Evaluating the Relationship between Ceramides and Depressive Symptoms in Coronary Artery Disease Patients

by

Adam Dinoff

A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Pharmacology and Toxicology, in the University of Toronto

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Master of Science

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University of Toronto

2016

Abstract

Depression is highly prevalent in individuals with coronary artery disease (CAD), and increases risk of mortality. Ceramides, a family of sphingolipid species, have been implicated in the pathophysiology of both CAD and depression due to their pro-inflammatory and pro-apoptotic characteristics. This study assessed the relationship between ceramides and depression in a CAD population. Linear regression models were used to assess the association between plasma ceramide concentrations and depressive symptoms, as measured by the Center for Epidemiological Studies Depression Scale (CESD). High performance liquid chromatography coupled electrospray ionization tandem mass spectrometry was used to measure ceramide species. Higher plasma concentrations of the ceramide species C16:0 (β=0.195, p=0.039) and C22:1 (β=0.199, p=0.039) were significantly associated with greater depressive symptoms. Plasma concentrations of C18:0 (β=0.108, p=0.257) and C20:0 (β=0.167, p=0.078) were not significantly associated with depressive symptoms. Findings suggest a potential role of specific ceramides in the pathophysiology of depression in CAD.
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer's Disease</td>
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<tr>
<td>AES</td>
<td>Apathy Evaluation Scale</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ASA</td>
<td>Acetylsalicylic Acid</td>
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<tr>
<td>aSMase</td>
<td>Acidic Sphingomyelinase</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary Artery Bypass Graft</td>
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<td>CAD</td>
<td>Coronary Artery Disease</td>
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<tr>
<td>CESD</td>
<td>Center for Epidemiological Studies Depression Scale</td>
</tr>
<tr>
<td>CMS</td>
<td>Chronic Mild Stress</td>
</tr>
<tr>
<td>CPS</td>
<td>Counts Per Second</td>
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<tr>
<td>CR</td>
<td>Cardiac Rehabilitation</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>CVA</td>
<td>Cerebrovascular Accident</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ESI-MS/MS</td>
<td>Electrospray Ionization Tandem Mass Spectrometry</td>
</tr>
<tr>
<td>FIASMA</td>
<td>Functional Inhibitor of Acidic Sphingomyelinase</td>
</tr>
<tr>
<td>GAD</td>
<td>Generalized Anxiety Disorder</td>
</tr>
<tr>
<td>GAD-7</td>
<td>Generalized Anxiety Disorder 7-Item Scale</td>
</tr>
<tr>
<td>GDS</td>
<td>Geriatric Depression Scale</td>
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<tr>
<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
</tr>
<tr>
<td>HAMD</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated Hemoglobin</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal Axis</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>-------------</td>
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<tr>
<td>HR</td>
<td>Heart Rate</td>
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<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IL-7</td>
<td>Interleukin-7</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
</tr>
<tr>
<td>MHx22:1</td>
<td>Monohexylceramide 22:1</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix Metalloproteinase</td>
</tr>
<tr>
<td>sMMSE</td>
<td>Standardized Mini Mental State Examination</td>
</tr>
<tr>
<td>nSMase</td>
<td>Neutral Sphingomyelinase</td>
</tr>
<tr>
<td>PSQI</td>
<td>Pittsburgh Sleep Quality Index</td>
</tr>
<tr>
<td>PTCA</td>
<td>Percutaneous Transluminal Coronary Angioplasty</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post-Traumatic Stress Disorder</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for the Diagnosis of DSM-V Disorders</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SMase</td>
<td>Sphingomyelinase</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SPT</td>
<td>Serine Palmitoyltransferase</td>
</tr>
<tr>
<td>SRRS</td>
<td>Social Readjustment Rating Scale</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>STAI</td>
<td>Spielberger State Trait Anxiety Inventory</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic Antidepressant</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient Ischemic Attack</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-Alpha</td>
</tr>
<tr>
<td>TRI</td>
<td>Toronto Rehabilitation Institute</td>
</tr>
<tr>
<td>VIF</td>
<td>Variance Inflation Factor</td>
</tr>
<tr>
<td>VO₂ Peak</td>
<td>Peak Oxygen Uptake</td>
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1 Introduction

1.1 Statement of Problem

Coronary artery disease (CAD) affects 1 in 6 Canadians over the age of 65 and is responsible for approximately 20% of all deaths in Canada (Statistics Canada 2006, 2009). CAD significantly affects one’s quality of life, with 60% of those with CAD reporting reduced physical activity and 30% reporting disability and unemployment due to illness (Public Health Agency of Canada 2009). CAD also represents a large economic burden, with estimates that CAD alone costs the Canadian economy approximately 10 billion dollars annually in physician services, hospital costs, missed work time and decreased productivity due to illness (Public Health Agency of Canada 2009). With our aging population we can only expect the burden of CAD to increase in the near future.

Depression is a common comorbidity of CAD and worsens prognosis in CAD patients (Celano and Huffman 2011, Dickens 2015, Rudisch and Nemeroff 2003). It is estimated that 15-20% of patients with CAD meet criteria for major depressive disorder (MDD), a prevalence that is 3-4 times greater than that in the general population (Marcus et al. 2012, Rudisch and Nemeroff 2003). Furthermore, it has been reported that 30-45% of CAD patients suffer from clinically significant depressive symptoms (Rudisch and Nemeroff 2003). Depression is an important comorbidity of CAD because it worsens prognosis and health-related quality of life in these patients (Bradley and Rumsfeld 2015, Celano and Huffman 2011, Lichtman et al. 2014). CAD patients with depression are approximately 1.5-3 times more likely to die due to cardiac-related causes than non-depressed CAD patients (Celano and Huffman 2011, Rudisch and Nemeroff 2003). In addition, CAD patients with depressive symptoms have a 50% greater risk of subsequent cardiac events such as myocardial infarction (MI) or stroke than those free of depressive symptoms (van Melle et al. 2004, Whooley et al. 2008). Clearly depression is important in CAD and must be addressed to reduce the large burden of CAD.
Many cases of depression, whether co-morbid with CAD or on their own, are treatment resistant (Souery et al. 2006). This is likely due to the heterogeneity in etiology and pathophysiology of different cases of depression (Belmaker and Agam 2008). Currently there are an array of different antidepressant medication classes, yet some cases of depression remain resistant to treatment after multiple attempts at treatment with a variety of different antidepressants (Souery et al. 2006). Understanding the mechanisms of depression will allow us to identify new therapeutic targets, potentially resulting in the creation of novel antidepressant classes. This should allow us to treat more cases of depression successfully.

Current treatment options for depression in individuals with CAD include pharmacotherapy (most commonly with selective serotonin reuptake inhibitors), psychotherapy, and physical activity (Lichtman et al. 2008). Multiple trials have evaluated the impact of antidepressant use on depression treatment in CAD (Taylor 2008). Sertraline, fluoxetine, citalopram, paroxetine, nortriptyline, and mirtazapine have all been shown to be safe to use in CAD patients; however, efficacies of these antidepressant drug treatments vary and appear to display only modest effect sizes in the treatment of depression and reduction of cardiac morbidity and mortality (Baumeister et al. 2011, Taylor 2008). Thus, there is the potential for more effective treatments of depression in CAD.

One potential novel therapeutic target for the treatment of depression in CAD is a family of sphingolipid species called ceramides (Gulbins et al. 2015, Kornhuber et al. 2014). Ceramides have been suggested as a novel antidepressant target due to their potential roles in reducing hippocampal neurogenesis and dysregulating the hypothalamic-pituitary-adrenal (HPA) axis.
Ceramides have also been implicated in the progression of CAD (Schissel et al. 1996), a chronic inflammatory condition, and it has been observed that aberrations in the metabolic pathways responsible for ceramide production may be associated with increased risk of CAD and depression (Gulbins et al. 2015, Kinnunen and Holopainen 2002).

Few studies have assessed the relationship between ceramides and depression in humans. A study by Gracia-Garcia et al. found elevated concentrations of the ceramide species C16:0, C18:0, C20:0, C24:1, and C26:1 in depressed compared to non-depressed individuals (Gracia-Garcia et al. 2011). The sample size of that study was small (n=46) and consisted of cognitively healthy elderly and elderly with Alzheimer’s disease (AD). Presence of AD may have modified the difference in ceramide concentrations between depressed and non-depressed individuals in that study, warranting further investigation into the relationship between ceramide concentrations and depression. Another study, by Demirkan et al., assessed correlations of ceramides with depressive symptoms in a community population (Demirkan et al. 2013). However, ceramides were not the primary predictor of depression in that study and assessment of a variety of other sphingolipids presented problems with multiple comparisons and thus false discoveries.

No studies to date have examined ceramides as correlates of depressive symptoms or predictors of depression in CAD. Ceramides may be a potential novel therapeutic target for depression, or a potential biomarker of depression. To determine this, more clinical studies are required to evaluate the relationship between ceramides, depressive symptoms, and depression in humans.
1.2 Purpose of Study and Objective

The primary objective of this study is to assess a novel mechanism suggested to be involved in the pathophysiology of both depression and CAD, with the goal of identifying potential biomarkers as well as novel therapeutic targets for the treatment of depression in CAD. In particular, this study will focus on ceramides, a family of sphingolipids that have been found to play crucial roles in cell signaling and other vital cell processes such as inflammation and apoptosis (Bikman and Summers 2011, Mencarelli and Martinez-Martinez 2013). This information may have diagnostic and treatment implications for depression and depressive symptoms in individuals with CAD as well as depression outside the context of CAD.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

1.3.1 Primary Hypothesis

Higher plasma concentrations of the ceramides C18:0 and C20:0 will be associated with greater depressive symptoms in individuals with CAD.

Rationale: C18:0 and C20:0 are the ceramide species most strongly implicated in depression in past clinical studies. In the study by Gracia-Garcia et al., plasma concentrations of C18:0 and C20:0 were significantly elevated in individuals with MDD compared to those with no depression (Gracia-Garcia et al. 2011). Other ceramide species were also found to be significantly elevated in individuals with MDD; however, C18:0 and C20:0 had the greatest effect sizes when compared between depressed and non-depressed individuals in that study (Gracia-Garcia et al. 2011). Elevation of plasma C18:0 has also been observed in another neuropsychiatric condition, post-traumatic stress disorder (PTSD) (Hammad et al. 2012). Additionally, C18:0 has been implicated in apoptosis (Senkal et al. 2007, Wang et al. 2012), a potential mechanism of neurodegeneration in depression (McKernan et al. 2011).
Furthermore, another clinical study reported associations between higher plasma concentration of C18:0 and C20:0 and greater depressive symptoms, providing further evidence for the importance of these species (Demirkan et al. 2013). However, these associations did not survive statistical significance after correcting for multiple comparisons. Based on those previous clinical studies and preclinical evidence, C18:0 and C20:0 appear to be the ceramide species likely to be strongly linked to depression in humans.

### 1.3.2 Secondary Hypotheses

Higher plasma concentrations of the ceramides C16:0 and C24:1 will be associated with greater depressive symptoms in individuals with CAD.

**Rationale:** These ceramide species were also found to be elevated in individuals with MDD in the previous clinical study by Gracia-Garcia et al. (Gracia-Garcia et al. 2011), but with smaller effect sizes.

Depressed individuals with CAD will have higher mean plasma concentrations of the ceramides C18:0, C20:0, C16:0, and C24:1 than non-depressed individuals with CAD.

**Rationale:** As stated previously, it has been reported in one clinical study that some ceramide species are elevated in individuals with depression (Gracia-Garcia et al. 2011). However, that study looked only at individuals with AD and cognitively healthy elderly individuals. No study has yet to assess differences in plasma ceramide concentrations between depressed and non-depressed individuals with CAD. Furthermore, replicating the results of the previous clinical study by Gracia-
Garcia et al. would strengthen the evidence that some ceramide species are elevated in individuals with depression.

### 1.3.3 Exploratory Hypotheses

Higher plasma concentrations of the ceramides C18:0 and C20:0 will predict presence of depression in individuals with CAD.

**Rationale:** This hypothesis will help evaluate whether these ceramide species may be clinically useful biomarkers of depression.

A number of other ceramide species and other sphingolipids were measured in this study. Ceramides are a large family of sphingolipids each with different chemical and physical properties (Bikman and Summers 2011, Christie 2014). Thus it is likely that different ceramides have different cellular and molecular targets, and therefore do not all contribute equally to depression if they do at all (Kolesnick 2002, Mathias et al. 1998). There are many sphingolipid species intimately linked to the metabolism of ceramides, such as sphingomyelins, sphingosine, and sphingosine-1 phosphate (Blachnio-Zabielska et al. 2015, Vethakanraj et al. 2015). Some of these other sphingolipid species have also been implicated in the pathophysiology of depression (Gulbins et al. 2015, Muller et al. 2015). As part of our exploratory analyses, all species measured in this study will be assessed as correlates of depressive symptoms and predictors of depression in our sample. In addition, differences in mean concentration of each species between depressed and non-depressed participants will be assessed.
1.4 Review of Literature

1.4.1 Coronary Artery Disease (CAD)

CAD is a prevalent and important disease in the Canadian and global populations (Public Health Agency of Canada 2009, Unsar et al. 2007). CAD is responsible for approximately 1 in 5 deaths in Canada and negatively affects quality of life (Public Health Agency of Canada 2009, Unsar et al. 2007). Many CAD patients report decreased mobility and physical activity, reduced capacity for activities of daily living, and unemployment due to illness (Public Health Agency of Canada 2009, Unsar et al. 2007). CAD also represents a significant economic burden, with estimates that CAD alone costs the Canadian economy 10 billion dollars each year (Public Health Agency of Canada 2009).

CAD is defined by a narrowing of one or more coronary arteries due to a build-up of fatty plaque deposits inside the artery wall, reducing blood flow to the heart. The process in which these fatty plaques are deposited inside the artery wall and grow in size is termed atherosclerosis (Hansson 2005). These plaques are formed under the endothelial layer of the blood vessel and are comprised of lipid deposits, macrophages and other immune cells, and a collagen rich extracellular matrix (Fuster et al. 1992, Jonasson et al. 1986).

The coronary arteries are vital to the delivery of oxygen and other important nutrients to heart tissue; and when blood flow in these arteries is compromised, tissue death or malfunction can occur (Fuster et al. 1992). This can result in angina, ischemic heart disease, or a myocardial infarction (MI) and subsequent death (Fuster et al. 1992). Treatment of CAD can consist of pharmacotherapy, revascularization procedures such as coronary artery bypass grafts (CABG) or percutaneous
transluminal coronary angioplasty (PTCA) and stents, exercise, and/or diet modification (Hansson 2005, Schuler et al. 1992, Serruys et al. 2009).

1.4.2 CAD and Depression

Major depression is defined by persistent feelings of sadness and reduced responsiveness to pleasurable stimuli, affecting how people think, feel and behave (Rapaport et al. 2002). It is estimated that 15-20% of individuals with CAD meet criteria for major depressive disorder (MDD) and that 30-45% of CAD patients experience clinically significant depressive symptoms (Rudisch and Nemeroff 2003). This is a prevalence 3-4 times greater than the prevalence of depression in the general population (Marcus et al. 2012). Literature on the relationship between CAD and Depression suggests a bidirectional relationship between these two diseases; CAD increases risk of depression and presence of depression increases risk of CAD (Bradley and Rumsfeld 2015, Goldston and Baillie 2008, Thomas et al. 2004).

There is an array of neurobiological and behavioral mechanisms that may explain this bidirectional relationship (Dickens 2015, Goldston and Baillie 2008, Parissis et al. 2007, Thomas et al. 2004). Depression is associated with a number of physiological imbalances such as elevated inflammation, increased platelet activation, elevated cortisol, autonomic dysfunction, and endothelial dysfunction (Bradley and Rumsfeld 2015). The result of these imbalances may lead to increased sympathetic tone resulting in higher blood pressure (BP), increased ventricular arrhythmias, reduced heart rate variability, and accentuation of atherosclerosis (Bradley and Rumsfeld 2015). Some or all of these physiological imbalances associated with depression may be explained by reduced serotonergic transmission resulting in subsequent amygdala dysfunction and increased HPA axis activity, both of which are common in depression (Bradley and Rumsfeld 2015, Dickens 2015, Goldston and Baillie
A number of behavioral factors likely contribute to the increased risk of CAD in depressed individuals (Goldston and Baillie 2008). Individuals with depression are less likely to exercise, less likely to comply with dietary restrictions, and more likely to smoke, all behaviors that may contribute to CAD (Anda et al. 1990, Lesperance et al. 1996, Paffenbarger et al. 1994, Wilson 1994).

Similar to depression, CAD is also associated with elevated levels of inflammation and inflammatory cytokines (Hansson 2005). Inflammatory responses are vital to the initiation and progression of atherosclerosis (Hansson 2005). Inflammatory cytokines such as C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-7 (IL-7), and fibrinogen have all been reported to be elevated in in patients with CAD (Hansson 2005). As mentioned, elevated inflammation is also observed in individuals with depression, and may be a link between CAD and MDD. Furthermore, increased platelet activation and thrombus formation are important pathological features of CAD (Goldston and Baillie 2008). These processes occur in the periphery of individuals with CAD, but may also be occurring simultaneously in the brain of these individuals. Conceivably, elevated platelet activity and thrombus formation may be reducing blood flow in regions of the brain of individuals with CAD, resulting in a lack of nutrient supply to these tissues. This in turn may result in processes contributing to the etiology of depression such as neuroinflammation and neurodegeneration (Hurley and Tizabi 2013). Indeed, increased platelet activation has been observed in depressed individuals with and without CAD (Goldston and Baillie 2008). It has also been reported that selective serotonin reuptake inhibitors (SSRIs) may reduce platelet activity in depressed individuals, further suggesting a role of platelet hyperactivity in depression (Musselman et al. 2000). CAD is a disease of the blood vessels that supply the heart, but the processes that occur in the coronary arteries of CAD patients may also be occurring in the cerebral vessels of these patients (Goldston and Baillie 2008). Atherosclerosis is a process that affects the entire vascular system (Goldston and
The relationship between CAD and depression is complex and likely involves many different factors.

Depression is associated with worse health outcomes in CAD (Barth et al. 2004, Dickens 2015). Depression increases risk of cardiac mortality by 1.5-3 times in CAD patients (Barth et al. 2004, Celano and Huffman 2011, Rudisch and Nemeroff 2003). Depressive symptoms have been shown to be strong predictors of low functional status and lower quality of life (Rumsfeld et al. 2003, Vaccarino et al. 2001). Furthermore, depression has been reported to increase the risk of future cardiac events such as a MI in CAD patients independently of traditional cardiac risk factors (Barth et al. 2004, Penninx et al. 2001). The negative impact of depression on cardiac outcomes increases with symptom severity (Lesperance et al. 2000, Penninx et al. 2001), increasing, for example, the risk of hospitalization due to complications of CAD in a dose-dependent manner (Rutledge et al.). Depression is highly prevalent in CAD and is a major factor jeopardizing health, recovery, and survival in these individuals.

Current treatments for depression in CAD include pharmacotherapy (most commonly with SSRIs), psychotherapy, and physical activity (Lichtman et al. 2008). A large proportion of CAD patients do not respond significantly to current interventions (Baumeister et al. 2011, Taylor 2008). In the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy (CREATE) trial, only 52.8% of CAD patients with depression responded to citalopram treatment after 12 weeks and only 35.9% of patients achieved remission (Lesperance et al. 2007). In the Enhancing Recovery in Coronary Heart Disease (ENRICHD) trial, only modest benefits of a psychosocial intervention on mental health and quality of life were observed (Mendes de Leon et al. 2006). Non-response to therapy is of particular clinical importance because it has been shown to be associated with
increased risk of future cardiac events, such as cardiac mortality and acute coronary syndrome (de Jonge et al. 2007). Taken altogether, the evidence suggests that depression in CAD is currently treated sub-optimally (Baumeister et al. 2011), highlighting a need to explore alternative or adjunctive therapeutic strategies.

1.4.3 Ceramides

Ceramides are a family of sphingolipid species consisting of a sphingoid base linked to a fatty acid via an amide bond (Bikman and Summers 2011). They are formed as key intermediates in the biosynthesis of all complex sphingolipids such as sphingomyelins and cerebrosides (Gangoiti et al. 2010). Ceramides are produced via three different pathways (Gulbins et al. 2015). They can be generated by de novo synthesis starting from palmitoyl-CoA and serine in a multi-step pathway (Chaurasia and Summers 2015). Ceramides can also be generated by the reacylation of their degradation product sphingosine, in what is known as the salvage pathway (Kitatani et al. 2008). Finally, the most common method of ceramide production is hydrolysis of sphingomyelins via sphingomyelinases (SMases) (Goni and Alonso 2002). Ceramides are a component of sphingomyelins and glycosphingolipids and are cleaved from these species by SMases (Kornhuber et al. 2014). SMases are characterized by the optimal pH they function in and thus acid sphingomyelinases (aSMases), neutral sphingomyelinases (nSMases) and alkaline sphingomyelinase exist (Goni and Alonso 2002).

Ceramides are mainly located in cell membranes and have numerous biological functions (Bikman and Summers 2011, Christie 2014, Gangoiti et al. 2010). Although numerically they only make up a minor component of cell membranes, they play vital roles in cell signaling by altering membrane stability and subsequently receptor stability and function (Christie 2014). Ceramides often aggregate
in specific regions of cell membranes termed ceramide-rich platforms where they permit 
oligomerization of receptors thereby amplifying or modifying cell signaling (Gangoiti et al. 2010). It 
is believed that ceramide-rich platforms modify both receptor- and stress-mediated cell signaling, 
and thus may influence various disease states (Stancevic and Kolesnick 2010). Ceramides may also 
influence membrane permeability via interactions with ion channels (Christie 2014).

One of the most important functions of ceramides, and their most studied function, is their role in 
the regulation of apoptosis, a vital process in which cells are deliberately killed to benefit the 
organism (Gangoiti et al. 2010). Some ceramides, in particular C16:0 and C18:0, have been observed 
to promote apoptosis in vitro (Herget et al. 2000, Huang et al. 2011, Senkal et al. 2007, Thomas et 
al. 1999). Consequently, aberrations in ceramide metabolism may have significant consequences in 
the regulation of apoptosis. Failure to properly regulate apoptosis may have catastrophic 
consequences including the initiation and progression of many disease states such as 
al. 2012). As important moderators of apoptosis, ceramides have been implicated in the 
pathophysiology of numerous disease states (Gangoiti et al. 2010).

1.4.4 Ceramides and CAD

Clinical evidence has demonstrated associations between aberrations in ceramide metabolism and 
increased risk of CAD as well as poorer prognoses in individuals with CAD (Ichi et al. 2006, Pan et al. 
2014, Schissel et al. 1996). Upon their production in blood vessels, ceramide species promote 
oxidation and aggregation of low-density lipoprotein (LDL) in the vessel wall, a necessary step in 
atherosclerosis (Schissel et al. 1996). It has also been observed that atherosclerotic lesions are 
enriched with ceramide species (Edsfeldt et al. 2016, Schissel et al. 1996). One proposed mechanism
by which ceramides are produced in vessel walls is via breakdown of sphingomyelin by SMases (Schissel et al. 1996). It has been observed that human atherosclerotic lesions are highly enriched with sphingomyelin (Schissel et al. 1996). Sphingomyelin is transported into arterial walls by atherogenic lipoproteins and subsequently hydrolyzed into ceramides via arterial wall SMases (Bismuth et al. 2008). Indeed, increased plasma sphingomyelin concentration has been shown to be an independent predictor of CAD in humans (Jiang et al. 2000).

Ceramide concentrations are also correlated with tumor necrosis factor-α (TNF-α) concentrations, an important inflammatory cytokine associated with CAD (Bismuth et al. 2008, Sawada et al. 2004). TNF-α has been shown to increase ceramide concentrations via activation of SMases (Sawada et al. 2004). Other inflammatory cytokines such as interleukin-2 (IL-2) and endostatin have been shown to increase ceramide content in heart tissue (Bismuth et al. 2008). Conversely, ceramides have been shown to promote production of some inflammatory cytokines such as IL-6 and CRP, thereby having direct pro-inflammatory effects and participating in the atherosclerotic process (Blake and Ridker 2001). Additionally, other species implicated in CAD such as homocysteine and matrix metalloproteinases (MMPs) have been shown to stimulate ceramide production (Bismuth et al. 2008).

Further evidence that ceramides play a role in atherosclerosis and CAD is exhibited when ceramide concentrations are pharmacologically reduced. Myriocin, a compound that inhibits ceramide production, has been found to reduce atherosclerotic lesion size in mice (Hojjati et al. 2005). These results must be taken with caution however as myriocin has also been shown to inhibit HMG CoA reductase, a key enzyme in the biosynthesis of cholesterol (Bismuth et al. 2008). Further, myriocin may be able to reduce concentrations of inflammatory proteins (Park et al. 2006). These two effects

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of myriocin may be the largest cause of the reduction in atherosclerotic lesion size rather than a reduction in ceramides. Altogether, there is considerable evidence that ceramides play a role in the pathophysiology of atherosclerosis and CAD.

1.4.5 Ceramides and Depression: Neurobiological Mechanisms

Ceramides are gaining prominence as a novel antidepressant target due to their potential roles in the pathophysiology of depression (Kornhuber et al. 2014). Commonly observed in depression is a reduction in adult hippocampal neurogenesis (Henn and Vollmayr 2004). Neurogenesis, the generation of new neurons, has only been observed to occur in two regions of the adult brain, the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles (Henn and Vollmayr 2004). Neurogenesis is dependent on the differentiation and proliferation of neuronal stem cells and progenitor cells into mature adult neurons (Ming and Song 2011). Ceramides have been shown to influence motility and apoptosis of neural stem cells and progenitor cells of the developing brain (Bieberich 2012). As discussed previously, ceramides may play a central role in apoptosis, thus it is feasible that an imbalance of ceramides may cause apoptosis of neural stem cells and/or glial cells (Wang et al. 2012) in the hippocampus thus hindering neurogenesis in this region.

Another pathophysiological feature commonly found in depression is hyperactivity of the HPA axis (Stetler and Miller 2011). The HPA axis is largely responsible for an organism’s response to stressful stimuli (Smith and Vale 2006). Dysregulation of this axis can result in over-adaptation and over-compensation to stressful stimuli and subsequent unnecessary cell injury and death (Pariante and Lightman 2008). The HPA axis is regulated in part by the hippocampus (Smith and Vale 2006). The hippocampus is thought to have a negative control over the HPA axis (Smith and Vale 2006).
Reduced hippocampal neurogenesis potentially caused by elevated ceramides may inhibit the hippocampi’s ability to negatively control the HPA axis resulting in HPA axis hyperactivity.

Glucocorticoids, such as cortisol, are important molecules involved in stress responses (Smith and Vale 2006). Concentrations of glucocorticoids are regulated by the HPA axis and overproduction of glucocorticoids as observed in individuals with depression may inhibit neurogenesis (Pariante and Lightman 2008). Thus a vicious cycle may emerge where reduced inhibitory control of the HPA axis via the hippocampus results in increased glucocorticoid concentrations and consequent inhibition of hippocampal neurogenesis (Pariante and Lightman 2008).

Elevated inflammation is another condition commonly observed in depression (Raison et al. 2006), as evidenced by elevated concentrations of multiple pro-inflammatory cytokines and acute phase proteins such as CRP and TNF-α in depressed individuals and those with depressive symptoms (Adibfar et al. 2016, Liu et al. 2016, Suarez et al. 2004). Whether or not this association is causal has yet to be determined (Raison et al. 2006). Treatment of depression with SSRIs has been observed to reduce inflammation in humans (Hannestad et al. 2011, Hiles et al. 2012). As mentioned, ceramides have been associated with various pro-inflammatory cytokines such as IL-6, TNF-α, and CRP (Bismuth et al. 2008, Sawada et al. 2004). Elevation of ceramide concentrations may contribute to depression and depressive symptoms via upregulation of pro-inflammatory cytokines resulting in neuroinflammation and consequent neurodegeneration (Kornhuber et al. 2014, Muhle et al. 2013).

Additionally, ceramides may alter monoamine neurotransmitter transport, in particular the transport of serotonin and dopamine (Riddle et al. 2003). A reduction in serotonergic and dopaminergic transmission has been strongly implicated in the pathophysiology of depression (Hirschfeld 2000). Many anti-depressant drugs regulate extracellular concentrations of these two
neurotransmitters and it is believed that this is the primary mechanism by which these drugs exert their effects (Hirschfeld 2000). The ceramide species C2 was reported to enhance the reuptake of serotonin which subsequently would lead to a reduction in serotonergic transmission (Riddle et al. 2003). Interestingly, this enhancement in the reuptake of serotonin appeared to be caused by an increased reuptake of serotonin via the dopamine transporter and not the serotonin transporter (Riddle et al. 2003). Furthermore, C2 was reported to inhibit dopamine reuptake, suggesting a positive effect on depression (Riddle et al. 2003). It is plausible that ceramides exert effects on monoamine transporters either directly via membrane formation of ceramide-rich platforms that alter receptor stability and function (Gangoiti et al. 2010), or indirectly via modification of intracellular kinases and phosphatases (Dobrowsky and Hannun 1992, Tanabe et al. 1998, Westwick et al. 1995). The effects of ceramide species on monoamine neurotransmitter transport requires further investigation as only one ceramide species has been studied and only one study has been conducted. However, this study provides evidence of the potential of ceramides to alter monoamine neurotransmitter signaling.

Further evidence that ceramides play a role in depression pathophysiology is that many antidepressants inhibit aSMase, an enzyme that produces ceramides via hydrolysis of sphingomyelin (Kornhuber et al. 2010). The term functional inhibitors of acid sphingomyelinase (FIASMA) was created by Kornhuber et al. and describes a group of pharmacological agents that inhibit, to varying degrees, the activity of aSMase (Kornhuber et al. 2010). All tricyclic antidepressants (TCAs) and many SSRIs such as fluoxetine and sertraline are FIASMAs (Kornhuber et al. 2010). Perhaps a reduction of ceramide production via inhibition of aSMase is one mechanism by which antidepressants exert their antidepressant effects. This reduction of ceramide concentrations caused by antidepressants that act as FIASMAs does not occur until two to three weeks after
administration of the antidepressant, a timeline consistent with the onset of action of antidepressants (Kornhuber et al. 2009). This is due to the fact that these antidepressants take two to three weeks to accumulate in sufficient amounts in the brain, in particular the lysosomes of neurons and glial cells, where they inhibit aSMase activity (Kornhuber et al. 2009). This consistency in the timeline of ceramide reduction and onset of antidepressant action suggests a role of ceramides in the pathophysiology of depression in humans. Although a reduction in ceramides may be a potential inadvertent mechanism by which antidepressants reduce depression, no drug to date exists that specifically targets ceramides in order to treat depression (Kornhuber et al. 2014).

Depression is a multifactorial disorder and likely presents a different pathophysiological profile in each individual (Belmaker and Agam 2008). This may explain why not all antidepressants reduce ceramide concentrations and why not all individuals respond to antidepressant therapy. The sum of neurobiological evidence suggests that ceramides may play a role in some cases of depression due to their pro-inflammatory and pro-apoptotic characteristics.

### 1.4.6 Ceramides and Depression: Evidence in Animals

In one of the most commonly used animal models of depression, the chronic mild stress (CMS) model, hippocampal ceramide concentrations were found to be doubled compared to control mice (Gulbins et al. 2013). This elevated ceramide content was accompanied by reduced neurogenesis and reduced neuronal maturation (Gulbins et al. 2013). Transgenic mice have been generated that either overexpress aSMase or have had the aSMase gene knocked out and therefore do not express aSMase (Gulbins et al. 2013). Mice overexpressing aSMase displayed greater ceramide production in the hippocampus, reduced neurogenesis, reduced neuronal maturation and reduced neuronal survival (Gulbins et al. 2013). Concomitantly, these mice displayed depression-like behavior on
several behavioral tests such as the forced swim test, the novelty suppressed feeding test, and the coat state assessment (Gulbins et al. 2013). Conversely, aSMase knockout mice presented with reduced ceramide concentrations in the hippocampus and displayed reduced depression-related behavior in some but not all behavioral tests when compared to wild type mice (Gulbins et al. 2013). In those experiments, it is not specified which ceramide species in particular are elevated, but rather that total ceramide concentrations are increased, thus making it difficult to assess which specific ceramide species are associated with depression.

Pharmacological evidence of ceramides’ role in depression is limited. Injection of C16:0 into the hippocampus of mice was shown to induce depression-like behavior (Gulbins et al. 2013). In CMS mice, antidepressants have been observed to reduce depression-like behavior; however, in CMS mice with their aSMase gene knocked out, amitriptyline did not reduce depression (Gulbins et al. 2013). This suggests that one mechanism by which some antidepressants exert their effects is via inhibition of aSMase and consequent reduction of ceramides. Furthermore, treatment of CMS mice with fendiline, a calcium channel blocker that is also a FIASMA and that has not been reported to be an antidepressant, was also shown to reduce hippocampal ceramide concentrations, increase hippocampal neurogenesis, and decrease depressive behaviors (Gulbins et al. 2013). Taken altogether there is substantial evidence from animal models that ceramide species are involved in depression pathophysiology; however, it should be noted that most of this evidence comes from one single group of researchers (Gulbins et al.), thus evidence is limited in that respect. Further animal research on ceramides’ role in depression is required to attempt to determine if a causal relationship exists and to elucidate the mechanisms by which ceramides may cause depression.
1.4.7 Ceramides and Depression: Clinical Evidence

Increasing recognition of the potential role of ceramides in depression has led to the first studies of ceramides and their relationship to depression in human subjects. In a study with cognitively healthy elderly and elderly with Alzheimer’s disease, plasma concentrations of the ceramide species C16:0, C18:0, and C20:0 were found to be significantly higher in individuals with current depression or individuals that had experienced a depressive episode within the last two years when compared to non-depressed individuals (Gracia-Garcia et al. 2011). C24:1 and C26:1 were also elevated in depression in that study (Gracia-Garcia et al. 2011). Effect sizes varied between each ceramide species, with C18:0 displaying the greatest elevation in depressed individuals and C20:0 showing the next greatest elevation (Gracia-Garcia et al. 2011). C16:0 showed the third greatest elevation in depressed individuals (Gracia-Garcia et al. 2011). That group also assessed the relationship between ceramide species and geriatric depression scale (GDS) scores but found no significant associations across all study participants or within each subgroup group (AD vs cognitively healthy). One limitation of that study was its relatively small sample size (n=46) which may explain why no associations were found between ceramides and GDS scores.

In a Dutch family-based lipidomics study the ceramide species C18:0 and C20:0 were once again found to be implicated in depression (Demirkan et al. 2013). C18:0 displayed a positive association with Center for Epidemiological Studies Depression Scale (CESD) scores while C20:0 displayed a positive association with Hospital Anxiety and Depression – Depression subscale scores (HADS-D) (Demirkan et al. 2013). The p-values for these associations were both less than 0.05; however, they were not considered statistically significant in that study as the threshold for significance was lowered to correct for multiple comparisons. Still, those associations should not be overlooked as
that study consisted of a very large sample size (n=742). Furthermore, C16:0 and C22:0 showed trending positive associations with HADS-D scores.

Finally, C18:0 has also been implicated in another psychiatric disorder, PTSD (Hammad et al. 2012). In a clinical study, C18:0 was observed to be significantly elevated in the plasma of individuals with PTSD compared to healthy controls (Hammad et al. 2012). The sample size of that study was very small (n=13); however, that finding further suggests the importance of C18:0 in psychiatric disorders. Clinical evidence of the role of ceramides in depression is limited but has implicated some ceramide species, most strongly C18:0 and C20:0. Further clinical research is warranted and required to assess the relationship between ceramides, depression, and depressive symptoms in order to determine if a reduction in ceramide concentrations in humans can alleviate depressive symptoms and treat depression.

### 1.4.8 Ceramides as a Link Between CAD and Depression

CAD may be a good environment to study the relationship between ceramides and depression as both ceramides and depression appear to be elevated in this population. Elevated ceramides may be a result of elevated inflammatory processes in CAD (Bismuth et al. 2008). TNF-α and other inflammatory cytokines implicated in CAD have been reported to increase peripheral ceramide concentrations (Bismuth et al. 2008, Sawada et al. 2004). Increased peripheral ceramide concentrations may result in increased ceramide concentrations in the brain (Zimmermann et al. 2001). Higher brain concentrations of ceramides may result in increased neuroinflammation and neurodegeneration due to their pro-inflammatory and pro-apoptotic characteristics (Kornhuber et al. 2009). These processes would explain the reduced hippocampal neurogenesis present in many
cases of depression and likely contribute to the pathophysiology of depression (Bakunina et al. 2015).

Depression is not only a neuropsychiatric disorder, but it is also a systemic disorder with both psychological and somatic manifestations (Kornhuber et al. 2014). Somatic manifestations of depression include but are not limited to fatigue, hyper- or hyposomnia, and abnormal appetite resulting in weight gain or loss (Kapfhammer 2006). Current theories of depression tend to focus on reduced monoaminergic transmission in the brain, which has faced problems in explaining the somatic symptoms of depression such as inflammation and coronary dysfunction (Hirschfeld 2000). Ceramides may be the key to explaining these somatic symptoms. Elevated ceramide concentrations appear to be present in some cases of depression (Gracia-Garcia et al. 2011). Elevated ceramides in the brain could reasonably result in elevated peripheral ceramide concentrations, which may contribute to some of the peripheral and somatic manifestations of depression (Kornhuber et al. 2014). As mentioned, ceramides have been shown to promote production of some inflammatory cytokines resulting in increased inflammation, a condition observed in many cases of depression (Blake and Ridker 2001, Kohler et al. 2015). Furthermore, increased ceramide concentrations in the periphery may promote oxidation and aggregation of LDL in vessel walls, accelerating atherosclerosis in the vessels of depressed individuals (Schissel et al. 1996).

As the relationship between CAD and depression appears to be bidirectional, and ceramides have been implicated in the pathophysiology of both diseases, a vicious cycle may exist in which one disease results in increased ceramide concentrations, initiating and/or exacerbating the other disease and resulting in a further increase in ceramide concentrations. Further research is required
to determine if ceramides play a causal role in these diseases, are a by-product of these diseases, or both.

1.4.9 Sphingolipids, CAD, and Depression

Sphingolipids are a diverse class of lipids containing a sphingoid base attached to an acyl group such as a fatty acid and are commonly present in brain cell membranes (Merrill et al. 2005, Muller et al. 2015). Lipid families in the sphingolipid class include sphingomyelins, sphingosines, cerebrosides, gangliosides, and ceramides (Merrill et al. 2005). Other sphingolipids aside from ceramides, such as sphingosines and sphingomyelins, may also be implicated in depression and CAD pathophysiology (Demirkan et al. 2013, Muller et al. 2015). Sphingolipids in combination with cholesterol play an important role in cell signaling by altering the affinity and function of receptors (Muller et al. 2015). Thus it is plausible that aberrations in sphingolipid metabolism may play a role in depression via alterations in monoaminergic neurotransmission and alterations in cellular response to stress (Jernigan et al. 2015, Muller et al. 2015).

1.5 Summary of Background

Substantial evidence exists implicating ceramides, a family of bioactive sphingolipids, in the pathophysiology of both CAD and depression. Evidence in both animals and humans has been accumulated that suggests abnormal ceramide concentrations play a role in the initiation and/or progression of both diseases (Kornhuber et al. 2014). The potential neurobiological mechanisms by which ceramides may cause depression are biologically plausible and focus on ceramide-induced neuro-inflammation and neurodegeneration. However, there are other hypothesized mechanisms by which ceramides may cause depression such as via altering monoaminergic neurotransmission (Riddle et al. 2003). Furthermore, many currently prescribed antidepressants lower ceramide
concentrations and the timeline by which they do so is consistent with the delayed therapeutic
effect of these drugs (Gulbins et al. 2013, Kornhuber et al. 2009). Aberrations in the metabolism of
other sphingolipids may also be linked to depression and may occur frequently in individuals with
CAD (Demirkan et al. 2013, Muller et al. 2015). Despite the current evidence, the extent and
consistency of the role of ceramides in the pathophysiology of depression and CAD is still not clear.
More studies are required, particularly in humans, to deduce the role of ceramides in these
pathophysiologies.

CAD may be a good environment to examine the relationship between ceramides and depressive
symptoms because both of these appear to be elevated in CAD (Bradley and Rumsfeld 2015, Schissel
et al. 1996). CAD is a prevalent and important disease in our population and represents a significant
detriment to quality of life and large economic burden (Public Health Agency of Canada 2009).
Depression and depressive symptoms are frequent in this population and reduce quality of life and
worsen prognoses in these individuals (Dickens 2015). No studies to date have examined ceramides
as correlates of depressive symptoms in CAD.
2. Methods

2.1 Patient Recruitment

All study participants were recruited from Toronto Rehabilitation Institute’s (TRI) cardiac rehabilitation (CR) program. Patients with CAD between the ages of 50-75 entering cardiac rehab were asked to participate in this study.

2.1.1 Inclusion and Exclusion Criteria

All study participants were required to have one or more of the following diagnoses as evidence of CAD: coronary angiographic evidence of ≥ 50% blockage in ≥ 1 major coronary artery, previous hospitalization for acute MI, a prior revascularization procedure (stent or CABG), or previous diagnosis of ischaemic heart disease. Furthermore, only participants with stable CAD, as defined by no hospitalizations for cardiac events such as an acute MI, unstable angina, or coronary revascularization procedure within the four weeks prior to their baseline assessment, were accepted into the study. Study participants were required to be between 50 and 75 years of age and speak and understand English in order to complete the assessments. All participants were required to be taking a statin medication due to their potential effects on mood and cognition (Kim et al. 2015, Power et al. 2015). This was not a hindrance to study recruitment as approximately 95% of CAD patients enrolled in TRI’s cardiac rehab program are on statins (Swardfager et al. 2010).

Conversely, use of antidepressants within 3 months of the baseline assessment constituted an exclusion criterion due to the impact of some but not all antidepressants on ceramide concentrations in the body (Gulbins et al. 2013, Gulbins et al. 2015, Kornhuber et al. 2014). Some but not all antidepressants have been shown to inhibit aSMase, resulting in decreased ceramide concentrations (Kornhuber et al. 2010). Thus, in order to standardize the study population, use of
antidepressants was excluded. Patients diagnosed with any of dementia, Parkinson’s, Huntington’s chorea, an autoimmune disease (e.g. multiple sclerosis, rheumatoid arthritis, Crohn’s disease), active cancer, impaired liver or kidney function or history of cerebrovascular accident (CVA), transient ischemic attack (TIA), brain tumor, subdural hematoma, epilepsy, or any other conditions likely to significantly impact mood or cognition were excluded from this study. Furthermore, patients with significant cognitive impairment (as assessed by a standardized mini mental state examination [sMMSE] of ≤ 24), bipolar disorder, or schizophrenia were also excluded. Lastly, use of antipsychotics also constituted an exclusion criterion due to their effects on mood and cognitive outcomes, as well as their potential effects on ceramide concentrations (Alexander et al. 2011, Hori et al. 2015, Kornhuber et al. 2008, Kornhuber et al. 2013).

2.2 Collection of Demographic and Medical Information

After obtaining consent, cardiac medical history and other relevant medical and surgical history was obtained from study participants and via TRI’s electronic and physical medical records. Cardiopulmonary and metabolic health indices (VO₂ Peak, resting HR, max HR, BP, height, weight, BMI, and body fat percentage) were also collected from TRI’s electronic medical records. Demographic information such as age, gender, marital status, employment status and socioeconomic status as well as smoking history was obtained verbally from study participants.

2.3 Assessments

Patients were scheduled to come in for their assessment and blood collection as close to the start of their CR program as possible. A number of different assessments were administered to participants.
2.3.1 Center for Epidemiological Studies Depression Scale (CESD)

The CESD is a 20-item self-reported scale used for measuring depressive symptoms (Radloff 1977). It consists of questions covering multiple domains of depressive symptoms including mood, cognitive and somatic symptom clusters (Radloff 1977). The CESD has been used in the past to measure depressive symptoms in CAD populations and has been shown to be a reliable measure of depressive symptoms in this population (Dowlati et al. 2010, McManus et al. 2005, Rudisch and Nemeroff 2003, Swardfager et al. 2010, Swardfager et al. 2011a, Swardfager et al. 2011b).

2.3.2 Structured Clinical Interview for the Diagnosis of DSM-V Disorders – Depression & Anxiety (SCID)

Presence of major depressive disorder (MDD) or minor depression was assessed using a structured clinical interview for the diagnosis of DSM-V disorders (SCID). Participants were classified as depressed if either minor or major depression was present. A separate SCID was also administered to assess for presence of generalized anxiety disorder (GAD).

2.3.3 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale is a 14-item self-reported scale with integrated depression and anxiety subscales (Zigmond and Snaith 1983). It was created for use in a medical outpatient population (such is the present study population) and has been validated for use in CAD populations (Martin et al. 2003, Zigmond and Snaith 1983).

2.3.4 Generalized Anxiety Disorder 7-Item Scale (GAD-7)

The Generalized Anxiety Disorder 7-Item Scale (GAD-7) is a short self-reported scale used to assess presence of GAD and its severity in clinical practice and research (Spitzer et al. 2006). GAD and
symptoms of anxiety were assessed in all participants in order to assess anxiety as a potential confounding factor in the relationship between ceramides and depressive symptoms (Demirkan et al. 2013, Hammad et al. 2012).

2.3.5 Spielberger State-Trait Anxiety Inventory (STAI)

The Spielberger State-Trait Anxiety Inventory consists of two 20-item self-reported scales used to measure presence and severity of state and trait anxiety (Julian 2011). STAI scores have been shown to be associated with clinician rated GAD severity (Hopko et al. 2000) and the trait subscale in particular has been found to be a strong predictor of anxiety disorders (Hishinuma et al. 2001). STAI scores were used as another measure of anxiety symptom severity in all participants.

2.3.6 Apathy Evaluation Scale (AES)

The Apathy Evaluation Scale is an 18-item self-reported questionnaire that assesses presence and severity of apathy (Marin et al. 1991). Apathy was assessed in all participants as it is often masked or mistaken for symptoms of depression and can erroneously increase depression scale scores due to convergence between measures of apathy and depression (Levy et al. 1998, Marin et al. 1993). Apathy or symptoms of apathy are common in depression and may also confound the relationship between ceramides and depressive symptoms (van Reekum et al. 2005).

2.3.7 Social Readjustment Rating Scale (SRRS)

The Social Readjustment Rating Scale (SRRS) is a 43-item self-reported questionnaire inquiring about potentially stressful life events and other sources of emotional stress that an individual has endured within the last year (Holmes and Rahe 1967). It is used to measure an individual’s current “life” or
emotional stress (Holmes and Rahe 1967). Emotional stress was assessed in this study as a potential confounding factor in the ceramides depression relationship.

2.3.8 Standardized Mini Mental State Examination (sMMSE)

The Mini Mental State Examination (sMMSE) is a short examination of global cognition used to assess for the presence and severity of cognitive impairment (Molloy and Standish 1997, Tombaugh and McIntyre 1992). Cognition and presence of cognitive impairment were assessed as confounders in the ceramides depression relationship (Carrasco et al. 2012, McIntyre and Lee 2015, Mielke et al. 2010, Saleem et al. 2013).

2.3.9 Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index is a self-reported questionnaire assessing various domains of sleep quality (Buysse et al. 1989). Poor sleep quality is common in depression (Benca and Peterson 2008, Hayashino et al. 2010) and may contribute to the development of CAD (Mooe et al. 2001, Twig et al. 2016). Sleep quality was assessed in all patients as another potential confounder of the relationship between ceramides and depression.

2.4 Assays

2.4.1 Blood Collection

A 12-hour fasting blood sample was collected from each participant. The majority of participants were scheduled to come to our lab for assessments and blood collection at 0900 h ± 1 h. This standardized time and fasting blood allowed for relative control of diurnal and dietary influences on ceramide and other lipid concentrations. Blood was drawn into EDTA containing vacutainer tubes in
order to collect plasma samples. Blood was then centrifuged at 1000 x g for 15 minutes, and plasma was immediately isolated, aliquoted, and stored at -80°C until analyzed.

2.4.2 Lipidomic Analysis

Sphingolipid species (including ceramides) were quantified using high performance liquid chromatography coupled electrospray ionization tandem mass spectrometry (HPLC ESI-MS/MS). All measurements were performed blind to clinical characteristics of the participants. Plasma samples were injected into a PerkinElmer HPLC (Waltham, Massachusetts, USA) equipped with a phenomenex, luna 100 x 2 mm, 5 µm, C18 column coupled with guard column containing identical packaging material (Phenomenex, Torrance, California, USA) using a PAL autosampler. A mobile phase consisting of 85% methanol, 15% H2O, and 5 mM ammonium formate was first injected for 0.5 min into the LC column for pre-equilibration. The column was then eluted with a second mobile phase consisting of 99% methanol, 1% formic acid, and 5 mM ammonium formate at a flow rate of 100.0 µl/min. The eluted sample was then injected into the ion source for detection and quantification of ceramides and sphingomyelins (m/z 264.4, 266.4 for ceramides and 184.4 for sphingomyelins respectively). Data was collected and processed by Analyst 1.4.2 software package. Ceramide concentrations were initially presented in counts per second (cps), but were converted to ng/ml by applying a standard curve.

2.5 Sample Size Calculation

Sample size was calculated based on effect size estimates from two reports of elevated ceramides in depression and other mood disorders (Gracia-Garcia et al. 2011, Hammad et al. 2012). Effect sizes ranged between 0.08 and 0.66 in those reports (Gracia-Garcia et al. 2011, Hammad et al. 2012). A medium effect size was chosen for this sample size calculation to reflect the average of effect sizes.
reported in the literature. Using IBM SPSS SamplePower 3.0 (Chicago, Illinois, USA) a sample size of 82 participants was calculated that allows a linear regression model to achieve 80% power to detect an effect size of \( r = 0.3 \) with an \( \alpha \)-level of 0.05. A target of 100 participants was set in order to account for the possibility of missing information such as undetectable ceramide concentrations or incompletion of the assessments. This sample size allows for the inclusion of 9 covariates in the statistical models used in this study.

2.6 Statistical Analyses

Plasma ceramide concentrations were log-transformed (base of 10) to account for a positively skewed non-normal distribution. Multiple linear regression models were used to assess whether higher plasma ceramide concentrations are associated with greater depressive symptoms, as measured by CESD score, after adjusting for age, gender, BMI, and total cholesterol. Normality of residuals in these models was assessed by visual inspection of normal probability plots. Analysis of covariance (ANCOVA) was used to assess differences in plasma ceramide concentrations between depressed and non-depressed study participants after adjusting for age. Binary logistic regression models were used to assess plasma ceramide concentrations as predictors of depression after adjusting for age. Age was the only covariate included in this model because the depressed group contained only 18 participants, allowing for the inclusion of only one covariate. All analyses were performed using IBM SPSS version 20. Associations between participant sociodemographic/clinical characteristics and log-transformed concentrations of ceramides C18:0 and C20:0 were found using analysis of variance (ANOVA) for categorical characteristics and bivariate correlations for continuous characteristics. For all analyses, a \( p \)-value \( \leq 0.05 \) was accepted as significant as this study was exploratory, being the first study to assess associations between ceramides and depressive symptoms in a CAD population.
2.6.1 Selection of Covariates

Covariates were chosen *a priori*. Potential multicollinearity of ceramides and covariates was assessed using variance inflation factor (VIF) and tolerance statistics. As general guidelines, covariates with a VIF greater than 2.5 and a tolerance below 0.4 were considered collinear.

2.6.1.1 Age

Age has been shown to be associated with depression and depressive symptoms (Mirowsky and Ross 1992, Wade and Cairney 1997). The nature of this relationship (i.e. linear or polynomial) is unclear (Mirowsky and Ross 1992, Wade and Cairney 1997). Our own lab has found that younger age was associated with greater depressive symptoms in CAD patients (Swardfager et al. 2008). In addition, age may be associated with a change in lipidome, altering ceramide concentrations (Hughes et al. 2012).

2.6.1.2 Gender

Depression is 1.5-3 times more prevalent in women than men (Ustun 2000). This difference may be attributable to differences in neurotransmitter and hormonal systems between genders (Halbreich and Lumley 1993). Differences in stress responses may also contribute to the higher prevalence of depression in females (Nolen-Hoeksema 2001). We have previously found that in a CAD population, females had greater depressive symptom severity than males (Swardfager et al. 2008). Gender may also be associated with differences in lipidomics and ceramide concentrations (Ishikawa et al. 2014).

2.6.1.3 Body Mass Index (BMI)

BMI has been reported to be associated with depression (de Wit et al. 2009, Noh et al. 2015). This association appears to be U-shaped with greatest prevalence of depression appearing in those who
are underweight (BMI < 18.5) and those who are obese (BMI ≥ 30) (de Wit et al. 2009, Martin-Rodriguez et al. 2016). In addition, ceramides may be elevated in obesity (Adams et al. 2004, Turpin et al. 2014).

2.6.1.4 Total Cholesterol

Low serum cholesterol concentration may be associated with depression (Aijanseppa et al. 2002, Manfredini et al. 2000); however, reports on whether total cholesterol concentration is associated with depression are inconsistent (Ergun et al. 2004). Ceramide and cholesterol metabolism may be linked as both species are lipids (Cutler et al. 2004, Ridgway 2000).
3. Results

3.1 Demographics and Clinical Characteristics of Study Participants

119 participants with coronary artery disease were recruited from TRI. Sociodemographic and clinical characteristics of all study completers, including anthropometric measurements, cardiopulmonary fitness measurements, cardiac diagnoses, concomitant medications, and psychometric measures are presented in Table 1. Data are presented as means and standard deviations for continuous variables or total number of people and percentage of study population for categorical variables.

Table 1  Sociodemographic and clinical characteristics of study participants (N=119)

<table>
<thead>
<tr>
<th></th>
<th>Association with log-transformed C18:0</th>
<th>Association with log-transformed C20:0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD or n (%)</td>
<td>F or r value</td>
</tr>
<tr>
<td>Sociodemographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.6 ± 6.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Gender (# of males)</td>
<td>101 (84.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>Ethnicity (# of Caucasians)</td>
<td>99 (83.2)</td>
<td>0.14</td>
</tr>
<tr>
<td>Total Years of Education</td>
<td>16.3 ± 3.5</td>
<td>-0.17</td>
</tr>
<tr>
<td>Marital Status (# married)</td>
<td>84 (70.6)</td>
<td>2.25</td>
</tr>
<tr>
<td>Employment (# employed)</td>
<td>65 (54.6)</td>
<td>2.67</td>
</tr>
</tbody>
</table>
### Annual Income ($Cdn)

| Annual Income ($Cdn) | 110 965 ± 150 662 | -0.21 | 0.03* | -0.10 | 0.28 |

### Vascular Risk Factors and Severity of CAD

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Value</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>29.1 ± 5.1</td>
<td>0.01</td>
<td>0.94</td>
<td>-0.03</td>
<td>0.74</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.4 ± 16.7</td>
<td>0.11</td>
<td>0.25</td>
<td>0.03</td>
<td>0.76</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>99.1 ± 12.2</td>
<td>0.07</td>
<td>0.47</td>
<td>0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>Body fat %</td>
<td>31.5 ± 10.4</td>
<td>0.01</td>
<td>0.91</td>
<td>-0.05</td>
<td>0.62</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>125.2 ± 17.3</td>
<td>0.04</td>
<td>0.64</td>
<td>0.11</td>
<td>0.23</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>77.1 ± 9.5</td>
<td>0.09</td>
<td>0.35</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>Hypertension (# of participants with comorbidity)</td>
<td>72 (60.5)</td>
<td>0.01</td>
<td>0.93</td>
<td>1.27</td>
<td>0.26</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>3.5 ± 0.8</td>
<td>-0.05</td>
<td>0.60</td>
<td>0.19</td>
<td>0.04*</td>
</tr>
<tr>
<td>History of Smoking</td>
<td>72 (60.5)</td>
<td>1.13</td>
<td>0.29</td>
<td>4.04</td>
<td>0.05*</td>
</tr>
<tr>
<td>Total Years Smoked</td>
<td>13.7 ± 16.2</td>
<td>0.08</td>
<td>0.42</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>Alcohol Consumption (# of drinks per week)</td>
<td>3.3 ± 4.6</td>
<td>-0.05</td>
<td>0.57</td>
<td>-0.09</td>
<td>0.34</td>
</tr>
<tr>
<td>Diabetes (# of participants with comorbidity)</td>
<td>20 (16.8)</td>
<td>2.15</td>
<td>0.15</td>
<td>0.36</td>
<td>0.55</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>HbA1c (mmol/L)</td>
<td>0.06 ± 0.01</td>
<td>-0.07</td>
<td>0.47</td>
<td>0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>Cumulative Stenosis of Major Coronary Arteries*</td>
<td>149.8 ± 67.5</td>
<td>0.07</td>
<td>0.52</td>
<td>0.08</td>
<td>0.43</td>
</tr>
</tbody>
</table>

### Cardiac History (# with history of disease)

<table>
<thead>
<tr>
<th>Myocardial Infarction (MI)</th>
<th>58 (48.7)</th>
<th>0.00</th>
<th>0.99</th>
<th>0.39</th>
<th>0.54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percutaneous Coronary Intervention (PCI – i.e. stent)</td>
<td>77 (64.7)</td>
<td>0.86</td>
<td>0.36</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td>Coronary Artery Bypass Graft (CABG)</td>
<td>40 (33.6)</td>
<td>0.18</td>
<td>0.68</td>
<td>3.80</td>
<td>0.054</td>
</tr>
</tbody>
</table>

### Cardiopulmonary Fitness

<table>
<thead>
<tr>
<th>VO₂ Peak (ml/kg/min)</th>
<th>21.0 ± 5.6</th>
<th>-0.13</th>
<th>0.15</th>
<th>-0.13</th>
<th>0.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Heart Rate (beats per minute)</td>
<td>122.0 ± 20.3</td>
<td>0.07</td>
<td>0.43</td>
<td>0.07</td>
<td>0.47</td>
</tr>
</tbody>
</table>

### Concomitant Medications (# of participants on medication)

<p>| Beta-Blockers | 95 (79.8) | 0.02 | 0.89 | 0.01 | 0.92 |</p>
<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Participants</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypertensives</td>
<td>84 (70.6)</td>
<td>0.01</td>
<td>0.93</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Platelet Inhibitors (including ASA)</td>
<td>115 (96.6)</td>
<td>0.10</td>
<td>0.75</td>
<td>0.22</td>
<td>0.64</td>
</tr>
<tr>
<td>Acetylsalicylic Acid (ASA)</td>
<td>111 (93.3)</td>
<td>0.17</td>
<td>0.68</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>Diuretics</td>
<td>19 (16.0)</td>
<td>2.16</td>
<td>0.14</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Calcium-channel Blockers</td>
<td>17 (14.3)</td>
<td>0.47</td>
<td>0.49</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>5 (4.2)</td>
<td>0.35</td>
<td>0.55</td>
<td>0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>Statins</td>
<td>119 (100.0)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Psychometrics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spielberger State-Trait Anxiety Inventory (STAI) – Trait Anxiety Raw Score</td>
<td>31.7 ± 9.4</td>
<td>0.06</td>
<td>0.51</td>
<td>0.03</td>
<td>0.76</td>
</tr>
<tr>
<td>Generalized Anxiety Disorder 7-Item Scale (GAD-7) Score</td>
<td>2.6 ± 3.6</td>
<td>0.02</td>
<td>0.85</td>
<td>0.08</td>
<td>0.37</td>
</tr>
<tr>
<td># of Participants with SCID-diagnosed GAD</td>
<td>5 (4.2)</td>
<td>0.94</td>
<td>0.39</td>
<td>0.11</td>
<td>0.90</td>
</tr>
<tr>
<td>Apathy Evaluation Scale (AES) Score</td>
<td>26.2 ± 7.9</td>
<td>0.08</td>
<td>0.38</td>
<td>0.12</td>
<td>0.19</td>
</tr>
<tr>
<td>Social Readjustment Rating Scale (SRRS) Score</td>
<td>214.8 ± 125.8</td>
<td>-0.04</td>
<td>0.68</td>
<td>0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Standardized Mini Mental State Examination (sMMSE) Score</td>
<td>29.0 ± 1.2</td>
<td>-0.17</td>
<td>0.06</td>
<td>-0.09</td>
<td>0.32</td>
</tr>
<tr>
<td>Pittsburgh Sleep Quality Index (PSQI) Score</td>
<td>5.8 ± 4.0</td>
<td>-0.06</td>
<td>0.56</td>
<td>0.04</td>
<td>0.71</td>
</tr>
</tbody>
</table>

5 participants were missing annual income information, 2 participants were missing body fat % measurement, 1 participant was missing BP measurement, 1 participant was missing hypertension information, 3 participants were missing total years smoked information, 1 participant was missing HbA1c measurement, 22 participants were missing cumulative stenosis information, 1 participant was missing VO2 Peak measurement, 1 participant was missing max heart rate measurement, 2 participants were missing STAI-Trait score, 3 participants were missing GAD-7 score, 16 participants were missing SCID-GAD information, 3 participants were missing AES score, 6 participants were missing SRRS score, and 4 participants were missing PSQI score.

*Cumulative stenosis of major coronary arteries was defined as combined % stenosis of left main, left anterior descending, left circumflex, and right main coronary arteries.

3.2 Depression Characteristics of Study Population

Depressive symptoms and presence of depression within the study population are summarized in Table 2 and Figure 1. Data are presented as means and standard deviations for continuous variables.
or total number of people and percentage of study population for categorical variables. The modal
total CESD score was 1, with a total score of 0 being the second most common score in the study population.

Table 2  Depression characteristics of all study participants (N=119)

<table>
<thead>
<tr>
<th></th>
<th>Association with log-transformed C18:0</th>
<th>Association with log-transformed C20:0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD or n (%)</td>
<td>F or r value</td>
</tr>
<tr>
<td>Center for Epidemiological Studies Depression Scale (CESD) Score</td>
<td>7.4 ± 7.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Hospital Anxiety and Depression Scale – Depression Subscale (HADS-D) Score</td>
<td>2.6 ± 2.5</td>
<td>0.19</td>
</tr>
<tr>
<td># of Participants with SCID-Diagnosed Depression</td>
<td>18 (15.1)</td>
<td>0.00</td>
</tr>
<tr>
<td>History of Depression</td>
<td>9 (7.6)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*2 participants were missing HADS-D score
3.3. Normalization of Ceramide Concentrations

Plasma ceramide concentrations were log-transformed to normalize distribution. Mean log-transformed C18:0 and C20:0 concentrations were 0.84 ± 0.25 [range = 0.16 – 1.49] and 1.93 ± 0.21 [range = 1.28 – 2.42] respectively. Figures 2 and 3 show changes in distribution of C18:0 and C20:0 concentrations respectively after log-transformation.

Figure 1  Distribution of CESD scores.
Figure 2  Normalization of C18:0 concentration via log-transformation (base of 10)

Figure 3  Normalization of C20:0 concentration via log-transformation (base of 10)
3.4 Addressing the Hypotheses

Bivariate analysis revealed a significant association between the ceramide species C22:1 and total CESD score, as well as trending associations between C20:0 and total CESD score, and C16:0 and total CESD score (Table 3). No other ceramide was significantly associated with total CESD score in bivariate analysis (Table 3).

<table>
<thead>
<tr>
<th>Ceramide</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>0.157</td>
<td>0.088</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.085</td>
<td>0.357</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.162</td>
<td>0.079</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.134</td>
<td>0.148</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.075</td>
<td>0.416</td>
</tr>
<tr>
<td>C26:0</td>
<td>0.000</td>
<td>0.997</td>
</tr>
<tr>
<td>C16:1</td>
<td>-0.034</td>
<td>0.712</td>
</tr>
<tr>
<td>C22:1</td>
<td>0.211</td>
<td>0.021*</td>
</tr>
<tr>
<td>C24:1</td>
<td>0.029</td>
<td>0.753</td>
</tr>
</tbody>
</table>

3.4.1 Primary Hypothesis

In this study sample, higher log-transformed plasma concentration of C18:0 was not associated with greater depressive symptoms, as measured by CESD score (Tables 4 & 5). Higher log-transformed plasma concentration of C20:0 showed a trending association with greater depressive symptoms,
after adjusting for age, gender, BMI, and total cholesterol (Tables 6 & 7). Neither model significantly predicted total CESD score (Tables 4 & 6). Multicollinearity was not present in either model.

Table 4  Summary of multiple linear regression model used to assess the association between log-transformed C18:0 concentration and CESD score.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted R²</th>
<th>F</th>
<th>df</th>
<th>p-value (≤0.025)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>-0.008</td>
<td>0.805</td>
<td>118</td>
<td>0.549</td>
</tr>
</tbody>
</table>

Table 5  Coefficients in the multiple linear regression model used to assess the association between log-transformed C18:0 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>t</th>
<th>p-value (≤0.025)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.599</td>
<td>0.550</td>
<td>0.550</td>
</tr>
<tr>
<td>Log-transformed C18:0</td>
<td>0.108</td>
<td>1.138</td>
<td>0.257</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.095</td>
<td>-1.005</td>
<td>0.317</td>
</tr>
<tr>
<td>Gender</td>
<td>0.034</td>
<td>0.348</td>
<td>0.729</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>0.109</td>
<td>1.150</td>
<td>0.253</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>0.053</td>
<td>0.550</td>
<td>0.583</td>
</tr>
</tbody>
</table>
Table 6  Summary of multiple linear regression model used to assess the association between log-transformed C20:0 concentration and CESD score.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted R²</th>
<th>F</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.008</td>
<td>1.189</td>
<td>118</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Table 7  Coefficients in the multiple linear regression model used to assess the association between log-transformed C20:0 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.348</td>
<td>-0.348</td>
<td>0.729</td>
</tr>
<tr>
<td>Log-transformed C20:0 concentration</td>
<td>0.167</td>
<td>1.781</td>
<td>0.078</td>
</tr>
<tr>
<td>Age</td>
<td>-0.081</td>
<td>-0.879</td>
<td>0.381</td>
</tr>
<tr>
<td>Gender</td>
<td>0.038</td>
<td>0.393</td>
<td>0.695</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>0.113</td>
<td>f1.198</td>
<td>0.234</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>0.015</td>
<td>0.153</td>
<td>0.879</td>
</tr>
</tbody>
</table>
Figure 4  The unadjusted association between log-transformed C20:0 plasma concentration and depressive symptom severity, as measured by the CESD, in 119 CAD patients.

3.4.2 Secondary Hypotheses

Higher log-transformed plasma concentration of C16:0 was significantly associated with greater depressive symptoms, as measured by CESD score, after adjusting for age, gender, BMI, and total cholesterol concentration (Tables 8 & 9). Log-transformed plasma concentration of C24:1 was not significantly associated with depressive symptoms in this study population (Tables 10 & 11). Neither model significantly predicted total CESD score (Tables 8 & 10). Multicollinearity was not present in either model.
Table 8  Summary of multiple linear regression model used to assess the association between log-transformed C16:0 concentration and CESD score.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted R²</th>
<th>F</th>
<th>Df</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.018</td>
<td>1.431</td>
<td>118</td>
<td>0.219</td>
</tr>
</tbody>
</table>

Table 9  Coefficients in the multiple linear regression model used to assess the association between log-transformed C16:0 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>T</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.618</td>
<td>-0.618</td>
<td>0.538</td>
</tr>
<tr>
<td>Log-transformed C16:0 concentration</td>
<td>0.195</td>
<td>2.087</td>
<td>0.039*</td>
</tr>
<tr>
<td>Age</td>
<td>-0.098</td>
<td>-1.059</td>
<td>0.292</td>
</tr>
<tr>
<td>Gender</td>
<td>0.046</td>
<td>0.470</td>
<td>0.639</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>0.127</td>
<td>1.348</td>
<td>0.180</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>0.070</td>
<td>0.727</td>
<td>0.469</td>
</tr>
</tbody>
</table>
Figure 5  The unadjusted association between log-transformed C16:0 plasma concentration and depressive symptom severity, as measured by the CESD, in 119 CAD patients.

Table 10  Summary of multiple linear regression model used to assess the association between log-transformed C24:1 concentration and CESD score.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>F</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>-0.019</td>
<td>0.561</td>
<td>118</td>
<td>0.730</td>
</tr>
</tbody>
</table>
Table 11  Coefficients in the multiple linear regression model used to assess the association between log-transformed C24:1 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>T</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.643</td>
<td>-0.643</td>
<td>0.522</td>
</tr>
<tr>
<td>Log-transformed C24:1 concentration</td>
<td>0.030</td>
<td>0.323</td>
<td>0.747</td>
</tr>
<tr>
<td>Age</td>
<td>-0.076</td>
<td>-0.815</td>
<td>0.417</td>
</tr>
<tr>
<td>Gender</td>
<td>0.032</td>
<td>0.320</td>
<td>0.750</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.110</td>
<td>1.149</td>
<td>0.253</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>0.046</td>
<td>0.469</td>
<td>0.640</td>
</tr>
</tbody>
</table>

There were no differences in mean log-transformed plasma concentrations of C16:0, C18:0, C20:0 and C24:1 between depressed and non-depressed participants in this study (Table 12).

Table 12  Differences in mean log-transformed ceramide concentrations between depressed and non-depressed study participants.

<table>
<thead>
<tr>
<th>Ceramide Species</th>
<th>Depressed participants (n=18)</th>
<th>Non-depressed participants (n=101)</th>
<th>F</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>1.310</td>
<td>1.277</td>
<td>1.208</td>
<td>0.274</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.843</td>
<td>0.840</td>
<td>0.101</td>
<td>0.752</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.955</td>
<td>1.923</td>
<td>0.459</td>
<td>0.499</td>
</tr>
<tr>
<td>C24:1</td>
<td>1.791</td>
<td>1.856</td>
<td>0.220</td>
<td>0.640</td>
</tr>
</tbody>
</table>
3.4.3 Exploratory Hypotheses

Neither log-transformed plasma concentrations of C18:0 nor C20:0 were a significant predictor of depression (Tables 13 & 14). Neither model significantly predicted presence of depression (Tables 13 & 14). Age was also not a significant predictor of depression in either model (Tables 13 & 14).

Table 13 Coefficients of binary logistic regression model used to assess log-transformed plasma concentration of C18:0 as a predictor of depression.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>Standard Error</th>
<th>p-value</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogC18:0</td>
<td>0.333</td>
<td>1.034</td>
<td>0.747</td>
<td>1.396 (0.184 – 10.601)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.055</td>
<td>0.042</td>
<td>0.187</td>
<td>0.947 (0.872 – 1.027)</td>
</tr>
<tr>
<td>Constant</td>
<td>1.448</td>
<td>2.571</td>
<td>0.573</td>
<td>4.254 (NA)</td>
</tr>
</tbody>
</table>

Note: Model $\chi^2 = 1.776$, $p = 0.412$; $R^2 = 0.015$ (Cox & Snell), 0.026 (Nagelkerke)

Table 14 Coefficients of binary logistic regression model used to assess log-transformed plasma concentration of C20:0 as a predictor of depression.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>Standard Error</th>
<th>p-value</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogC20:0</td>
<td>0.898</td>
<td>1.289</td>
<td>0.486</td>
<td>2.455 (0.196 – 30.685)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.054</td>
<td>0.041</td>
<td>0.186</td>
<td>0.947 (0.874 – 1.026)</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.048</td>
<td>3.425</td>
<td>0.989</td>
<td>0.953 (NA)</td>
</tr>
</tbody>
</table>

Note: Model $\chi^2 = 2.163$, $p = 0.339$; $R^2 = 0.018$ (Cox & Snell), 0.031 (Nagelkerke)

In this study we measured concentrations of 9 different ceramide species, 10 sphingomyelins, 4 dihydro sphingomyelins, 10 monohexylceramides, and an additional 10 other sphingolipid species (43 species in total). In exploratory analyses, higher log-transformed C22:1 concentration was significantly associated with greater depressive symptoms, after adjusting for all covariates (Tables 15 and 16). Multicollinearity was not present in any model used in the exploratory analyses.
### Table 15  Summary of multiple linear regression model used to assess the association between log-transformed C22:1 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Adjusted $R^2$</th>
<th>$F$</th>
<th>df</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.018</td>
<td>1.443</td>
<td>118</td>
<td>0.214</td>
</tr>
</tbody>
</table>

### Table 16  Coefficients in the multiple linear regression model used to assess the association between log-transformed C22:1 concentration and CESD score.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>t</th>
<th>p-value (≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>0.412</td>
<td>0.681</td>
</tr>
<tr>
<td>Log-transformed C22:1</td>
<td>0.199</td>
<td>2.100</td>
<td>0.038</td>
</tr>
<tr>
<td>Log-transformed C22:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.087</td>
<td>-0.949</td>
<td>0.345</td>
</tr>
<tr>
<td>Gender</td>
<td>0.025</td>
<td>0.260</td>
<td>0.795</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.077</td>
<td>0.802</td>
<td>0.424</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>0.009</td>
<td>0.089</td>
<td>0.930</td>
</tr>
</tbody>
</table>
Monohexylceramide 22:1 (MHxC22:1) was also implicated in depression in this study. Although not a significant predictor in any statistical model used in this analyses, MHxC22:1 showed a trend in both regression models, and was the only species to show a trend for a difference in mean log-transformed concentration between depressed and non-depressed study participants. Mean log-transformed MHxC22:1 concentration was higher in depressed than non-depressed participants (1.029 vs 0.907 respectively). Results for MHxC22:1 in all statistical models are shown in Table 17.
Table 17  Results of all models containing log-transformed MHxC22:1 concentration

<table>
<thead>
<tr>
<th>Model</th>
<th>β, B, or F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Linear Regression</td>
<td>β = 0.169</td>
<td>0.080</td>
</tr>
<tr>
<td>Binary Logistic Regression</td>
<td>B = 1.769</td>
<td>0.079</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>F = 3.128</td>
<td>0.080</td>
</tr>
</tbody>
</table>

No other species was significantly associated with depressive symptoms nor found to be a significant predictor of depression in this study. No other species was found to be significantly elevated, or significantly lowered in depressed compared to non-depressed participants.

3.5 Post Hoc Analyses

In bivariate correlational analysis or one-way ANOVA, total years of education and history of smoking were found to be significantly associated with log-transformed C20:0 concentration (Table 1). These two variables were added to the multiple linear regression model used in section 3.4.1 to assess whether higher log-transformed C20:0 plasma concentration is associated with greater depressive symptoms. Addition of these two covariates eliminated the trending association found between higher log-transformed C20:0 concentration and CESD score (β = 0.151, p =0.127). Neither additional covariate was a significant predictor of CESD score in the new model (total years of education: β = -0.014, p = 0.947; history of smoking: β = 0.076, p = 0.440).

Bivariate correlational analysis revealed that annual income was associated with log-transformed C16:0 concentration in this study sample. Thus, annual income was added as a covariate to the
multiple linear regression model used to assess whether higher log-transformed C16:0 concentration is associated with greater depressive symptoms. After adjusting for annual income, higher log-transformed C16:0 was no longer significantly associated with higher CESD score (β = 0.189, p = 0.057); however, a trending association was still present. Annual income was not a significant predictor of CESD score (β = -0.042, p = 0.668) in this model.

In bivariate correlational analysis or ANOVA, total years of education, waist circumference, HbA1c, AES score, sMMSE score, and presence of diabetes were significantly associated with log-transformed C22:1 concentration. These variables were added to the multiple linear regression model used to assess whether higher log-transformed C22:1 concentration is associated with greater depressive symptoms. Addition of these covariates eliminated the significant association between higher log-transformed C22:1 concentration and CESD score (β = 0.089, p = 0.341). Of the additional covariates added to the model, only AES score was a significant predictor of CESD score (β = 0.555, p = 0.000).
4 Discussion and Conclusions

4.1 Summary of Findings

To our knowledge, the present study is the first to investigate the association between ceramides and depressive symptoms in a CAD population. Furthermore, it is the first study to investigate differences in ceramide concentrations between depressed and non-depressed CAD patients. Ceramides and other sphingolipid species have been implicated in the pathophysiology of both CAD and depression (Bismuth et al. 2008, Demirkan et al. 2013, Muller et al. 2015, Pan et al. 2014, Schissel et al. 1996). This, combined with the high prevalence of depression in CAD (3-4 times greater than in the general population) (Rudisch and Nemeroff 2003), may make CAD a good environment to evaluate the potential role of ceramides in depression. Based on a previous clinical study that found elevated ceramides in depressed compared to non-depressed individuals (Gracia-Garcia et al. 2011), it was hypothesized that higher concentrations of C18:0 and C20:0 would be most strongly associated with depressive symptoms in individuals with CAD. These two species were chosen for the primary hypothesis because they were found to have the greatest effect sizes when differences in concentrations of ceramides between depressed and non-depressed individuals were assessed by Gracia-Garcia et al. (Gracia-Garcia et al. 2011). In this study, we report that C18:0 was not associated with depressive symptoms in this study sample and that higher C20:0 concentration showed a trending association with greater depressive symptoms. Neither species was significantly elevated in depressed individuals in this study sample.

In addition to C18:0 and C20:0, the previous clinical study on ceramides and depression found significantly elevated plasma concentration of the ceramide species C16:0 in depressed participants, and an elevation of C24:1 (Gracia-Garcia et al. 2011). In this study, C16:0 was found to be
significantly associated with depressive symptoms, but C24:1 was not. Neither species was found to be significantly elevated in depressed individuals nor a significant predictor of depression.

In exploratory analyses, 39 other sphingolipid species, including 5 other ceramides, sphingomyelins, and monohexylceramides, were assessed as correlates of depressive symptoms. Aberrant sphingolipid metabolism has been postulated to be a mechanism of the pathophysiology of both CAD and depression (Jernigan et al. 2015, Kinnunen and Holopainen 2002, Muller et al. 2015, Pan et al. 2014, Schissel et al. 1996), thus evaluation of a multitude of sphingolipid species allows for the greater possibility of detecting an abnormality in sphingolipid metabolism. In this study we found that higher C22:1 concentration was associated with greater depressive symptoms. Monohexylceramide 22:1 (MHxC22:1) was also implicated in depression in this study. A trending association was found between higher MHxC22:1 concentration and greater depressive symptoms, as well as MHxC22:1 as a predictor of depression in binary logistic regression analysis. In addition, a trend was observed for increased MHxC22:1 in depressed compared to non-depressed study participants. Aside from MHxC22:1, no species in this study predicted presence of depression in binary logistic regression analysis, nor was significantly elevated in depressed participants. This study provides evidence for a potential role of C16:0, C20:0, C22:1, and MHxC22:1 in the pathophysiology of depression in CAD.

4.2 Interpretation of Results

Although C18:0 was not found to be associated with depressive symptoms in this study, more evidence is needed to rule out the possibility of its contribution to depression in the context of CAD. On the other hand, C20:0 showed more promising results for a role in depression in those with CAD. Although it did not predict presence of depression, a trending association was observed between
C20:0 concentration and depressive symptoms in this study sample. Neither species was found to be significantly elevated in depressed participants; however, this result may have been influenced by the small number of depressed participants in the study sample. An exclusion criterion in this study was the current use of antidepressants, as some but not all antidepressants can lower ceramide concentrations. Thus to standardize the study population, those taking antidepressants were excluded. This exclusion criterion made it harder to recruit depressed participants. Perhaps a greater prevalence of depressed study participants would have altered the results of this study. Although we did not find strong evidence for a role of these two ceramide species in depression in those with CAD, more research is needed to determine whether or not these species contribute to the pathophysiology of depression in CAD and/or depression in those without CAD.

In this study, we explored the association between 7 other ceramide species and depressive symptoms and depression. Of these 7 species, two were found to be significantly associated with depressive symptoms, C16:0 and C22:1. C16:0 has been implicated in apoptosis (Cremesti et al. 2001, Herget et al. 2000, Thomas et al. 1999). Concentrations of C16:0 were found to be elevated in radiation and Fas-induced apoptotic cells (Thomas et al. 1999). Application of C16:0 to a mouse embryonic carcinoma cell line was observed to induce apoptosis in these cells (Herget et al. 2000). It is important to note however that results from an animal carcinoma cell line may not translate to healthy human cells. Furthermore, C16:0 has been shown to be required for the initiation of Fas-induced apoptosis (Cremesti et al. 2001, Grassme et al. 2001). With regards to C22:1, little is known about its biological function. These results are promising for a potential role of C16:0 and C22:1 in depression in CAD, but it cannot be concluded that these species are involved in the pathophysiology of depression from this study. As discussed, multiple ceramides and other sphingolipid species were assessed in this study and as a result we must be wary of false discoveries.
when assessing multiple associations. More evidence exists for a role of C16:0 in depression, as C16:0 has been shown to be pro-apoptotic and thus may contribute to neurodegeneration in depression. More evidence is needed to determine whether these and other ceramides are involved in the pathophysiology of depression.

With respect to the other sphingolipid species assessed in this study, the same conclusions can be made. Although MHxC22:1 was implicated in depression in this study, again we must be wary of multiple comparisons. MHxC22:1 was the only species assessed in this study that was found to be significantly elevated in depressed participants, although only a trending association was observed. MHxC22:1 is rarely mentioned in the sphingolipid literature, thus little is known about its function. It is closely related to C22:1, being the same molecule as C22:1 but with the addition of a sugar molecule. As C22:1 was also found to be associated with depressive symptoms in this study, this suggests that the chain length and presence of the double bond in the fatty acid are responsible for the biological action of these two species. These results suggest MHxC22:1 as one of the more promising targets for future studies of the potential role of ceramides in the pathophysiology of depression.

In this study, our results differed from those of Gracia-Garcia et al. who found significantly elevated plasma concentrations of the ceramide species C16:0, C18:0, and C20:0 in depressed participants compared to non-depressed participants (Gracia-Garcia et al. 2011). In this study, none of those ceramides were found to be significantly elevated in the plasma of depressed compared to non-depressed individuals with CAD. These negative results in this study may have been influenced by a lack of depressed participants and/or the difference in size between the subgroups of depressed and non-depressed participants. The size of the non-depressed subgroup in this study was
approximately 5 times greater than the depressed subgroup; however, this prevalence of depression is representative of the general CAD population. Furthermore, the presence of CAD in all study participants may explain why our results differed from those of Gracia-Garcia et al. It is possible that AD and CAD have different effects on ceramide metabolism, offering an explanation to why our results differed. Since ceramides have been suggested to play a role in the pathophysiology of CAD, perhaps individuals with CAD have elevated plasma ceramides compared to healthy controls making the distinction in ceramide concentrations between depressed and non-depressed individuals difficult to observe in this population. Another possible explanation for the difference in results between the two studies is that concomitant neurological pathologies, such as depression and AD, may represent an enhanced neurodegenerative state. Thus, significant elevation of ceramides may only be observed in advanced neurodegenerative states such as those individuals with depression and comorbid neuropathological abnormalities (Filippov et al. 2012).

Moreover, Gracia-Garcia et al. classified depression as any individual diagnosed with depression in the past two years or currently diagnosed with depression, whereas this study classified depression as only individuals currently diagnosed with depression. Inclusion of those with a recent history of depression but not current depression in the depressed group may have significantly impacted mean ceramide concentration in this group if past depression led to elevated ceramide concentrations that remained high post-depression. If increased ceramide concentrations are reactionary to depressed mood, those with current but not past depression may not have had sufficient time for ceramide concentrations to increase. Longitudinal analyses of changes in ceramide concentrations and mood would be valuable to assess the temporal relationship between ceramides and depression as well as to better define the causality of this relationship. The differing results between this study and the Gracia-Garcia study may be due to small sample sizes,
differences in methodology, or may indicate a distinction between depression in the context of AD or in an elderly population and depression in the context of CAD.

4.3 Limitations and Recommendations for Future Studies

Perhaps the greatest limitation of the present study is a lack of depressed participants recruited. As mentioned, current use of antidepressants was an exclusion criterion for this study as some but not all antidepressants can alter ceramide concentrations, and this criterion was implemented to standardize the study population. In addition, we did not set goals to recruit a certain amount of depressed participants, rather we recruited consecutive CAD patients from TRI regardless of their depression status. However, the prevalence of depression and depressive symptoms in the study population we recruited were representative of the general CAD population. Future clinical studies assessing the relationship between ceramides and depression should aim to recruit a target number of depressed participants in order to have a greater chance of observing an association between ceramides and depression.

Conversely, the presence of depressive symptoms and subthreshold depression that was found in this study population may have offered a good environment to study the relationship between ceramides and depression. Depressive symptoms and subthreshold depression in CAD have been shown to be clinically important, as they may predict mortality (Barefoot et al. 2000) and lack of adherence to treatment (Rieckmann et al. 2006). Thus, a relationship between ceramides and depressive symptoms in CAD is clinically important and would suggest a role for ceramides in the pathophysiology of MDD.
Another limitation of the present study is that depression and depressive symptoms are complex psychological phenomena that are often dynamic and difficult to measure. The CESD only assesses depressive symptoms over the past week of time and is thus sensitive to abnormally stressful weeks and events that might alter mood acutely but not chronically. This may result in scores that do not reflect the average depressive symptoms an individual has been experiencing over the past month, year, or beyond. If in fact ceramides are associated with depressive symptoms, this limitation of the CESD would not pose a problem if ceramide concentrations and mood are responsive to one another, and thus mirror changes in the other relatively rapidly.

Furthermore, the CESD is a subjective, patient-rated scale and thus may suffer from measurement error due to inaccurate responses from study participants. Use of an objective, observer-rated scale, such as the Hamilton Depression Rating Scale (HAMD) (Hamilton 1960), may have resulted in increased accuracy of depressive symptom measurement and thus better reflect depressive symptom presence and severity in study participants. The SCID is observer-rated and assesses for presence of depression over the past month and thus results in a more accurate classification of depression, but still suffers from a relatively small temporal timeline that may not reflect an individual’s mood over the past year or beyond. To address this potential limitation, previous studies have categorized depression as any individual diagnosed with depression in the past year (Danese et al. 2008) or two (Gracia-Garcia et al. 2011) along with all individuals currently diagnosed with depression in the study. The CESD is a commonly used tool for the measurement of depressive symptoms (Crichton et al. 2016, Swardfager et al. 2008) but no depression rating tool is perfect and we must take into consideration that our measurements of depressive symptoms are not perfectly reflective of the real depressive symptoms each participant is experiencing. Thus, measurement error may have contributed to the results of this study.
Use of statin medications by all study participants may have obscured the relationship between ceramides, depressive symptoms, and depression in this study. One study showed that pravastatin attenuated ceramide induced cytotoxicity in mouse cerebral endothelial cells, possibly via an upregulation of vascular endothelial growth factor (VEGF) expression (Chen et al. 2005). Thus, the possibility exists that statins may block potential depressogenic effects of ceramides. In our study, statin dose was only associated with plasma concentrations of two ceramide species (C16:0 and C26:0) and was not associated with total CESD score. Moreover, most CAD patients are on a statin medication (Swardfager et al. 2010), thus exclusion of statin use would hinder study recruitment greatly.

Furthermore, as this study is cross-sectional and observational we cannot infer any causal relationships between the ceramides found to be implicated in depression in this study. Longitudinal studies are warranted to determine if changes in ceramide concentrations reflect changes in depressive symptoms, and if an increase in ceramide concentrations is associated with incidence of depression and/or depressive symptoms. In addition, interventional studies in animals would provide strong evidence that ceramides can cause depressive symptoms and depression. Studies in which different ceramide species are injected into different brain regions of rats or other animals could help determine if ceramides contribute to the etiology and pathophysiology of depression and in particular which ceramide species contribute the greatest to depression.

Further studies in animals can be done to determine if plasma concentration of ceramides reflects brain concentrations. Another limitation of the present study is that we are assessing plasma ceramides under the assumption that plasma ceramide concentrations reflect brain concentrations;
however, in reality this may not be the case. One study in rats observed increases in both plasma and brain cortex concentrations of ceramides after injection with lipopolysaccharide (LPS), and showed that the ceramide species C6 was able to cross the rat blood-brain barrier (BBB) (Zimmermann et al. 2001). However, evidence that plasma and brain concentrations of ceramides are associated, and that ceramides can cross the human BBB is scarce. Theoretically, ceramides should be able to cross the human BBB, as being lipids they are lipid-soluble (Banks 2009). Although ceramides in plasma may contribute to the pathophysiology of depression, past research and biological plausibility suggest that if ceramides play a role in depression, they exert their depressogenic effects in the brain rather than in the periphery (Kornhuber et al. 2014, Muller et al. 2015). Unfortunately, it is not feasible to extract brain tissue from living humans, but future post-mortem studies should be undertaken to assess brain concentrations of ceramides in depressed individuals compared to those who were free of depression.

In any lipidomics study there is a possibility for error in the measurement of species. Errors in ceramide and other sphingolipid measurements in this study could have arisen from errors in measurement technology or errors in the handling of specimen.

Finally, there is a limitation to the statistical analyses performed in this study, as the CESD is not the ideal dependent variable for linear regression analysis. Firstly, CESD scores are highly positively skewed, with frequent total scores of 0 and 1 present in this study population (Figure 1). Additionally, CESD score is not a truly continuous variable, rather it is a scalar variable. These two conditions pose challenges for the use of CESD as a dependent variable in a linear regression model; however, we believe that linear regression analysis is robust enough to overcome these characteristics of the CESD.
4.4 Conclusion

From the results of this study alone, we cannot conclude that ceramides do or do not play a role in the pathophysiology of depression in CAD or in individuals without CAD. Evidence that C16:0 and C22:1 plasma concentrations are associated with depressive symptoms in those with CAD was found, but this must be replicated in other samples to be taken as strong evidence that these ceramides are associated with depression. C20:0 and MHxC22:1 were also implicated in depression in this study. It is promising that two ceramide species that were implicated in depression in the study by Gracia-Garcia et al. were found to be associated with depressive symptoms in this study; however, we did not find evidence for an elevation of these species in depressed individuals similar to that which was found in the Gracia-Garcia study. Further clinical and pre-clinical research on the potential role of ceramides in depression is warranted.

If ceramides are found to be implicated in depression, novel pharmacotherapies aimed at reducing ceramide concentrations may be an effective strategy in treating depression and depressive symptoms in those with or without CAD. Already, candidate drugs that reduce ceramide concentrations exist (Cinar et al. 2014, Kornhuber et al. 2010, Miyake et al. 1995). Myriocin, an antibiotic derived from certain fungi, has been shown to be a potent inhibitor of serine palmitoyltransferase (SPT), an important enzyme in the de novo synthesis of ceramides (Miyake et al. 1995). Myriocin has been shown to induce the regression of atherosclerotic lesions in mice (Hojjati et al. 2005, Park et al. 2008), but its effects on depression and depressive symptoms have not been investigated. JD5037, a peripherally restricted inverse agonist of the cannabinoid receptor CB1, attenuated diet-induced increases in ceramide concentrations in mice with high-fat diet-induced obesity (Cinar et al. 2014). This effect of JD5037 on ceramide concentrations occurred via inhibition of SPT, as well as reduced expression of ceramide synthases, enzymes responsible for
ceramide production, and increased expression of ceramidases (Cinar et al. 2014), enzymes responsible for ceramide degradation. Finally, a term for functional inhibitors of acidic sphingomyelinas, FIASMAs, created by Kornhuber et al., reflects drugs that reduce ceramide concentrations via inhibition of aSMase (Kornhuber et al. 2010). Many FIASMAs have other primary pharmacological targets aside from aSMase and are approved for use in humans to treat various diseases (Kornhuber et al. 2010). Importantly, these approved FIASMAs are minimally toxic and therefore have the potential to be used in humans to treat depression (Kornhuber et al. 2010). Indeed, treatment of CMS mice with fendiline, a calcium channel blocker that is also a FIASMA, was shown to reduce ceramide concentrations and depressive behaviors in these mice (Gulbins et al. 2013).

There is a wealth of potential pharmacological targets for ceramide reduction, including SPT, SMases, ceramide synthases and ceramidases (Cinar et al. 2014, Kornhuber et al. 2010). Novel pharmacotherapies for the treatment of depression and depressive symptoms may result in improved patient outcomes and improved health-related quality of life for those who suffer from treatment-resistant depression or depressive symptoms with or without CAD. Furthermore, ceramide concentrations may one day be useful biomarkers for depression, guiding more successful diagnoses and therapies for depressed patients.
References


Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology 2015.

Banks WA. Characteristics of compounds that cross the blood-brain barrier. BMC Neurol 2009; 9 Suppl 1: S3.


Molloy DW, Standish TI. A guide to the standardized Mini-Mental State Examination. Int Psychogeriatr 1997; 9 Suppl 1: 87-94; discussion 143-50.


Public Health Agency of Canada C. Tracking Heart Disease and Stroke in Canada. 2009.


List of Publications and Abstracts

Meeting/Conference Abstracts


**Dinoff A, Saleem M, Herrmann N, Khan M, Haughey N, Oh PI, Mielke M, Lanctôt KL (2016)** Evaluating the Relationship between Ceramides and Depressive Symptoms in Coronary Artery Disease (CAD) Patients. *Visions in Pharmacology 2016.* Department of Pharmacology & Toxicology, University of Toronto (Toronto, ON).

List of Awards and Sources of Funding

**Scholarships**

2014/2015 – University of Toronto Fellowship

2015/2016 – University of Toronto Fellowship

**Travel Awards**

2016 – SGS Conference Grant
Appendices

Appendix A: Research Ethics Board Approval
To: Dr. Krista Lanciot
Psychiatry
Room FG05

From: Dr. Philip Hebert

Date: November 11, 2011

Subject: The Heart-Mind Connection: Evaluating the Association between Ceramides and Cognitive Decline in Coronary Artery Disease

Project Identification Number: 279-2011
Approval Date: November 11, 2011
Expiry Date: November 11, 2012

The Research Ethics Board of Sunnybrook Health Sciences Centre has conducted a Delegated Board review of the research protocol referenced above and approved the involvement of human subjects on the above captioned date. The quorum for approval did not involve any member associated with this project.

The approval of this study includes the following documents:

- Protocol dated August 15, 2011
- Informed Consent Form dated November 3, 2011
- The following scales/tools received October 18, 2011
  - CES-D
  - STAI-S
  - STAI-T
  - SCID
  - CVLT-II Standard Form
  - BVMT-R
  - Digit Symbol – Coding
  - Stroop Neuropsychological Screening Test (Victoria)
  - Trails Making Test – Part A and B
  - CVLT-II Delayed Recall Trials
  - BVMT-R Delayed Recall Trials
  - FAS and Animal Naming Test
  - Word Fluency
  - AES-C
  - SRRS
  - Pittsburgh Sleep Quality Index

The Research Ethics Board of Sunnybrook Health Sciences Centre operates in compliance with the Tri-Council Policy Statement 2nd edition, ICH GCP Guidelines, Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Products Regulations, and Part 3 of the Medical Devices Regulations. All Health Canada regulated trials at Sunnybrook are conducted by a Qualified Investigator.

Fully affiliated with the University of Toronto
All correspondence with the REB must include the assigned Project Identification Number. The REB requires immediate notification of all internal serious adverse events and significant deviations. Study continuation beyond one year requires submission of a renewal form prior to the expiry date or a study completion report must be received to close the file with the REB.

All REB approved studies may be subject to review by the Sunnybrook Quality Assurance and Education Program and, as Principal Investigator, you are responsible for the ethical conduct of this study. Approval by the Sunnybrook REB entails compliance with current legislation outlined in the Ontario Personal Health Information Protection Act (PHIPA) and all policies and guidelines established by Sunnybrook. All applicable contracts and agreements must be submitted to Sunnybrook Legal Services before this research may be initiated.

Philip C. Hébert, MD PhD FCFPC
Chair, Research Ethics Board

OR

Miriam Shuchman, MD
Vice-Chair, Research Ethics Board
January 30, 2012

Dr. Krista Lancetot
Sunnybrook Health Sciences Centre
Psychopharmacology/Psychiatry
2075 Bayview Avenue
North York, Ontario
M4N 3M5

Dear Dr. Lancetot:

RE: TRI REB #: 11-058
The Heart-Mind Connection: Evaluating the Association between Ceramides and Cognitive Decline in Coronary Artery Disease

The Toronto Rehabilitation Institute Research Ethics Board has reviewed the above-named submission. Any concerns and requested revisions have been addressed to the satisfaction of the REB. The protocol version 1.1, dated January 2012 is approved for use for the next 12 months. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

Also approved are the following documents:
- Information and Consent Form version 1.2, dated January 23, 2012
- Budget received December 2, 2011
- Acknowledgement of receipt of reimbursement for participation in study, received December 2, 2011
- Data Collection Forms received December 2, 2011
  - Mini Mental State Examination (MMSE)
  - Montreal Cognitive Assessment (MOCA)
  - Digit Symbol – Coding
  - Trails Making Test, Part A and B
  - Brief Visuospatial Memory Test – Revised (BCMT-R)
  - Brief Visuospatial Memory Test – Revised (BCMT-R) Delayed Recalls Trials
  - Centre for Epidemiological Studies – Depression Scale (CES-D)
  - State – Trait Anxiety Inventory – State (STAI-S)
  - State – Trait Anxiety Inventory – Trait (STAI-T)
  - Social Readjustment Rating Scale (SRRS)
  - Pittsburgh Sleep Quality Index
  - Structured Clinical Interview For the DSM – IV – Depression Module (SCID)
  - California Verbal Learning Test II (CVLT-II) – Standard Form
  - California Verbal Learning Test II (CVLT-II) – Delayed Recalls Trials
  - Stroop Neuropsychological Screening Test (Victoria)

TRI REB conforms with the Tri-Council Policy Statement (TCPS2): Ethical Conduct for Research Involving Humans and Ontario Privacy Legislation PIPPA

Toronto Rehab is a teaching and research hospital fully affiliated with the University of Toronto.
Page 2
January 30, 2012
Dr. Krista Lancot
TRI REB #: 11-058

- FAS and Animal Naming Test
- Word Fluency
- Apathy Evaluation Scale – Self
- Daily Food Diary

If, during the course of the research, there are any serious adverse events, changes in the approved protocol or consent form or any new information that must be considered with respect to the study, these should be brought to the immediate attention of the Board.

Best wishes for the successful completion of your project.

Yours sincerely,

[ ] Paul Oh MD, MSc, FRCPC, FACP
Chair, Research Ethics Board
Toronto Rehabilitation Institute

[ ] Ann Heesters BEd, BA, MA, PhD (ABD)
Vice Chair, Research Ethics Board
Toronto Rehabilitation Institute

January 30, 2012
Date of Initial REB Approval

January 30, 2013
Expiry Date of REB Approval

TRI REB conforms with the Tri-Council Policy Statement (TCPS2): Ethical Conduct for Research Involving Humans and Ontario Privacy Legislation PIIPA
Appendix B: Informed Consent Form
Supported by a grant from the Canadian Institute of Health Research

The Heart-Mind Connection: Evaluating the Association between Ceramides and Cognitive Decline in Coronary Artery Disease

Subject Information and Consent

INFORMED CONSENT:

You are being invited to participate in a research study conducted at the Toronto Rehabilitation Institute and Sunnybrook Health Sciences Centre under the supervision of the above investigators. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood. Participation is completely voluntary and you are free to withdraw from the study at any time. A description of this study follows.

This form explains the purpose of this research study, provides information about the study procedures, possible risks and benefits, and the rights of participants. Please read this form carefully and ask any questions you may have. Please ask the study staff or one of the investigators to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

INTRODUCTION

You are being asked to consider participating in this study because you have coronary artery disease (CAD) and because you are taking part in the Toronto Rehabilitation Institute’s Cardiac Rehabilitation Program. As a greater proportion of Canadians reach older ages, there is a need to maintain cognitive function (memory and learning ability) later in life. The knowledge from this study will help us to better understand memory decline in patients with coronary artery disease.
WHY IS THIS STUDY BEING DONE?
The purpose of this study is to investigate how certain substances in the blood can affect thinking. It has recently been discovered that certain byproducts of fat breakdown involved in the development of CAD, called ceramides, can harm brain cells. This study is being conducted to determine if there is a relationship between the levels of ceramides in the blood and memory decline. In addition, relationships between ceramides and other aspects of brain function, such as thinking speed and the ability to plan and sort information will be explored.

WHAT WILL HAPPEN DURING THIS STUDY?
If you choose to participate in this study, we will notify your TRI physician and your TRI-Cardiac rehab team of your involvement. This study will not interfere with any of the usual care received in rehab or from your family physician.

Baseline Visit:
If you agree to participate in this study, we would ask to review information that you have provided to the rehab team including demographic data (age, gender and diagnoses), what medications you are using, and the results of your exercise tests in the past year. If you agree to participate, you will be asked to undergo an assessment with a trained researcher that will take about 2 hours. This will include assessments of memory and thinking speed, and a screening interview for depression or substance abuse. We are assessing depressive symptoms as it is not uncommon for CAD rehab patients to show signs of depression. You will be asked to complete a few simple depression questionnaires assessing your mood and anxiety. For the cognitive scales you will be asked to complete a few verbal and visual tasks and reproduce a few simple shapes on paper. With your permission, we would notify your Toronto Rehabilitation team if the results of this interview suggest you might benefit from the resources that are already in place to assist subjects showing signs of depression or cognitive impairment. These resources include the opportunity to make appointments with a psychologist on staff at the Toronto Rehab. At this baseline visit approximately 2½ tablespoons of blood will be drawn.

If the results from the interview or blood sample show clinical abnormalities, with your permission, we will contact your physician at TRI.

Visit 2 (3 months) and Visit 3 (6 months):
After the initial baseline visit, you would return for 2 in-clinic visits, each lasting approximately 2 hours. Visits 2 and 3 will take place 3 months and 6 months after your initial visit. At each visit you will be asked to complete a number of paper and pencil assessment questionnaires. If you choose to participate in this research study, it will be necessary to collect some fasting blood samples for analysis. At visits 2 and 3 another approximately 2½ tablespoons of blood will be drawn. All blood samples will be identified by a unique number only (not your name). All samples will be analyzed for only these markers needed for the study and then destroyed once the assay is complete.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
It is anticipated that about 129 people recruited from the Toronto Rehabilitation Institute will participate in the study conducted with Sunnybrook Health Sciences Centre. The length of this study for participants is 6 months. The entire study is expected to take about 3 years to complete and the results should be known in 3½ years.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
If you decide to participate in this study you will be asked to do the following:

Attend 3 visits at Sunnybrook Health Sciences Centre (2075 Bayview Avenue, Room EG04). Each visit will last approximately 2 hours. You will be asked to complete a number of questionnaires, as well as give a blood sample at each visit.
WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?
There are no medical risks to you from participating in this study, as this is an observational study and does not involve a medical intervention but taking part in this study may make you feel uncomfortable.

Blood draw: As with any blood test, you may experience slight discomfort or bruising.

Cognitive testing: You may experience mental stress as a result of memory or timed tasks.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?
You may or may not benefit directly from participation in this study. Your participation may or may not help other people with coronary artery disease in the future. Knowledge gained from this study may be helpful to subjects in the future in the management of depressive symptoms or cognitive changes resulting from heart disease. As mentioned, the results may suggest that you would benefit from existing Toronto Rehabilitation Institute resources. The study results will be published, and if you wish, we will be happy to forward to you a copy of any publication(s) that may arise from this work.

CAN PARTICIPATION IN THIS STUDY END EARLY?
You can choose to end your participation at any time. If you withdraw voluntarily from the study, the information about you that was collected before you left the study will still be used. No new information about you will be collected without your permission.

WHAT ARE THE COSTS OF PARTICIPATING IN THIS STUDY?
Participation in this study will not involve any additional costs to you.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?
You will not be paid to participate in this study. However you will be reimbursed $23.00 for parking expenses each time you visit Sunnybrook for the purposes of this study.

DO THE INVESTIGATORS HAVE ANY CONFLICTS OF INTEREST?
There are no conflicts of interest to declare related to this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?
All participants in a research study have the following rights:

1. You have the right to have this form and all information concerning this study explained to you and if you wish translated into your preferred language.

2. Participating in this study is your choice (voluntary). You have the right to choose not to participate, or to stop participating in this study at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future care at the Toronto Rehabilitation Institute.

3. You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study. If you have any questions about this study you may contact the person in charge of this study (Principal Investigator) Dr. Lanctôt, Department of Psychiatry at 416-480 6100 x2241. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Paul Oh, Chair of the Toronto Rehabilitation Institute Research Ethics Board at (416) 597-3422 x 5263.

4. You have the right to have any information about you and your health that is collected, used or disclosed for this research study to be handled in a confidential manner.

If you decide to participate in this study, the investigator(s) and study staff will look at your personal health information and collect only the information they need for this study. “Personal health
information” is health information about you that could identify you because it includes information such as your;

- name,
- address,
- telephone number,
- date of birth,
- new and existing medical records, or
- the types, dates and results of various tests and procedures.

The following people may come to the hospital to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

Representatives of the TRI Research Ethics Board, a group of people who oversee the ethical conduct of research studies at TRI.

Access to your personal health information will take place under the supervision of the Principal Investigator. In addition, any study data about you that is sent outside of the hospital will have a code and will not contain your name or address, or any information that directly identifies you. “Study data” is information about you that is collected for the research study, but that does not directly identify you. Study data that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator(s), study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be completely eliminated.

When the results of this study are published, your identity will not be disclosed. The Principal Investigator will keep any personal information about you in a secure and confidential location for at least 5 years and then destroyed as required by Sunnybrook policy.

5. By signing this consent form, you do not give up any of your legal rights.

6. You have the right to receive a copy of this signed and dated informed consent form before participating in this study. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff.

7. You have the right to access, review and request changes to your personal health information.

8. You have the right to be informed of the results of this study once the entire study is complete.

Contacts:

If you have any questions about this study or for more information you may contact the Study Coordinator (416-480-6100 x3185), Dr. Krista Lanctôt (416-480-6100 x2241) or Dr. Paul Oh (416-597-3422 x5263).

If you have questions about your rights as a research participant, or about any ethical issues relating to this study, you can contact the Vice Chair of the UHN Rehabilitation Medicine and Sciences Research Ethics Board at (416) 597-3422 x3081 or the Sunnybrook Health Sciences Centre Research Ethics Board at (416) 480-4276.
DOCUMENTATION OF INFORMED CONSENT

Full Study Title: The Heart-Mind Connection: Evaluating the Association between Ceramides and Cognitive Decline in Coronary Artery Disease

Name of Participant: ________________________________________

Participant/Substitute decision-maker
By signing this form, I confirm that:
• This research study has been fully explained to me and all of my questions answered to my satisfaction
• I understand the requirements of participating in this research study
• I have been informed of the risks and benefits, if any, of participating in this research study
• I have been informed of any alternatives to participating in this research study
• I have been informed of the rights of research participants
• I have read each page of this form
• I authorize access to my personal health information, medical record and research study data as explained in this form
• I have agreed to participate in this study or agree to allow the person I am responsible for to participate in this study

_______________________ ___________ ________      ___________________
Name of participant/Substitute decision-maker (print)        Signature        Date

Person obtaining consent
By signing this form, I confirm that:
• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

_______________________ ___________ ________    ___________________
Name of Person obtaining consent (print)                             Signature                             Date

Statement of Investigator
I acknowledge my responsibility for the care and well-being of the above participant, to respect the rights and wishes of the participant as described in this informed consent document, and to conduct this study according to all applicable laws, regulations and guidelines relating to the ethical and legal conduct of research.

_______________________ ___________ ________
Name of Investigator (print)          Signature                   Date
Appendix C: Center for Epidemiological Studies - Depression Scale (CESD)
CENTRE FOR EPIDEMIOLOGICAL STUDIES-DEPRESSION SCALE (CES-D)

Instructions:
This questionnaire contains 20 statements. Please read each item carefully and circle the one answer that best describes how many days you felt or behaved this way during the past week.

Less than 1 Day (Rarely or Never)                                    1-2 Days (Some or Little of the Time)
3-4 Days (Occasionally or a Moderate amount of Time)                  5-7 Days (Most or All of the Time)

1. I was bothered by things that usually don’t bother me  |  <1 | 1-2 | 3-4 | 5-7
2. I did not feel like eating; my appetite was poor |  <1 | 1-2 | 3-4 | 5-7
3. I felt that I could not shake off the blues even with help from my family or friends |  <1 | 1-2 | 3-4 | 5-7
4. I felt that I was just as good as other people |  <1 | 1-2 | 3-4 | 5-7
5. I had trouble keeping my mind on what I was doing |  <1 | 1-2 | 3-4 | 5-7
6. I felt depressed |  <1 | 1-2 | 3-4 | 5-7
7. I felt that everything I did was an effort |  <1 | 1-2 | 3-4 | 5-7
8. I felt hopeful about the future |  <1 | 1-2 | 3-4 | 5-7
9. I thought my life had been a failure |  <1 | 1-2 | 3-4 | 5-7
10. I felt fearful |  <1 | 1-2 | 3-4 | 5-7
11. My sleep was restless |  <1 | 1-2 | 3-4 | 5-7
12. I was happy |  <1 | 1-2 | 3-4 | 5-7
13. I talked less than usual |  <1 | 1-2 | 3-4 | 5-7
14. I felt lonely |  <1 | 1-2 | 3-4 | 5-7
15. People were unfriendly |  <1 | 1-2 | 3-4 | 5-7
16. I enjoyed life |  <1 | 1-2 | 3-4 | 5-7
17. I had crying spells |  <1 | 1-2 | 3-4 | 5-7
18. I felt sad |  <1 | 1-2 | 3-4 | 5-7
19. I felt that people disliked me |  <1 | 1-2 | 3-4 | 5-7
20. I could not get “going” |  <1 | 1-2 | 3-4 | 5-7