DOI:10.2337/diacare.29.02.06.dc05-1353

Tabachnick, B. G. & Fidell, L. S. *Using Multivariate Statistics.* 4th Edition. New York, Allyn & Bacon, 2001.

Tan, S. A., Tan, L. G., & Berk, L. S. (2007). Effects of rosuvastatin and pioglitazone on monocyte chemotactic protein-1, cytokines, c-reactive protein and hdl-cholesterol in diabetic dyslipidemia. Journal of Clinical Lipidology, 1 (2), 160-161. DOI:10.1016/j.jacl.2007.03.030

Temelkova-Kurktschiev, T., Siegert, G., Bergmann, S., Henkel, E., & Koehler, C. et al. (2002). Subclinical inflammation is strongly related to insulin resistance but not to impaired insulin secretion in a high risk population for diabetes. *Metabolism*, 51 (6), 743-749. DOI:10.1053/meta.2002.32804

Wakabayashi, I. & Masuda, H. (2006). Association of acute-phase reactants with arterial stiffness in patients with type 2 diabetes mellitus. *Clinica Chimica Acta*, 365, 230-235. DOI:10.1016/j.cca.2005.08.023

Wancata, J., Alexandrowicz, R., Marquart, B., Weiss, M., & Friedrich, F. (2006). The criterion validity of the Geriatric Depression Scale: A systematic review. *Acta Psychiatrica Scandinavica*, 114, 398-410. DOI:10.1111/j.1600-0447.2006.00888.x

Wichers, M. & Maes, M. (2002).The psychoneroimmuno-pathophysiology of cytokine-induced depression in humans. *International Journal of Neuropsychopharmacology*, 5, 375-388. DOI:10.1017/S1461145702003103

Winkler, G., Salamon, F., Salamon, D. Speer, G., & Simon, K. et al. (1998). Elevated serum tumor necrosis factor-alpha levels can contribute to the insulin resistance in type II (non-insulin-dependent).diabetes and in obesity. *Diabetologia*, 41, 860-862. DOI:10.1007/s001250051000

Yu, H., Sheu, W. H., Song, Y., Liu, H., & Lee, W. (2004). C-reactive protein and risk factors for peripheral vascular disease in subjects with type 2 diabetes mellitus. *Diabetic Medicine*, 21, 336-341. DOI:10.1111/j.1464-5491.2004.01144.x

Appendix A: Supporting Figures

Figure 1. Conceptualization of Inflammation, Type 2 Diabetes Mellitus, and Depression

Figure 2. ANCOVA: Mean IL-6 Levels According to Diabetes and Depressed Mood Status (CES-D ≥ 20)

Figure 3. ANCOVA: Mean CRP Levels According to Diabetes and Depressed Mood Status (CES-D ≥ 20)

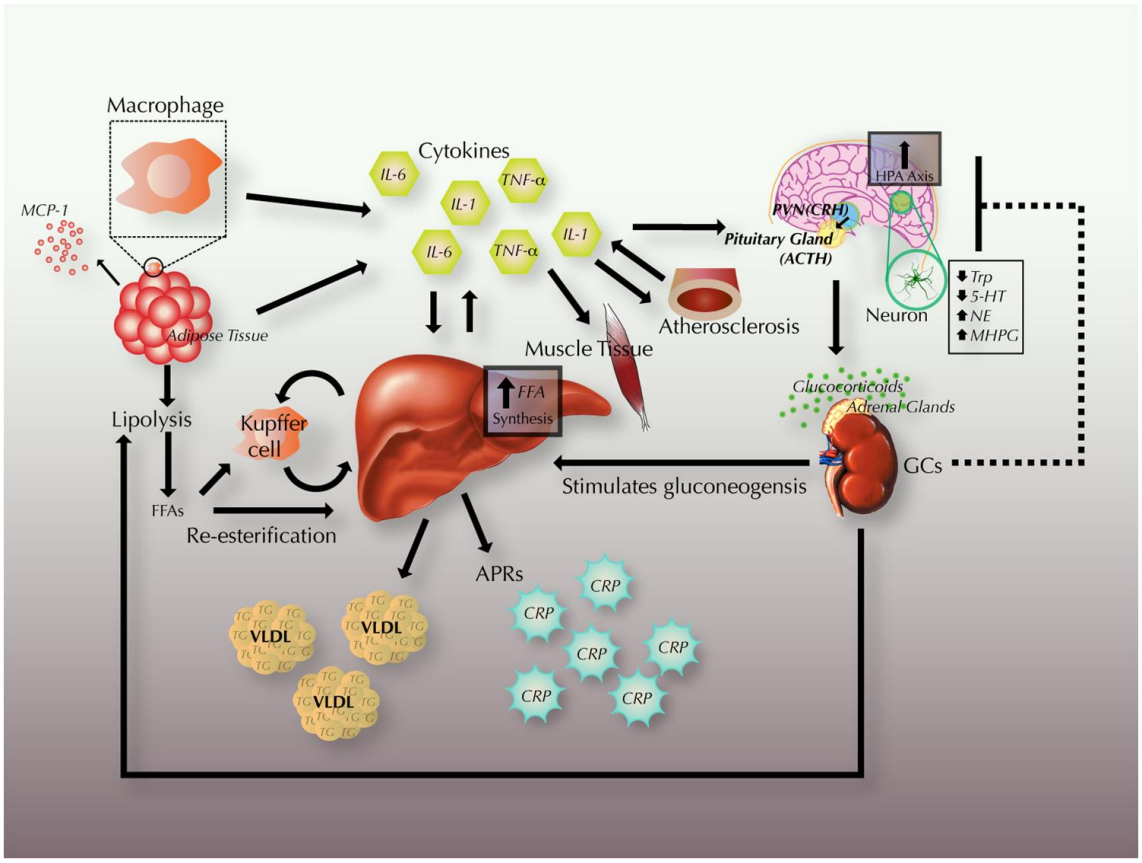

Figure 1. Conceptualization of Inflammation, Type 2 Diabetes Mellitus, and Depression

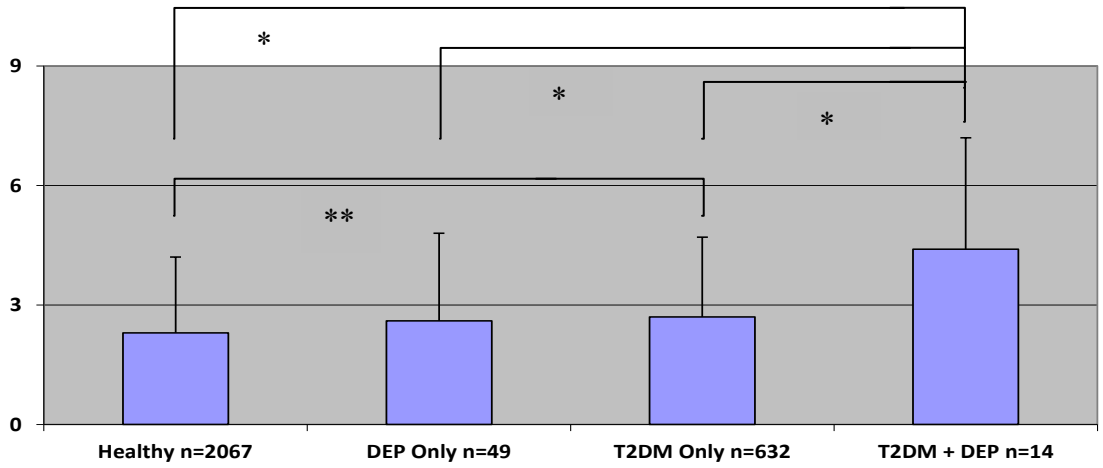

Figure 2. Mean IL-6 (pg/mL) levels (±S.D.) according to diabetes and elevated depressive syptoms (CES-D ≥ 20). Cell sizes may differ from the overall sample size due to variation in the availability of IL-6 for each participant.

*p<.05
**p<.0001

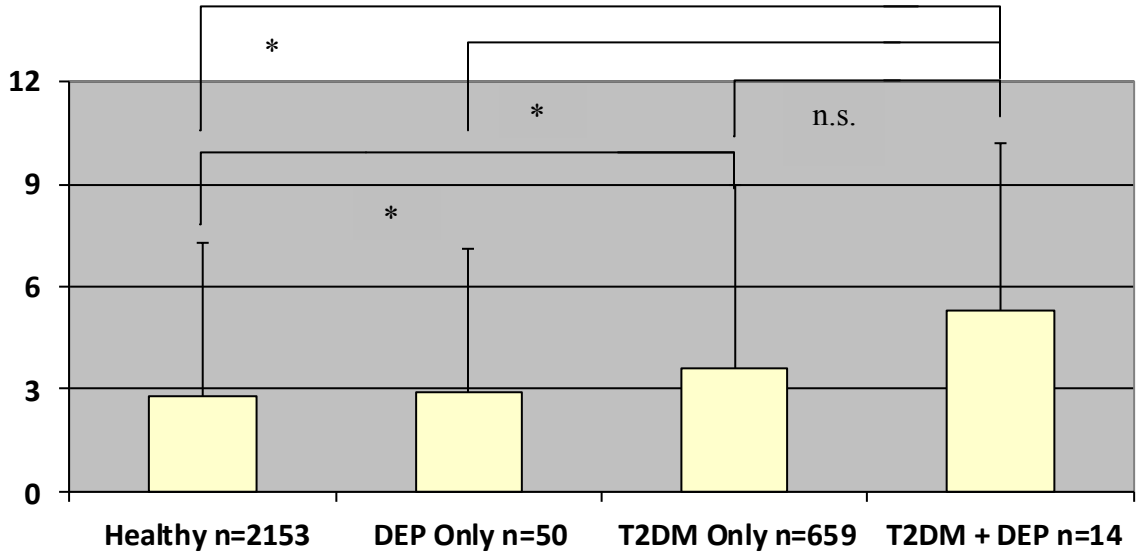

Figure 3. Mean CRP (mg/L) levels (±S.D.) according to diabetes and elevated depressive symptoms (CES-D ≥ 20). Cell sizes may differ from the overall sample size due to variation in the availability of CRP for each participant.

*p<.05

Note. Patients with CRP levels > 3.0 mg/L are considered to be at high risk for cardiovascular events (American Heart Association, 2010).

Appendix B: Supplementary Analyses

A series of supplemental analyses were conducted during this investigation to examine differences in levels of IL-6, TNF-α, and CRP between participants with and without T2DM, as well as participants with and without elevated depressive symptoms. These supplemental analyses were included here to replicate prior Health ABC study findings (de Rekeneire et al., 2006; Penninx et al., 2003) within this sample. This was done as a way to show that the levels of inflammatory markers for participants used during this investigation were comparable to the levels of inflammatory markers observed in prior studies.

Differences in Inflammatory Markers by T2DM Status

Given that plasma levels of inflammatory markers were non-normally distributed, median values (i.e., 25^{th}-75^{th} percentile) are reported below, and continuous level comparisons are based on log-transformed values. For IL-6, TNF-α, and CRP, continuous levels were significantly higher in participants with T2DM than those without T2DM (Table 11). The computed effect sizes ranged from $d = 0.23$ for TNF-α to $d = 0.33$ for IL-6. According to Cohen's (1992) guidelines for effect sizes in the behavioral sciences, these are small to medium effects.

Table 11. Differences in Inflammatory Markers by T2DM Status

	No T2DM (n=2307)	T2DM (n=707)	t	p[a]	*d*
IL-6 (pg/mL) Median (25^{th}-75^{th} percentile)	1.7 (1.2 – 2.6)	2.2 (1.5 – 3.2)	-7.40	<.0001	0.33
TNF-α (pg/mL) Median (25^{th}- 75^{th} percentile)	3.1 (2.4 – 4.0)	3.4 (2.6 – 4.5)	-5.25	<.0001	0.23
CRP (mg/L) Median (25^{th}- 75^{th} percentile)	1.6 (1.0 – 2.9)	2.2 (1.2 – 3.8)	-6.70	<.0001	0.29

[a]p-values based on *t* tests for log-transformed continuous inflammatory marker variables.

Differences in Inflammatory Markers by Depressed Mood Status

Given that plasma levels of inflammatory markers were non-normally distributed, median values (i.e., 25^{th}-75^{th} percentile) are reported below, and continuous level comparisons are based on log-transformed values. Continuous levels of IL-6 and TNF-α were significantly ($p<.05$) higher in participants with elevated depressive symptoms than those without minimal depressive symptoms (Table 12). The computed effect sizes were $d = 0.21$ for TNF-α and $d = 0.18$ for IL-6. According to Cohen's (1992) guidelines for effect sizes in the behavioral sciences, these are small effects. Continuous levels of CRP were marginally elevated among those with elevated depressive symptoms compared to those with minimal depressive symptoms.

Table 12. Differences in Inflammatory Markers by Depressed Mood Status

	Minimal Depressive Symptoms (n=2872)	Elevated Depressive Symptoms (n=142)	t	p[a]	*d*
IL-6 (pg/mL) Median (25^{th}-75^{th} percentile)	1.8 (1.3 – 2.8)	2.0 (1.4 – 3.1)	-2.09	<.05	0.18
TNF-α (pg/mL) Median (25^{th}- 75^{th} percentile)	3.2 (2.5 – 4.1)	3.4 (2.7 – 4.5)	-2.28	<.05	0.21
CRP (mg/L) Median (25^{th}- 75^{th} percentile)	1.7 (1.0 – 3.1)	2.0 (1.2 – 3.6)	-1.87	0.06	0.16

[a]p-values based on *t* tests for log-transformed continuous inflammatory marker variables.

Supplemental analyses were conducted during this investigation to determine which cut-score on the CES-D (i.e., CES-D ≥ 16 or CES-D ≥ 20) would be most appropriate for defining elevated depressive symptoms in the study groups because many of the physical symptoms of depressed mood (e.g., fatigue, weight/appetite fluctuations, & sleep disturbances) often overlap with symptoms of chronic illness (e.g., T2DM). The use of a cut-score of ≥ 16 is traditionally accepted as the standard threshold for establishing elevated depressive symptoms on the CES-D (Haringsma et al., 2004; Beekman et al., 1997). The sensitivity (96.2%) and specificity (23.8%) of using the CES-D cut-score ≥ 16 for detecting clinically relevant depressive symptoms among older adults (ages 55-85) has been reported by Haringsma and colleagues (2004). Upon review of the following supplemental analyses, it was determined that a higher cut-off score of ≥ 20 on the CES-D was preferred over the traditional cut-off score of ≥ 16 in order to reduce false-positive rates of clinically meaningful depressive symptoms among individuals with diabetes.

Differences in Inflammatory Markers by T2DM and Depressed Mood Status (CES-D ≥ 16)

After adjustment for race, gender, percent body fat, triglycerides, smoking status, and lung disease, the interaction between T2DM and elevated depressive symptoms on levels of CRP (mg/L) was significant, $F(1, 2875)=7.3$, $p<.05$, $\eta^2=.002$. Figure 4 shows that the adjusted marginal means for CRP were significantly higher ($p<.05$) among those

with T2DM+DEP compared to all other groups (4.6±3.7 versus 3.6±5.5 [T2DM Only], 2.7±3.4 [DEP Only], & 2.8±4.6 [Healthy]).

After adjustment for race, gender, study site, percent body fat, and heart and lung disease, the interaction between T2DM and elevated depressive symptoms on levels of IL-6 (pg/mL) was non-significant, $F(1, 2765)=1.1$, $p=0.29$. In addition, after adjustment for gender, percent body fat, and heart and kidney disease, the interaction between T2DM and elevated depressive symptoms on levels of TNF-α (pg/mL) was non-significant, $F(1, 2715)=0.25$, $p=0.61$.

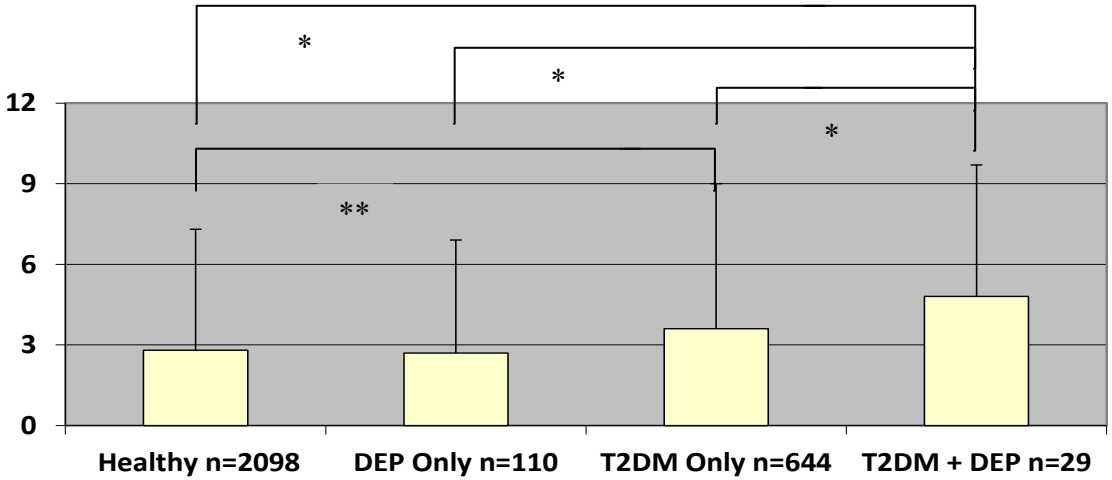

Figure 4. Mean CRP (mg/L) levels (±S.D.) according to diabetes and elevated depressive symptoms (CES-D ≥ 16). Cell sizes may differ from the overall sample size due to variation in the availability of CRP for each participant.

*p<.05
**p<.01

Note. Patients with CRP levels > 3.0 mg/L are considered to be at high risk for cardiovascular events (American Heart Association, 2010).

Risk of Depressed Mood (CES-D ≥ 16) at Baseline Associated with High Inflammation among those with T2DM

When elevated depressive symptoms was defined as CES-D ≥ 16, T2DM participants with high levels of IL-6 (Table 13), TNF-α (Table 14), or CRP (Table 15) were not significantly more likely to have elevated depressive symptoms than those with low levels of IL-6, TNF-α, or CRP after adjustment for gender, education level, marital status, and statin usage.

Table 13. Adjusted Risk of Depressed Mood (CES-D ≥ 16) Associated with High Levels of IL-6 in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	0.03	0.41	0.01	1.03	0.46-2.32
Education	-0.41	0.17	5.76*	0.67	0.48-0.93
Marital Status	0.30	0.23	1.67	1.35	0.86-2.11
Statin Usage	-1.39	1.03	1.82	0.25	0.03-1.88
IL-6	0.80	0.72	1.24	2.23	0.54-9.17

Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 631) = 6.92$; p = 0.54	ROC = 0.72	Max-rescaled $R^2 = 0.08$

*p<.05

Table 14. Adjusted Risk of Depressed Mood (CES-D ≥ 16) Associated with High Levels of TNF-α in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	0.02	0.42	0.00	1.02	0.45-2.33
Education	-0.32	0.16	4.21*	0.72	0.53-0.99
Marital Status	0.24	0.23	1.03	1.27	0.80-2.00
Statin Usage	-1.40	1.03	1.84	0.25	0.03-1.86
TNF-α	1.06	1.07	0.99	2.89	0.36-23.53

Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 620) = 10.78$; p = 0.21	ROC = 0.68	Max-rescaled $R^2 = 0.06$

*p<.05

Table 15. Adjusted Risk of Depressed Mood (CES-D ≥ 16) Associated with High Levels of CRP in T2DM

Predictors	B	S.E.	Wald	OR	95% CI
Gender	-0.02	0.41	0.00	0.98	0.43-2.21
Education	-0.33	0.16	4.59*	0.72	0.53-0.97
Marital Status	0.27	0.22	1.47	1.31	0.85-2.02
Statin Usage	-1.44	1.03	1.95	0.24	0.03-1.79
CRP	0.81	0.49	2.72	2.25	0.86-5.88

Hosmer-Lemeshow Goodness-of-Fit Test	$X^2(8, 640) = 3.73$; $p = 0.88$	ROC = 0.72	Max-rescaled $R^2 = 0.08$

*p<.05

Thesis and Dissertation Services