The Relationship Between Markers of Oxidative Stress and Depressive Symptoms in Coronary Artery Disease Patients

Alex Adibfar, BSc

A thesis submitted in conformity with the requirements for the degree of Master of Science

Graduate Department of Pharmacology and Toxicology

University of Toronto

© Copyright by Alex Adibfar (2016)
The Relationship Between Markers of Oxidative Stress and Depressive Symptoms in Coronary Artery Disease Patients

Alex Adibfar, BSc
Graduate Department of Pharmacology and Toxicology
University of Toronto
2016

Abstract

Depression is highly prevalent and associated with worse prognoses in patients with coronary artery disease (CAD), but underlying mechanisms remain unclear. Oxidative stress, and ensuing lipid peroxidation, may be implicated in both depressive and cardiovascular pathologies but has yet to be assessed in CAD. This study investigated the relationship between depressive symptoms and the lipid peroxidation markers 8-isoprostane (8-ISO), 4-hydroxy-2-nonenal (4-HNE), and lipid hydroperoxides (LPH) in 204 CAD patients. Serum lipid peroxidation markers were analyzed using spectrophotometric assays. Higher concentrations of 8-ISO, a late-stage marker of oxidative damage to lipids, predicted greater depressive symptom severity measured by the Center for Epidemiological Studies Depression Scale ($\beta=0.265$, $p<0.001$) in a linear regression. A lower lipid peroxidation ratio [LPH/(4-HNE+8-ISO)], indicating conversion of early- to late-stage lipid peroxidation, also predicted greater depressive symptoms ($\beta=-0.236$, $p=0.001$). Thus, lipid peroxidation may be an important correlate of depressive symptoms in CAD and should be further explored.
Acknowledgements

The past two years at Sunnybrook have been a truly wonderful experience. First and foremost, I would like to sincerely thank Drs. Krista Lanctôt and Nathan Herrmann for their invaluable mentorship. Dr. Lanctôt, I am truly inspired by your high standard for critical thinking and ethical research. The more I grow, both academically and personally, the more I appreciate your accomplishments and ongoing contributions to your field. While you instilled in me the importance of being a self-directed learner who strives to find the most critical gaps in the literature, your kind words of encouragement and open-door policy are what I will remember and cherish most. Dr. Herrmann, you embody so many qualities I aspire to – from your steadfast professionalism to your effortless humorous tone – and I really appreciate your genuine support and valuable clinical insights into every piece of work I have done at Sunnybrook. It was an honour to see you achieve a major, and much-deserved, milestone in your incredible career.

To the whole Andreazza lab, thank you for putting up with me. Dr. Andreazza, I will always look back fondly on our long and laughter-filled meetings. Your warmth is contagious, and I anticipate nothing but more great things from your budding career. Gustavo, thank you dearly for being such an unwavering source of help and entertainment. You have the rare gift of making basic science research bearable, and even mildly enjoyable, for someone like me.

To everyone at the Neuropsychopharmacology Research Group, thank you for making these two years fly by. Since I’ve been so lucky to be part of such an amazing group, I’ll put all the Adam / Alex mix-ups in the past. Most of you have been tremendously supportive mentors and a few of you did a good job of actually making me seem like a helpful mentor at times, but all of you have contributed to a wonderful work and social environment. Mahwesh, I can’t thank you enough for all your help amidst the long (and usually inconveniently timed) phone calls. Only you can understand that I really mean it when I say I couldn’t have done it without you. Your patience and generosity never cease to amaze and impress me, and I know that you will find success and fulfilment in whatever endeavours you choose to pursue in the future.

Finally, I want to thank my mother, Maryam Adibfar, for investing an immeasurable amount of time, effort, and love into my upbringing. I have yet to meet anyone with more strength and dedication than you, and I’ll never be able to repay you for all you’ve done for me… So let’s count this as a start.
# Table of Contents

Abstract ....................................................................................................................................... ii  
Acknowledgements ................................................................................................................... iii  
List of Tables ................................................................................................................................ viii  
List of Figures ................................................................................................................................ ix  
List of Appendices ..................................................................................................................... x  
List of Abbreviations .................................................................................................................. xi  
Introduction ................................................................................................................................. 1  
1.1 Statement of Problem ........................................................................................................... 1  
1.2 Purpose of the Study and Objective ..................................................................................... 2  
1.3 Statement of Research Hypotheses and Rationale for Hypotheses ....................................... 2  
  1.3.1 Primary Hypothesis ........................................................................................................ 2  
  1.3.2 Secondary Hypothesis .................................................................................................... 3  
  1.3.3 Exploratory Analyses ..................................................................................................... 3  
1.4 Review of the Literature ....................................................................................................... 5  
  1.4.1 Coronary Artery Disease .............................................................................................. 5  
  1.4.2 Depression and CAD .................................................................................................... 6  
  1.4.3 Current Treatments for Depression in CAD ................................................................. 7  
  1.4.4 Oxidative Stress ............................................................................................................ 9  
    1.4.4.1 General Concepts and Key Mediators ................................................................. 10  
    1.4.4.2 Lipid Peroxidation ................................................................................................. 11  
  1.4.5 Oxidative Stress and CAD .......................................................................................... 13  
  1.4.6 Oxidative Stress and Depression ................................................................................ 15  
    1.4.6.1 Relevance to Neurobiological Mechanisms ........................................................ 15
1.4.6.1.1 Monoaminergic neurotransmission .................................................................16
1.4.6.1.2 HPA axis activity ..........................................................................................17
1.4.6.1.3 Neurogenesis and neuroplasticity ..............................................................18
1.4.6.1.4 Mitochondrial function ...............................................................................19
1.4.6.1.5 Inflammation ..............................................................................................20
1.4.6.1.6 Platelet-activating factor activity ................................................................23

1.4.6.2 Current Evidence for Oxidative Stress in Depression ........................................24

1.4.6.3 Evidence for Selected Biomarkers ..................................................................26

1.5 Summary of Background ........................................................................................29

Materials and Methods ..............................................................................................30

2.1 Study Design ..........................................................................................................30

2.2 Eligibility Criteria ....................................................................................................30

2.3 Demographics and Medical History ........................................................................31

2.4 Assessments ............................................................................................................31

2.4.1 Center for Epidemiological Studies Depression Scale (CES-D) .......................32

2.4.2 Structured Clinical Interview for Depression (SCID) ........................................32

2.5 Blood Collection and Assays ..................................................................................33

2.5.1 Lipid Hydroperoxides (LPH) Assay ..................................................................33

2.5.2 4-Hydroxy-2-nonenal (4-HNE) Assay ................................................................34

2.5.3 8-Isoprostane (8-ISO) Assay .............................................................................34

2.6 Statistical Analyses .................................................................................................35

2.6.1 Covariates ............................................................................................................35

2.6.1.1 Age ..................................................................................................................35

2.6.1.2 Gender ............................................................................................................36
2.6.1.3 BMI .................................................................36
2.6.1.4 Smoking Status ................................................36
2.6.1.5 Antidepressant Medication Use ..........................36
2.6.1.6 Use of Anti-Oxidant Supplements ......................37
2.7 Sample Size Calculation ........................................37

Results ........................................................................38

3.1 Participant Recruitment ........................................38
3.2 Participant Characteristics ......................................39
3.3 Normalization of Lipid Peroxidation Marker Concentrations .....................................................41
3.4 Addressing the Hypotheses ....................................42
  3.4.1 Primary Hypothesis ............................................42
  3.4.2 Secondary Hypothesis ........................................45
  3.4.3 Exploratory Analyses .........................................47
    3.4.3.1 Exploratory Hypothesis 1: 4-HNE ....................47
    3.4.3.2 Exploratory Hypothesis 2: LPH .......................49
    3.4.3.3 Exploratory Hypothesis 3: Ratios ....................51
3.5 Post-hoc Analyses ..................................................54

Discussion and Conclusion ........................................56
4.1 Summary of Findings .............................................56
4.2 Interpretation of Results ........................................56
4.3 Limitations ..........................................................60
  4.3.1 Methodological ...............................................60
  4.3.2 Mechanistic Considerations ............................63
4.4 Future Directions ..................................................65
List of Tables

Table 1. Clinical studies assessing lipid peroxidation as measured by LPH, 4-HNE, and 8-ISO concentrations (n = 14) .................................................................................................................................27

Table 2. Demographic and clinical characteristics of study participants (n = 204) ........................................39

Table 3. Depression characteristics of study participants (n = 204) .........................................................41

Table 4. Overview of serum lipid peroxidation marker concentrations (n = 204) .................................. 41

Table 5. Summary of the linear regression model examining the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204) ..................44

Table 6. Final model in the linear regression analysis examining the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204) ..............44

Table 7. log-transformed 8-ISO concentrations in the depressed and non-depressed groups of CAD patients according to the depression module of the SCID (n = 203) ........................................46

Table 8. Coefficients of an ANCOVA analysis detecting differences in mean log-transformed 8-ISO concentrations between depressed and non-depressed CAD patients according to the SCID (n = 203) ..............................................................................................................47

Table 9. Summary of the linear regression model examining the association between log-transformed 4-HNE concentration and CES-D score in CAD patients (n = 204) ......................48

Table 10. Final model in the linear regression analysis examining the association between log-transformed 4-HNE concentration and CES-D score in CAD patients (n = 204) .................48

Table 11. Summary of the linear regression model examining the association between log-transformed LPH concentration and CES-D score in CAD patients (n = 196) .........................50

Table 12. Final model in the linear regression analysis examining the association between log-transformed LPH concentration and CES-D score in CAD patients (n = 196) ..............50

Table 13. Summary of the linear regression model examining the association between early- to late-stage lipid peroxidation ratio and CES-D score in CAD patients (n = 196) ..............53

Table 14. Final model in the linear regression analysis examining the association between early- to late-stage lipid peroxidation ratio and CES-D score in CAD patients (n = 196) ..............53

Table 15. Summary of the post hoc linear regression models including potential confounders of the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204) .................................................................................................................................55
List of Figures

Figure 1. Participant flow through each stage of study recruitment ........................................... 38

Figure 2. Distribution of log-transformed serum 8-ISO concentration ........................................... 42

Figure 3. The association between log-transformed 8-ISO concentration and depressive symptoms measured by the CES-D in CAD patients (n = 204) ............................................................... 43

Figure 4. Normal P-P plot of regression standardized residuals .................................................. 45

Figure 5. Boxplot illustrating differences in log-transformed 8-ISO concentrations of CAD patients divided into SCID-classified depression groups (n = 203) ............................................................... 46

Figure 6. The association between log-transformed 4-HNE concentration and depressive symptoms measured by the CES-D in CAD patients (n = 204) ............................................................... 47

Figure 7. The association between log-transformed LPH concentration and depressive symptoms measured by the CES-D in CAD patients (n = 196) ............................................................... 50

Figure 8. Distribution of processed early- to late-stage lipid peroxidation marker ratio .......... 52

Figure 9. The association between processed early- to late-stage lipid peroxidation ratio and depressive symptoms measured by the CES-D in CAD patients (n = 196) .......... 53
List of Appendices

Appendix A – Research Ethics Board Approval ................................................................. 91
Appendix B – Informed Consent Form ........................................................................... 96
Appendix C – Assessments .......................................................................................... 103
List of Abbreviations

AA          Arachidonic acid
ACE         Angiotensin-converting enzyme
ACS         Acute coronary syndrome
ANCOVA      Analysis of covariance
ANOVA       Analysis of variance
AP-1        Activator protein 1
ARB         Angiotensin receptor blocker
ATCH        Adrenocorticotropic hormone
ATP         Adenosine triphosphate
BDNF        Brain-derived neurotrophic factor
BMI         Body mass index
CABG        Coronary artery bypass graft
CAD         Coronary artery disease
CAT         Catalase
CES-D       Center for Epidemiological Studies Depression Scale
CNS         Central nervous system
CREATE      Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy
CR          Cardiac rehabilitation
CRH         Corticotropin-releasing hormone
COX         Cyclooxygenase
CVRF        Cardiovascular risk factor
DHA         Docosahexaenoic acid
ELISA       Enzyme-linked immunosorbent assay
ENRICHD     Enhancing Recovery in Coronary Heart Disease
EPA         Eicosapentaenoic acid
ETC         Electron transport chain
GPX         Glutathione peroxidase
GR          Glucocorticoid receptor
HPA         Hypothalamic-pituitary-adrenal
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDO</td>
<td>Indoleamine 2,3-dioxygenase</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intracellular adhesion molecule</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>KYN</td>
<td>Kynurenine</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LOX</td>
<td>Lipoxygenase</td>
</tr>
<tr>
<td>LPH</td>
<td>Lipid hydroperoxides</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine oxidase</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>MIND-IT</td>
<td>Myocardial Infarction and Depression – Intervention Trial</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>mtDNA</td>
<td>mitochondrial DNA</td>
</tr>
<tr>
<td>NAC</td>
<td>N-acetylcysteine</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor κB</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>PAF</td>
<td>Platelet-activating factor</td>
</tr>
<tr>
<td>PAF-AH</td>
<td>PAF-acetylhydrolase</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Platelet-endothelial cellular adhesion molecule</td>
</tr>
<tr>
<td>PFC</td>
<td>Pre-frontal cortex</td>
</tr>
<tr>
<td>PON-1</td>
<td>Paraoxonase</td>
</tr>
<tr>
<td>PTCA</td>
<td>Percutaneous transluminal coronary angioplasty</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>Redox</td>
<td>Reduction-oxidation</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SADHART</td>
<td>Sertraline Antidepressant Heart Attack Randomized Trial</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM IV Axis I Disorders</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic antidepressant</td>
</tr>
<tr>
<td>THC</td>
<td>Trillium Health Centre</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TRI</td>
<td>Toronto Rehabilitation Institute</td>
</tr>
<tr>
<td>TRP</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cellular adhesion molecule</td>
</tr>
<tr>
<td>VO\textsubscript{2}</td>
<td>Volume of oxygen</td>
</tr>
<tr>
<td>XO</td>
<td>Xanthine oxidase</td>
</tr>
<tr>
<td>4-HHE</td>
<td>4-hydroxy-2-hexenal</td>
</tr>
<tr>
<td>4-HNE</td>
<td>4-hydroxy-2-nonenal</td>
</tr>
<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>8-ISO</td>
<td>8-isoprostane</td>
</tr>
<tr>
<td>8-oxodG</td>
<td>8-hydroxy-2’-deoxyguanosine</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Statement of Problem

Coronary artery disease (CAD) is highly prevalent (Public Health Agency of Canada 2009) and commonly features co-morbid depression (Dowlati et al. 2010b, McManus et al. 2005, Rudisch and Nemeroff 2003, Swardfager et al. 2010, Swardfager et al. 2011a, Swardfager et al. 2011b). Unresolved depression in CAD presents a clinically important problem, as each condition appears to drive the other, conferring worse outcomes to afflicted individuals (Nemeroff and Goldschmidt-Clermont 2012). Currently available treatment options including pharmacological, psychotherapeutic, and exercise therapies are modestly and variably efficacious in CAD patients (Glassman et al. 2002, Grosso et al. 2014, Lesperance et al. 2007, Mazereeuw et al. 2016 (In Press), Mendes de Leon et al. 2006, Rutledge et al. 2013, Strik et al. 2000). The optimization of existing antidepressant therapies and discovery of novel ones are hampered by the poorly understood relationship between depression and CAD. Thus, more work is required to elucidate this complex relationship and effectively address this clinical need.

Mounting evidence implicates oxidative stress, and particularly lipid peroxidation, as a potential mechanism underlying the etiopathology of both depression and CAD (Bonomini et al. 2008, Maes et al. 2011a, Vogiatzi et al. 2009). Indeed, oxidative stress may act as an integrator that promotes several aberrant processes associated with depression in CAD including serotonergic imbalance, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, impaired neurogenesis and neuroplasticity, mitochondrial dysfunction, chronic low-grade inflammation, and platelet-activating factor (PAF) hyperactivity. These dysregulated mechanisms may collectively contribute to the neurodegeneration and depression commonly seen in CAD. Oxidative damage to lipids also produces measurable and biologically active
products that may reflect depressive pathophysiology but have yet to be assessed in this patient population (Adibfar et al. 2016). Therefore, lipid peroxidation may be an important correlate of depressive symptoms and depression in CAD that provides mechanistic insights and clinically useful biomarkers.

1.2 Purpose of the Study and Objective

This primary objective of this study is to evaluate the cross-sectional association between markers of oxidative damage to lipids and depressive symptoms in a CAD population. This information will help to ascertain whether oxidative stress, and ensuing lipid peroxidation, is a clinically important correlate of depression in a high-risk population. As this study will also assess the importance of the relative abundance of early- versus late-stage lipid peroxidation markers in relation to depressive symptoms, its findings may also reveal mechanistic insights into the relationship between oxidative stress and depression.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

1.3.1 Primary Hypothesis

**Hypothesis 1:** Higher concentrations of 8-ISO will predict greater depressive symptom severity, as measured by the CES-D, in CAD patients.

*Rationale:* 8-isoprostane (8-ISO), a late-stage product of oxidative damage to lipids (Morrow et al. 1990, Porter et al. 1995), is considered to be the gold standard for measuring oxidative stress *in vivo* (Milne et al. 2011, Moore and Roberts 1998, Niki 2008, Roberts et al. 2005) and is accordingly the most thoroughly studied marker in the clinical literature [Table 1]. Furthermore, its biological activity as a potent vasoconstrictor, both peripherally and in the brain microvasculature (Basu 2008, Gniwotta et al. 1997, Kromer and Tippins 1996), suggests that it may be involved in the etiopathology of depression and CAD. Depressive symptoms
were selected as a primary outcome measure, because they are particularly prevalent in CAD (Burg and Abrams 2001, Carney et al. 1987, Frasure-Smith et al. 1993, 1995b, Lesperance and Frasure-Smith 2003, Patten et al. 2006, Schleifer et al. 1989) and may reveal subtle associations between oxidative stress and depression. The 20-item Center for Epidemiological Studies Depression Scale (CES-D) (Radloff 1977) was chosen to measure depressive symptom severity, as it has been validated as a reliable assessment instrument in several studies with CAD patients (Dowlati et al. 2010b, McManus et al. 2005, Rudisch and Nemeroff 2003, Swardfager et al. 2010, Swardfager et al. 2011a, Swardfager et al. 2011b).

### 1.3.2 Secondary Hypothesis

**Hypothesis 2:** CAD patients with major or minor depression, diagnosed by the SCID, will have greater concentrations of 8-ISO compared to those without depression.

**Rationale:** While lipid peroxidation parameters have been shown to be elevated in depressed patients versus controls in various study cohorts [Table 1], it remains to be seen whether the same relationship holds in an aging CAD population with high baseline levels of oxidative stress. Using the researcher-rated depression module of the Structured Clinical Interview for DSM IV Axis Disorders (SCID) (First et al. 1996) in addition to the patient-rated CES-D allows for a more comprehensive evaluation of both depression and depressive symptoms in our sample and validates the clinical significance of elevated CES-D scores. The secondary analysis will also provide an assessment of depression measured over a longer, more reliable timespan of 1 month instead of 1 week for the CES-D [Appendix C].

### 1.3.3 Exploratory Analyses

**Exploratory hypothesis 1:** Higher concentrations of 4-HNE will predict greater depressive symptom severity, as measured by the CES-D, in CAD patients.
**Rationale:** 4-hydroxy-2-nonenal (4-HNE), another distinct late-stage marker of lipid peroxidation (Esterbauer et al. 1991, Porter et al. 1995), is thought to be involved in the pathogenesis of CAD (Hoff et al. 1989) and may promote apoptotic signalling pathways related to neurodegeneration (Davalli et al. 2016, Maurya et al. 2016, Wolkowitz et al. 2011b). It has also been shown to be elevated in depressed patients in two clinical studies featuring patients without CAD [Table 1] and will therefore be assessed to evaluate its promise as a marker of depressive symptoms in the CAD population.

**Exploratory hypothesis 2:** Higher concentrations of LPH will predict greater depressive symptom severity, as measured by the CES-D, in CAD patients.

**Rationale:** Lipid hydroperoxides (LPH), early-stage markers of lipid peroxidation (Porter et al. 1995), are present in atherosclerotic lesions (Suarna et al. 1995) and have been found to predict adverse cardiovascular events in patients with CAD (Walter et al. 2008). Furthermore, they have also been found to be elevated in relation to depression in two clinical studies featuring patients without CAD [Table 1]. As such, their association with depressive symptoms will be assessed in a CAD population.

**Exploratory hypothesis 3:** Lower lipid peroxidation ratios [LPH / (4-HNE + 8-ISO)], indicating greater lipid peroxidation progression, will predict greater depressive symptom severity, as measured by the CES-D, in CAD patients.

**Rationale:** Since oxidative stress and its damage to lipids is implicated in both depressive and cardiovascular etiopathologies, CAD patients with greater depressive symptoms may be particularly burdened with altered lipid composition. A recent study conducted by our
colleagues used a ratio of early- to late-stage lipid peroxidation markers to reflect the degree of oxidative damage to lipids (Scola et al. 2016). As such, it would be mechanistically and clinically informative to discern whether CAD patients with greater depressive symptoms feature a characteristic biomarker profile that indicates lipid peroxidation progression.

1.4 Review of the Literature

1.4.1 Coronary Artery Disease

CAD is a highly prevalent and debilitating condition affecting approximately 1.3 million Canadians and estimated to be responsible for 1 in every 5 deaths in Canada (Public Health Agency of Canada 2009). It is characterized by stenosis, or narrowing, of one or more of the coronary arteries (by at least 50% of vessel diameter) that perfuse the myocardium. Stenosis arises from a process known as atherosclerosis, in which leukocytes, lipid deposits, calcium, and a collagen-rich extracellular matrix form subendothelial atherosclerotic plaques that thicken the arterial wall, compromising blood flow and oxygen supply to the heart (Fuster et al. 1992). Well-documented cardiovascular risk factors (CVRFs) such as smoking, hypertension, obesity, dyslipidemia, and type II diabetes mellitus that are known to promote the development of atherosclerosis are frequently seen in the CAD population (Criqui 1986, Feeman 2004).

Clinically, atherosclerosis and subsequent thrombosis may eventually precipitate angina, myocardial infarction (MI), and even death (Falk et al. 1995, Fuster et al. 1990). While CAD can be non-invasively managed pharmacologically (Smith et al. 2011) and/or with lifestyle changes such as dietary modifications and physical exercise (Schuler et al. 1992), patients who have severe occlusions and/or are at high risk for MI may be required to undergo revascularization interventions, namely coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA) (Serruys et al. 2009).
1.4.2 Depression and CAD

Depression, a term that includes both major and minor depression, is characterized by persistent feelings of sadness and/or anhedonia as well as a variety of symptoms including fatigue, changes in appetite, and suicidal ideation (Rapaport et al. 2002). Minor depression differs from major depression in that it involves fewer criterion symptoms. Both major and minor depression impose a large socioeconomic burden (Judd et al. 1996) and are associated with negative health outcomes including impaired quality of life (Hofer et al. 2005, Stafford et al. 2007) and increased risk of suicide (Bostwick and Pankratz 2000, Lester 1993). Depression afflicts approximately 8% of Canadians (Bland et al. 1988, Patten et al. 2006) and has an annual prevalence of nearly 5% in the general population (Parikh et al. 2001). In contrast, 15-20% of individuals with CAD suffer from major depression in the first year following an acute coronary syndrome (ACS) (Patten et al. 2006) – a three- to four-fold increase compared to the general population – and an additional 30-45% of CAD patients meet criteria for minor depression (Celano and Huffman 2011, Sowden and Huffman 2009).

In addition to its high prevalence, depression is also associated with worse clinical outcomes in CAD. Both major and minor depression have been reported to increase the risk of subsequent cardiac events, such as MI, independently of traditional CVRFs in stable CAD (Frasure-Smith et al. 1993, 1995a, Ladwig et al. 1991, Lesperance and Frasure-Smith 2000, Wassertheil-Smoller et al. 1996), post-CABG (Blumenthal et al. 2003), or post-MI patients (Ahern et al. 1990, Frasure-Smith et al. 1993, 1995a, b, Ladwig et al. 1991). Greater symptom severity is also known to exacerbate the negative impact of depression on cardiac prognosis (Lesperance and Frasure-Smith 2000, Penninx et al. 2001). For example, hospitalization risk due to CAD-related complications increases with depressive symptom severity in dose-dependent fashion (Rutledge et al. 2006). Depressive symptoms, including those experienced
transiently and at subclinical levels, are associated with increased CAD severity and progression (Stewart et al. 2007) as well as increased incidence of future major depressive episodes (Patten et al. 2012). Findings from the Heart and Soul Study revealed that CAD patients experiencing depressive symptoms featured additional symptom burden, greater physical impairment, and worse overall quality of life compared to their non-depressed counterparts (Ruo et al. 2003). Furthermore, depressive symptoms present a barrier to effective secondary prevention, negatively interacting with such outcomes as psychosocial rehabilitation (Mayou et al. 1978, Stern et al. 1977) as well as adherence to cardiac medications (Blumenthal et al. 1982) and exercise interventions (Swardfager et al. 2011b). Thus, unresolved depression or subthreshold depressive symptoms in CAD represent a clinically important problem.

1.4.3 Current Treatments for Depression in CAD

Selective serotonin reuptake inhibitors (SSRIs) are presently the first-line medication for depression in CAD (Mavrides and Nemeroff 2013). Tricyclic antidepressants (TCAs) are not recommended for CAD patients due to their noted cardiotoxicity, while other antidepressant classes such as monoamine oxidase (MAO) inhibitors, norepinephrine-dopamine reuptake inhibitors, and serotonin-norepinephrine reuptake inhibitors have yet to be thoroughly studied in the context of CAD (Mavrides and Nemeroff 2013). Two large-scale randomized controlled trials (RCTs), the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy (CREATE) (Lesperance et al. 2007) and the Sertraline Antidepressant Heart Attack Randomized Trial (SADHART) (Glassman et al. 2002), established the safety of the SSRIs citalopram and sertraline, respectively, in CAD patients with major depression. However, they found limited efficacy, reporting response rates of 53% in CREATE and 67% in SADHART, with only modest differences between SSRI- and placebo-treated groups. Moreover, 64% of patients in the CREATE study continued to suffer
from depressive symptoms after 12 weeks of citalopram treatment (Lesperance et al. 2007). A third RCT revealed similar findings with fluoxetine (Strik et al. 2000). The Myocardial Infarction and Depression – Intervention Trial (MIND-IT) (Honig et al. 2007), which included CAD patients with both major and minor depression, found that mirtazapine, a tetracyclic antidepressant, conferred a modest therapeutic benefit comparable to that of the SSRIs, with a response rate of 47%.

While SSRIs are relatively safe compared to other classes such as the TCAs, they are not without drawbacks. For example, weight gain associated with SSRI use may exacerbate the adverse cardiometabolic profiles that already pose substantial burden in the CAD population (Beyazyuz et al. 2013). Citalopram use has been linked to a dose-dependent QT interval prolongation (Cooke and Waring 2013). SSRIs can also increase the bioavailability of beta-blocker medications, commonly used by CAD patients, by inhibiting the cytochrome P450-2D6 isozyme (Spina et al. 2008). Other undesirable side effects such as nausea, diarrhea, diaphoresis, and sexual dysfunction present barriers to compliance (Chemali et al. 2009). Thus, conventional antidepressant pharmacotherapy features significant limitations with respect to efficacy and tolerability in CAD patients.

The potential antidepressant benefit of ω-3 polyunsaturated fatty acids (PUFAs) has also been explored and reviewed in recent meta-analyses. It has been shown that ω-3 PUFA preparations consisting of at least 60% eicosapentaenoic acid (EPA) may be efficacious, with reported response rates ranging from 45% to 62% (Grosso et al. 2014, Martins et al. 2012, Sublette et al. 2011). However, a recent RCT conducted by our group that used an EPA-enriched formulation found no statistically significant differences in depressive symptom reduction between ω-3 PUFA- and placebo-treated CAD patients over 12 weeks of a supervised exercise intervention (Mazereeuw et al. 2016 (In Press)). Therefore, current
evidence suggests that the antidepressant benefits of ω-3 PUFA supplementation in CAD are variable and modest.

The antidepressant effects of supervised exercise interventions have also been assessed and meta-analyzed, both in somatically healthy individuals (Rimer et al. 2012) and in those with CAD (Gellis and Kang-Yi 2012, Rutledge et al. 2013). While the existing literature points to a modest benefit of exercise in mitigating depressive symptoms, many challenges continue to hinder the optimization of this intervention, particularly in the context of CAD. Notably, depressed patients with CAD are less likely to adhere to their CR program (Swardfager et al. 2011b), and not all compliers experience remission from depressive symptoms (Milani and Lavie 2007). Furthermore, there is no streamlined consensus regarding the optimal duration, intensity, and methodology (aerobic and/or resistance training) of CR.

Psychotherapy has been explored as another non-pharmacological avenue to treat depression in CAD, but current evidence has shown little clinical promise. Indeed, the Enhancing Recovery in Coronary Heart Disease (ENRICHD) (Mendes de Leon et al. 2006) and CREATE (Lesperance et al. 2007) studies found minimal and no antidepressant benefit, respectively, in CAD patients receiving adjunctive cognitive behavioural therapy compared to those receiving standard care.

Currently available treatments for depression in CAD are modestly and variably effective. As failure to achieve and sustain remission has been associated with poor longitudinal outcomes including increased risk of ACS and mortality (Friedmann et al. 2006, Januzzi et al. 2000, Lesperance and Frasure-Smith 2000, Penninx et al. 2001), this limitation presents a clinically important problem that invites investigation into the optimization of existing and discovery of novel antidepressant therapies.

1.4.4 Oxidative Stress
1.4.4.1 General Concepts and Key Mediators

Reactive oxygen species (ROS) are chemically unstable compounds that usually contain free radicals (i.e., unpaired electrons) and can react with a vast array of nearby cellular macromolecules including carbohydrates, lipids, proteins, and nucleic acids (Droge 2003, Liemburg-Apers et al. 2015, Marnett 2002, Porter et al. 1995). ROS production occurs intracellularly under normal physiological conditions, particularly by the mitochondrial electron transport chain (ETC) during oxidative phosphorylation (Lenaz 2001), and the reduction-oxidation (redox) reactions in which they participate lead to various functional changes such as post-translational protein modifications and regulation of gene expression, which play a homeostatic signalling role in cell division, proliferation, and apoptosis as well as immune defense against pathogens (Siwik and Colucci 2004, Zweier and Talukder 2006). As pro-oxidant reactions are normally balanced by the detoxifying action of endogenous and exogenous anti-oxidants, which quench ROS and/or reduce oxidized biomolecules, this process is normally beneficial (Sies 1997). However, an imbalance caused by increased ROS generation and/or impaired anti-oxidant activity results in oxidative stress, a condition in which excess ROS exert toxicity in their microenvironment by disrupting the finely regulated redox signalling pathways in which they are involved (Halliwell 1996, Scandalios 2005).

The most notable ROS produced in humans are non-radical hydrogen peroxide (H₂O₂), the superoxide anion (O₂•⁻), and the highly reactive hydroxyl radical (OH•) (Sies 1997). While ROS differ in terms of their molecular composition or presence of a free radical, they all share the ability to eventually promote free radical-mediated reactions. For example, superoxide does not directly possess particularly harmful properties, but it can react with nitric oxide (NO) to form the potent reactive nitrogen species (RNS), peroxynitrite (ONOO⁻) (Beckman et al. 1990). Moreover, superoxide can be enzymatically converted to hydrogen peroxide via the
catalytic action of superoxide dismutase (SOD) in the presence of its copper or zinc co-factors. If the resultant hydrogen peroxide is not successfully reduced to water and molecular oxygen by the anti-oxidant enzyme catalase (CAT), it can form the highly reactive and cytotoxic hydroxyl radical in the presence of ferrous iron through a process known as the Fenton reaction (Liochev and Fridovich 1994). As the high reactivity and short half-life of these compounds render direct ROS quantification challenging in biological systems, oxidative stress is generally measured by assessing oxidative damage to proteins, lipids, and nucleic acids, which gives rise to more stable markers that can be reliably measured in vivo (Halliwell and Whiteman 2004).

1.4.4.2 Lipid Peroxidation

Of the macromolecular classes that can be attacked by ROS, lipids are particularly vulnerable to oxidative modification due to the presence of their unsaturated bonds (Porter et al. 1995). This autocatalytic process, termed lipid peroxidation, generates lipid hydroperoxides (LPH) from fatty acids as its primary products through a number of distinct mechanisms. One of these mechanisms is non-enzymatic, free radical-mediated oxidation. In brief, free radical-mediated lipid peroxidation generally proceeds in three major stages: initiation, propagation, and termination (Porter et al. 1995). Initiation consists of hydrogen abstraction from a methylene carbon of a fatty acid (LH) by a ROS, such as the hydroxyl radical, to form a lipid radical (L•). Lipid radicals combine with molecular oxygen to produce a lipid peroxyl radical (LOO•), which in turn can abstract a hydrogen atom from another nearby fatty acid side chain to yield LPH (LOOH), considered to be the primary products of lipid peroxidation, while generating another lipid peroxyl radical. Since a new radical is formed and many LPH can be formed in a chain reaction initiated by a single radical, this stage is aptly termed propagation. Termination results from the combination of any two free radical species, or of a free radical
species with an anti-oxidant, to quench the unpaired electrons. Oxidation of membrane lipids can also proceed via the enzymatic action of lipoxygenase (LOX) and cyclooxygenase (COX), which oxidize the ω-6 PUFA, arachidonic acid (AA), into pro-inflammatory compounds such as thromboxanes, leukotrienes, and prostaglandins (Funk 2001).

LPH are considered primary or intermediary products of lipid peroxidation, because they themselves are inherently unstable. Indeed, if they are not effectively neutralized by anti-oxidant defenses, they are susceptible to fragmentation into a number of various products in subsequent free radical-mediated reactions (Porter et al. 1995). Among these products are a group of reactive aldehydes, including the intensively studied 4-hydroxy-2-nonenal (4-HNE), which is the main product formed by the lipid peroxidation of ω-6 PUFAs such as AA and linoleic acid (Esterbauer et al. 1991). These compounds are end-products of lipid peroxidation but are highly reactive, most notably forming protein adducts by reacting with cysteine sulphydryl groups, histidine imidazole groups, and lysine amino groups to produce cytotoxic effects locally (Dalleau et al. 2013, Esterbauer et al. 1991). LPH can alternatively be processed in a number of reactions to yield a family of prostaglandin (PG)-like compounds known as the isoprostanes, which are primarily produced via free-radical mediated peroxidation of AA that is completely independent of the COX and LOX pathways (Morrow et al. 1990). F2-isoprostanes comprise 64 isomeric compounds that resemble PGF2α in structure and include the thoroughly studied 8-epi PGF2α, also known as 8-isoprostane (8-ISO). F2-isoprostanes are formed by the reduction of the labile bicyclic endoperoxide-containing H2-isoprostanes. Other related isoprostane sub-families deriving from H2-isoprostanes are the D2-isoprostanes and E2-isoprostanes, which spontaneously rearrange to form J2-isoprostanes and A2-isoprostanes, respectively (Davies et al. 2004). Unlike LPH and reactive aldehydes, isoprostanes – particularly 8-ISO – are chemically stable and thus widely considered to be the ideal marker to
measure oxidative stress in vivo (Milne et al. 2011, Moore and Roberts 1998, Niki 2008, Roberts et al. 2005). They are far from biologically inert, however, as they exert numerous biological actions via receptor-dependent and independent mechanisms (Milne et al. 2008, Musiek et al. 2005).

1.4.5 Oxidative Stress and CAD

Mounting evidence suggests that oxidative stress is intimately involved in the development and progression of atherosclerosis. ROS induce endothelial dysfunction, an important early step in atherogenesis, by reacting with NO, thereby reducing its bioavailability and impairing the vasodilatory response, as well as promoting related processes such as platelet aggregation and monocyte recruitment (Forstermann and Munzel 2006). ROS also trigger the oxidative modification of low-density lipoproteins (LDL) to oxidized LDL (ox-LDL), which are more readily engulfed by macrophages than their reduced counterparts (Quinn et al. 1987). The resultant activated foam cells increase pro-inflammatory cytokine production, which triggers further monocyte recruitment and infiltration as well as smooth muscle cell migration and proliferation (Bonomini et al. 2008). Thus, oxidative stress actively participates in immune activation in the vasculature. Moreover, oxidative stress mediated by hydrogen peroxide promotes tyrosine kinase phosphorylation, leading to increased endothelial permeability and enhanced binding of neutrophils to the endothelium (Vepa et al. 1999). Redox signalling also plays a role in the pro-atherogenic role of oxidative stress. Specifically, ROS induce the production of nuclear factor κB (NF-κB) and activator protein 1 (AP-1), transcription factors that act in smooth muscle cells to increase expression of notable cellular adhesion molecules such as vascular cellular adhesion molecules (VCAM-1); intracellular adhesion molecules (ICAM-1); platelet-endothelial cellular adhesion molecules (PECAM-1); E-, L-, and P-selectins; and pro-inflammatory cytokines (Vogiatzi et al. 2009). Oxidative stress therefore acts
as a key pro-atherogenic mediator that acts in concert with other dysregulated processes such as inflammation, endothelial dysfunction, and platelet hyperactivity to promote the development and progression of atherosclerosis.

ROS can be enzymatically generated in the vasculature by various sources including xanthine oxidase (XO) (Spiekermann et al. 2003), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Griendling et al. 2000, Guzik et al. 2000), myeloperoxidase (MPO) (Daugherty et al. 1994, Spickett et al. 2000), LOX (Cyrus et al. 1999), nitric oxide synthase (NOS) (Vasquez-Vivar et al. 1998), and the mitochondrial respiratory chain (Madamanchi et al. 2005). They can also arise from deficiencies in a large number of enzymatic anti-oxidants such as SOD, CAT, glutathione peroxidase (GPX), peroxiredoxin, and thiol-disulfide oxidoreductases (Hamilton et al. 2004, Wassmann et al. 2004). Clinically, evidence implicating oxidative stress in atherosclerosis has been provided by studies demonstrating elevated pro-oxidant (Gocmen et al. 2008a, Kaneda et al. 2002, Kaya et al. 2012, Maes et al. 2010) and impaired anti-oxidant (Cebi et al. 2011, Gocmen et al. 2008b, Yildiran et al. 2011) activity in individuals with cardiovascular disease. In regard to lipid peroxidation specifically, elevated concentrations of several key markers including LPH, 4-HNE, and 8-ISO have been found in atherosclerotic lesions (Gniwotta et al. 1997, Leonarduzzi et al. 2005, Vassalle et al. 2003) and in patients with CAD (Buffon et al. 2000, Gocmen et al. 2008a, Kovacs et al. 1997, Singh et al. 1995, Vassalle et al. 2003). Moreover, the end-products of oxidative damage to lipids are not only not only indicators of but also contributors to oxidative stress in CAD. Indeed, 4-HNE exacerbates atherosclerosis by modifying ε-lysine residues on apolipoprotein B-100 of LDL, thereby facilitating their uptake by macrophages (Hoff et al. 1989). 8-ISO is a potent vasoconstrictor (Gniwotta et al. 1997, Kromer and Tippins 1996) with demonstrated pro-atherogenic properties. For example, 8-ISO has been shown to
increase scavenger receptor A and matrix metalloproteinase activity \textit{in vitro}, resulting in foam cell formation (Scholz et al. 2004). Pre-clinical research has also linked 8-ISO to thromboxane A$_2$-mediated platelet activation (Kromer and Tippins 1996, Minuz et al. 1998), proliferation of vascular smooth muscle cells and fibroblasts (Takahashi et al. 1992), and heightened endothelin 1 expression in aortic endothelial cells (Yura et al. 1999).

Further implicating oxidative stress in CAD are the anti-oxidant properties of several pharmacological cardiovascular agents. For example, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), which act to reduce the activity of the renin-angiotensin system, have been shown to inhibit NADPH oxidase, improve endothelial function, and reduce risk of ACS in both pre-clinical and clinical studies (Lee and Leeson 2006, Tousoulis et al. 2006). Conversely, anti-oxidant therapies have shown promise in halting the progression of atherosclerosis in many pre-clinical studies (Carew et al. 1987, Iuliano et al. 2000, Kita et al. 1987, Lynch et al. 1996). However, reports from clinical trials assessing the potential atheroprotective benefits of dietary anti-oxidant supplementation have been far more discordant (Vogiatzi et al. 2009). Nonetheless, there are multiple lines of evidence implicating oxidative stress, and lipid peroxidation in particular, as a mediator of a number of dysregulated processes thought to collectively drive cardiovascular pathology.

\textbf{1.4.6 Oxidative Stress and Depression}

\textit{1.4.6.1 Relevance to Neurobiological Mechanisms}

The brain comprises 2\% of total body weight but accounts for 20\% of total oxygen consumption. Due to its high metabolic rate and relatively scarce anti-oxidant capacity, it is particularly susceptible to ROS-induced damage (Smith et al. 2007). It is therefore unsurprising that oxidative stress is thought to be involved in a host of neuropsychiatric conditions including depression (Maes et al. 2011a), Alzheimer’s disease (Smith et al. 2007),
and schizophrenia (Koga et al. 2015). With respect to depression in particular, oxidative stress is associated with a number of aberrant processes such as monoamine imbalance, HPA axis dysregulation, impaired neurogenesis and neuroplasticity, mitochondrial dysfunction, immune activation, and PAF hyperactivity, which are complex and inter-related mechanisms that have been proposed to partly underlie the etiopathology of depression.

1.4.6.1.1 Monoaminergic neurotransmission

An imbalance in the monoaminergic system, particularly impaired serotonergic neurotransmission, is a well-established mechanism involved in the pathogenesis of depression (Coppen 1967, Schildkraut 1965). Indeed, several conventional antidepressants, most notably SSRIs, are thought to exert their therapeutic effects primarily by restoring serotonin (5-HT) deficits (Hirschfeld 2000). As 5-HT has anti-oxidant properties (Khanzode et al. 2003), reduced 5-HT represents an impairment in anti-oxidant capacity and favours oxidative stress. Additionally, ROS may act in concert with pro-inflammatory cytokines such as interferon (IFN)-γ to activate indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the breakdown of tryptophan (TRP), an essential amino acid and 5-HT precursor, into the neurotoxic catabolite kynurenine (KYN), thereby depleting TRP and reducing 5-HT levels (Maes et al. 2011b). ROS-induced damage to membrane PUFAs can also modulate membrane structure and fluidity, potentially influencing the surface expression of 5-HT receptors (Maes et al. 1999a, Peet et al. 1998). Clinically, 5-HT levels have been correlated with depressive symptom severity and concentrations of 8-hydroxy-2′-deoxyguanosine (8-oxodG), a reliable marker of oxidative damage to DNA, in a small study of 24 young women with depressive symptoms (Iida et al. 2011). Naringenin, an anti-oxidant flavonoid found in grapefruit that has been shown to reduce oxidative damage to DNA in experimental models, has recently demonstrated antidepressant-like effects in mice while increasing brain 5-HT, norepinephrine,
brain-derived neurotrophic factor (BDNF), and glucocorticoid receptors (GRs) (Yi et al. 2010, Yi et al. 2012, Yi et al. 2014). There is therefore evidence to suggest that oxidative stress may, in part, promote depression by compromising serotonergic neurotransmission.

1.4.6.1.2 HPA axis activity

Another pathophysiological mechanism thought to be associated with depression is dysregulation of the HPA axis, which plays an important role in mediating the stress response and is regulated in part by the hippocampus (Moylan et al. 2013). Reduced hippocampal neurogenesis, a commonly observed feature in depression, can impair the hippocampus’ inhibitory control of the HPA axis, resulting in hyperactivity characterized by elevated secretion of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and downstream glucocorticoids such as cortisol (De Kloet et al. 1991, Sapolsky 1996). Chronic exposure to cortisol further impairs hippocampal neurogenesis, thus exacerbating HPA axis dysregulation. While our group did not find elevated hair cortisol concentration in CAD patients with depressive symptoms (Dowlati et al. 2010b), the role of HPA axis alteration is well-documented in depression as evidenced by clinical studies demonstrating higher CRH neuronal activation and elevated concentrations of salivary, plasma, and urinary cortisol in depressed patients (Adibfar et al. 2016, Nemeroff and Vale 2005). Furthermore, intracerebroventricular CRH administration can precipitate depressive symptoms (Holsboer et al. 1992), while chronic antidepressant treatment has been shown to up-regulate glucocorticoid receptor (GR) expression, thereby restoring cortisol-mediated negative feedback and reducing HPA axis hyperactivity (Pariante 2004). Importantly, there is a wealth of evidence suggesting that glucocorticoids induce neuronal oxidative stress by enhancing mitochondrial respiration and oxidative phosphorylation. Indeed, acute corticosteroid treatment has been shown to increase oxidative stress in a dose-dependent manner in rat hippocampal slice cultures (You et
al. 2009). This effect, which was concomitant with up-regulation of the pro-oxidant enzyme NAPDH oxidase and down-regulation of the anti-oxidant enzyme GPX, was abrogated by pre-treatment with the anti-oxidant, N-acetylcysteine (NAC). Cell culture studies have also found that exposure to sulforaphane, an anti-oxidant phytochemical found in cruciferous vegetables, mitigates suppression of the Nrf2-dependent anti-oxidant signalling pathway caused by exposure to glucocorticoids (Kratschmar et al. 2012, Lin et al. 2014). Additionally, ROS can impair the expression and nuclear translocation of GRs, and this inhibitory effect is also attenuated in the presence of exogenous anti-oxidants (Okamoto et al. 1999). Together, those findings highlight the abundance of research implicating ROS as molecular regulators of the neuroendocrine system and suggest that oxidative stress may contribute to the HPA axis hyperactivity observed in depression.

1.4.6.1.3 Neurogenesis and neuroplasticity

The “neurotrophic model of depression” posits that impaired neurogenesis and neuroplasticity are key contributors to depressive pathobiology. It stems from the premise that reduced hippocampal BDNF activity impairs the viability and differentiation potential of stem cells in the subgranular zone of the dentate gyrus and of those in the subventricular zone of the lateral ventricles, which project to the pre-frontal cortex (PFC) (Castren et al. 2007, Duman and Monteggia 2006). While those changes have not been shown to precipitate depressive episodes, there are multiple lines of evidence implicating BDNF in depression. First, lower serum concentrations of BDNF are consistently found in unmedicated patients with depression (Brunoni et al. 2008, Polyakova et al. 2015). Second, mice treated with peripherally-administered BDNF demonstrate reduced depressive-like behaviour and elevated hippocampal neurogenesis (Shirayama et al. 2002, Siuciak et al. 1997). This finding suggests that circulating BDNF may reflect what is taking place centrally and that its clinical promise may exceed that
of a simple biomarker. Third, treatment with antidepressants has been shown to stimulate hippocampal neurogenesis in animals and increase serum BDNF concentrations in depressed patients (Polyakova et al. 2015). In addition to its neurotrophic properties, BDNF possesses anti-inflammatory (Jiang et al. 2010, Jiang et al. 2011) and anti-oxidant features (Wu et al. 2015) that may, in part, account for its antidepressant efficacy. Conversely, oxidative stress might interfere with BDNF expression, and therefore compromise neuronal excitability and plasticity, by impairing N-methyl-D-aspartate (NMDA) channel function (Zou and Crews 2006) and decreasing the DNA-binding activity of AP-1 (Gaiddon et al. 1996). Further linking oxidative stress to BDNF is the recent finding that the anti-oxidant polyphenol resveratrol, found in the skin of red grapes and blueberries, was shown to ameliorate depressive-like behaviour while up-regulating hippocampal BDNF levels in corticosterone-treated treated mice (Ali et al. 2015). Thus, emerging evidence suggests that oxidative stress may negatively interact with BDNF-mediated neurogenesis and neuroplasticity, the impairment of which are associated with the pathophysiology of depression.

1.4.6.1.4 Mitochondrial function

Mitochondria are the chief energy source of cells, as they house the oxidative phosphorylation machinery. They are accordingly responsible for the majority of adenosine triphosphate (ATP) generation – and electron leakage, which leads to ROS production – resulting from aerobic respiration as well as other metabolic pathways such as the urea cycle and β-oxidation of fatty acids (Liu et al. 2002). Due to its high energetic demand and limited anti-oxidant capacity, the central nervous system (CNS) is particularly reliant on proper mitochondrial function and vulnerable to its dysregulation. Impaired mitochondrial function adversely affects key processes, such as intracellular calcium homeostasis, and is associated with numerous neurodegenerative disorders including depression (Federico et al. 2012, Tobe
Indeed, animal studies have shown that antidepressants can up-regulate mitochondrial energy production (Scaini et al. 2011) and that NAC can simultaneously improve mitochondrial function and reduce depressive-like behaviour (Wright et al. 2015). While the investigation of mitochondrial dysfunction in clinical studies remains in its infancy, emerging evidence supports a role for mitochondrial dysfunction in depression. Individuals with depression have demonstrated decreased brain energy generation (Baxter et al. 1985, Gardner et al. 2003a, Gardner et al. 2003b, Gardner and Boles 2008a, b) as well as alterations in mitochondrial size, distribution, and function (Gardner and Boles 2011). Clinical studies have also found that depressed patients exhibit lower expression of mitochondrial DNA (mtDNA)-encoded transcripts (Ben-Shchar and Karry 2008, Shao et al. 2008) and significantly greater mitochondrial oxidative damage (Chang et al. 2015) compared to their non-depressed counterparts. Additionally, reduced ETC complex I function has been reported in the post-mortem PFC of depressed patients by our colleagues (Andreaazza et al. 2010). Those clinical findings are of particular relevance to oxidative stress, because ROS can oxidize mtDNA and increase mitochondrial permeability, leading to mitochondrial swelling and release of pro-apoptotic mediators such as cytochrome c, which is released upon the rupture of the mitochondrial outer membrane (Moylan et al. 2013). Excess ROS can also modulate the intracellular signal transduction pathways in which mitochondria are involved, thereby causing intracellular calcium dyshomeostasis. The aforementioned processes collectively underscore the potential importance of mitochondrial-derived hyperproduction of ROS in promoting neurodegeneration and depression.

1.4.6.1.5 Inflammation

Perhaps the most thoroughly characterized aberrant process that may link oxidative stress to depression is a chronic low-grade inflammatory state in the absence of acute injury or
pathogen (Maes 2008). A multitude of pre-clinical and clinical studies have found that depression is associated with increased concentrations of pro-inflammatory cytokines and activation of the cell-mediated immune (CMI) system (Liu et al. 2016). Indeed, a meta-analysis conducted by our group showed that concentrations of interleukin (IL)-6 and tumour necrosis factor (TNF)-α were consistently elevated in depressed patients free of co-morbid disease (Dowlati et al. 2010a). Additional studies revealed numerous pro-inflammatory features related to CMI activation in depression, namely the levels of plasma soluble IL-2 receptor (Liu et al. 2012), the distribution of T-cell types (Maes et al. 1990), and increased production of IFN-γ (Maes et al. 1994), which notably stimulates IDO (MacKenzie et al. 1999). Accordingly, kynurenine metabolites of the IDO pathway, which promote oxidative stress by stimulating NADPH oxidase (Dykens et al. 1987, Goldstein et al. 2000, Murakami et al. 2006, Okuda et al. 1998, Rios and Santamaria 1991, Rosell et al. 2011), have also been found to be associated with depression (Gabbay et al. 2010) and greater depressive symptom severity (Swardfager et al. 2009). Another recent meta-analytic review with 18,527 study participants found that higher CRP and IL-6 concentrations were associated with a significantly greater risk of subsequent depressive symptoms (Valkanova et al. 2013).

Neopterin, another important peripheral inflammatory marker that promotes oxidative stress by potentiating the harmful effects of ROS such as hydrogen peroxide (Murr et al. 1994, Weiss et al. 1993), has also been found to be elevated in depressed patients (Maes et al. 2012b).

Evidence from observational studies has been corroborated by a growing number of interventional studies indicating that exogenous administration of pro-inflammatory compounds such as cytokines (Bonaccorso et al. 2002, Caraceni et al. 1998, Malaguarnera et al. 1998, Pavol et al. 1995, Raison et al. 2010) and lipopolysaccharide endotoxin injections (Brydon et al. 2009, Strike et al. 2004) can induce depressive symptoms. Conversely, several
studies have suggested that conventional antidepressants may have negative immunoregulatory properties, suppressing CMI activation and lowering pro-inflammatory cytokine concentrations (Diamond et al. 2006, Maes et al. 1999b, Szuster-Ciesielska et al. 2003), while anti-inflammatory agents have shown promise in treating depressive symptoms (Liu et al. 2016). Additionally, the aforementioned findings measuring peripheral markers are now being complemented with direct measurements of central inflammation as evidenced by a recent study using positron emission tomography, which found that those undergoing major depressive episodes exhibited elevated translocator protein density, a marker of microglial activation and neuroinflammation, in numerous brain regions compared to their non-depressed counterparts (Setiawan et al. 2015).

Importantly, oxidative stress and inflammation may fuel one another in a feed-forward manner. As previously mentioned, ROS can induce the production of transcription factors such as NF-κB and AP-1 to stimulate pro-inflammatory cytokine expression (Moylan et al. 2014). Conversely, pro-inflammatory cytokines contribute to oxidative stress by activating macrophages and microglia, which release ROS in their local environment as part of the cytotoxic immune response against invading pathogens (Maes et al. 2012a). Perhaps the most intriguing clinical findings linking inflammation to oxidative stress in depression are those showing that depression features autoimmune responses directed against neo-epitopes that are rendered immunogenic due to damage by ROS and RNS. During inflammation-associated depression, it is thought that lipid and proteins with epitopes that are normally unrecognized by the immune system can be oxidatively or nitrosatively modified to form neo-epitopes, which may elicit an immunoglobulin (Ig)G- or IgM-mediated autoimmune response, thereby further exacerbating inflammation (Maes et al. 2011a). Indeed, such autoimmune reactions have been demonstrated to be greater in depressed patients for a variety of neo-epitopes including
increased IgG or IgM antibodies against serum ox-LDL, which is particularly relevant to atherosclerosis (Maes et al. 2010); phosphatidyl inositol (Pi) (Maes et al. 2013), an important cell membrane constituent and lipid messenger involved in maintaining proper serotonergic neurotransmission (Akin et al. 2004, Ananthanarayanan et al. 2005); conjugated oleic acid (Maes et al. 2013), which influences membrane fluidity and integrity (Calder et al. 1994); and the lipid peroxidation products azelaic acid and MDA (Maes et al. 2013). Interestingly, it has also been shown that these increased autoimmune responses are also associated with chronicity of depression. That finding might indicate that chronic low-grade inflammation occurring in tandem with increased ROS and RNS production may increase the propensity to acquire immunogenicity directed against several modified neo-epitopes. Moreover, the ensuing inflammatory response may promote the progression of oxidative stress and further degrade functionally active lipids and proteins. In brief, there is a great breadth and depth of evidence implicating inflammation as an important mediator of the relationship between oxidative stress and depression.

1.4.6.1.6 Platelet-activating factor activity

Platelet-activating factors (PAFs) are a family of potent pro-inflammatory phospholipids that have recently been linked to depression (Mazereeuw et al. 2013). Specifically, our group found that higher plasma concentrations of the PAFs phosphocholine (PC)(O-12:0/2:0), PC(O-14:1/2:0), PC(O-17:3/2:0), and PC(O-18:3/2:0) were significantly associated with depressive symptom severity in a sample of 26 depressed patients with CAD (Mazereeuw et al. 2015b). In addition to their demonstrated pro-inflammatory properties, these lipid species are also associated with oxidative stress. Indeed, PAFs have been shown to stimulate ROS and RNS production (Klabunde and Anderson 2002, Kubes et al. 1991, Kurose et al. 1996, Schmidt et al. 1992, Schwappach et al. 1995). Conversely, the RNS peroxynitrite
can potentiate the pro-inflammatory and pro-oxidant actions of PAFs by attacking PAF-acetylhydrolase (PAF-AH), an enzyme responsible for PAF inactivation (MacRitchie et al. 2007). Despite the relative dearth of research on this novel family of lipidomic biomarkers, it appears that PAFs share a bi-directional relationship with oxidative stress that may partially account for their potential involvement in the etiopathology of depression.

1.4.6.2 Current Evidence for Oxidative Stress in Depression

Just as the vasculature is vulnerable to oxidative stress due to its high lipid content and oxygen consumption, the CNS is also highly susceptible to oxidative damage due to its high metabolic rate and its abundance of lipids, which are major constituents of neuronal membranes as well as the myelin sheaths that help conduct efficient neuronal signalling (Joshi and Pratico 2014). In particular, the peroxidation of membrane phospholipids containing ω-6 PUFAs such as AA yields harmful compounds regardless of the specific metabolic pathway involved. AA can be metabolized to pro-inflammatory series thromboxanes, leukotrienes and prostaglandins via COX-mediated enzymatic oxidation (Funk 2001). Alternatively, it can be processed via non-enzymatic, free radical-mediated lipid peroxidation to yield LPH and, eventually, end-stage metabolites such as 4-HNE and 8-ISO should anti-oxidant defenses be unable to neutralize LPH (Porter et al. 1995). Elevated levels of these compounds may not only reflect dysregulations in the aforementioned processes that promote oxidative stress but also confer pro-atherogenic and depressogenic effects. Indeed, 4-HNE readily forms protein adducts and can induce pro-apoptotic signalling pathways when present in high concentrations (Csala et al. 2015, Dalleau et al. 2013). 8-ISO is also a potent vasoconstrictor in the brain microvasculature that induces the formation of thromboxane in the endothelium, which then contracts the vascular smooth muscle and causes endothelial cell death (Basu 2008). Thromboxane, in turn, can induce 8-ISO generation and produce a positive feedback loop of
vascular damage. Thus, high levels of these AA-derived metabolites may exacerbate depressive pathology.

Evidence implicating oxidative stress in depression has been indirectly provided by a multitude of animal and clinical studies demonstrating elevated levels of biomarkers of oxidative damage to macromolecules and pro-oxidant enzymes, as well as impaired anti-oxidant defenses characterized by reduced levels of enzymatic and non-enzymatic anti-oxidants, in relation to depression. Indeed, recent meta-analyses have revealed that depressed patients, compared to controls, have significantly higher serum concentrations of the ROS peroxide \( \text{O}_2^\cdot \), pro-oxidant enzymes XO and MAO, and 8-oxodG, a marker of oxidatively modified DNA; and significantly lower serum concentrations of the anti-oxidant-enhancing enzyme paraoxonase (PON-1) as well as the non-enzymatic anti-oxidants albumin, zinc, and uric acid (Black et al. 2015, Jimenez-Fernandez et al. 2015, Liu et al. 2015). Moreover, treatment with antidepressants normalized the levels of the majority of these markers. Corroborating these findings are port-mortem studies showing excess pro-oxidant (Che et al. 2010, Michel et al. 2010) and impaired anti-oxidant (Gawryluk et al. 2011, Michel et al. 2007) activity in the brain tissue of patients with depression. In regard to lipid peroxidation markers specifically, our group recently conducted a meta-analytic review indicating increased lipid peroxidation as measured by LPH, ox-LDL, malondialdehyde (MDA), and 8-ISO in depressed patients free of co-morbid disease compared to their non-depressed counterparts (Mazereeuw et al. 2015a). Antidepressant pharmacotherapy was also associated with a significant reduction in these markers in this study.

Those clinical findings have been complemented by a number of pre-clinical studies in which rodents are subjected to chronic stress paradigms designed to induce a depressive-like state characterized by anhedonic and amovitational behaviour. Such experimental models have
demonstrated reductions in anti-oxidant activity as measured by SOD, CAT, glutathione, and GPX concentrations (Che et al. 2015, Cline et al. 2015, Lucca et al. 2009a, Zafir and Banu 2007) and elevations in central lipid peroxidation as measured by MDA concentrations (Che et al. 2015, Lucca et al. 2009a, Lucca et al. 2009b) in several key brain regions underlying emotion including the hippocampus and PFC. Furthermore, treatment with antidepressants has been found to concomitantly abrogate depressive-like behaviour and normalize oxidative stress parameters (Garg and Kumar 2008, Kumar and Garg 2009, Zafir and Banu 2007, Zafir et al. 2009). Collectively, those findings not only confirm data from clinical studies but also suggest that a pro-oxidant state in the CNS may mirror that of the periphery.

1.4.6.3 Evidence for Selected Biomarkers

To date, a number of studies have assessed LPH, 4-HNE, and 8-ISO in relation to depression or depressive symptoms [Table 1]. Importantly, many of these studies failed to control for medication use or anti-oxidant supplementation and none was comprised of a CAD population, inviting investigation into lipid peroxidation markers in relation to depressive symptoms in CAD.
Table 1. Clinical studies assessing lipid peroxidation as measured by LPH, 4-HNE, and 8-ISO concentrations (n = 14).

<table>
<thead>
<tr>
<th>Study</th>
<th>Marker</th>
<th>Patient Population</th>
<th>Sample size T (D / C)</th>
<th>Age (years)</th>
<th>Gender (% male)</th>
<th>Medium</th>
<th>Diagnostic Method</th>
<th>Assay</th>
<th>Significant association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black et al. (2016)</td>
<td>8-ISO</td>
<td>Community adults</td>
<td>2,968 (460 / 2,508)</td>
<td>40 ± 4</td>
<td>46%</td>
<td>Plasma</td>
<td>CES-D</td>
<td>GC / MS</td>
<td>+/-</td>
</tr>
<tr>
<td>Chung et al. (2009)</td>
<td>8-ISO</td>
<td>Fibromyalgia patients</td>
<td>48 (28 / 20)</td>
<td>46 ± 12</td>
<td>2%</td>
<td>Urine</td>
<td>CES-D</td>
<td>GC / MS</td>
<td>-</td>
</tr>
<tr>
<td>Chung et al. (2013)</td>
<td>8-ISO</td>
<td>Young adults</td>
<td>54 (18 / 36)</td>
<td>32 ± 8</td>
<td>33%</td>
<td>Urine</td>
<td>HAM-D; POMS</td>
<td>GC / MS</td>
<td>+</td>
</tr>
<tr>
<td>Dimopoulos et al. (2008)</td>
<td>8-ISO</td>
<td>Community elderly</td>
<td>66 (33 / 33)</td>
<td>66 ± 3</td>
<td>39%</td>
<td>Plasma</td>
<td>GDS</td>
<td>ELISA</td>
<td>+</td>
</tr>
<tr>
<td>Milaneschi et al. (2013)</td>
<td>8-ISO</td>
<td>Community elderly</td>
<td>M: 1027 (31 / 996) F: 948 (52 / 896)</td>
<td>75 ± 3</td>
<td>52%</td>
<td>Urine</td>
<td>GDS</td>
<td>RIA</td>
<td>+</td>
</tr>
<tr>
<td>Panee et al. (2015)</td>
<td>4-HNE</td>
<td>HIV patients and Meth users</td>
<td>123 (100 / 23)</td>
<td>41 ± 2</td>
<td>92%</td>
<td>CSF</td>
<td>CES-D</td>
<td>ELISA</td>
<td>+/-</td>
</tr>
<tr>
<td>Pomara et al. (2012)</td>
<td>8-ISO</td>
<td>Community elderly</td>
<td>47 (28 / 19)</td>
<td>67 ± 6</td>
<td>53%</td>
<td>CSF</td>
<td>SCID</td>
<td>ELISA</td>
<td>+</td>
</tr>
<tr>
<td>Rawdin et al. (2013)</td>
<td>8-ISO</td>
<td>Young adults</td>
<td>39 (19 / 20)</td>
<td>37 ± 11</td>
<td>36%</td>
<td>Plasma</td>
<td>SCID</td>
<td>GC / MS</td>
<td>-</td>
</tr>
<tr>
<td>Segal et al. (2012)</td>
<td>8-ISO</td>
<td>SLE patients</td>
<td>122 (71 / 51)</td>
<td>42 ± 2</td>
<td>9%</td>
<td>Plasma</td>
<td>CES-D</td>
<td>GC / MS</td>
<td>+</td>
</tr>
<tr>
<td>Selley (2004)</td>
<td>4-HNE</td>
<td>Healthy adults</td>
<td>50 (25 / 25)</td>
<td>47 ± 11</td>
<td>50%</td>
<td>Plasma</td>
<td>SCID</td>
<td>GC / MS</td>
<td>+</td>
</tr>
<tr>
<td>Tsuboi et al. (2004)</td>
<td>LPH</td>
<td>Community adults</td>
<td>75 (n/a)</td>
<td>55 ± 10</td>
<td>0%</td>
<td>Serum</td>
<td>CES-D; GHQ</td>
<td>Hb-MB</td>
<td>+</td>
</tr>
<tr>
<td>Vargas et al. (2013)</td>
<td>LPH</td>
<td>Outpatients</td>
<td>340 (140 / 201)</td>
<td>18 – 60</td>
<td>34%</td>
<td>Plasma</td>
<td>HAM-D</td>
<td>FOX</td>
<td>+/-</td>
</tr>
<tr>
<td>Wolkowitz et al. (2011)</td>
<td>8-ISO*</td>
<td>Young adults</td>
<td>35 (18 / 17)</td>
<td>37 ± 11</td>
<td>34%</td>
<td>Serum</td>
<td>SCID</td>
<td>GC / MS</td>
<td>-</td>
</tr>
<tr>
<td>Yager et al. (2010)</td>
<td>8-ISO</td>
<td>Young adults</td>
<td>145 (73 / 72)</td>
<td>29 ± 9</td>
<td>17%</td>
<td>Serum</td>
<td>HAM-D</td>
<td>ELISA</td>
<td>+</td>
</tr>
</tbody>
</table>
*Oxidative stress defined as 8-ISO / Vitamin C ratio

Abbreviations: C = controls; CSF = cerebrospinal fluid; D = depressed patients; ELISA = enzyme-linked immunosorbent assay; F = females; FOX = modified ferric oxide-xylene orange assay; GC / MS = gas chromatography / mass spectrometry; GDS = Geriatric Depression Scale; GHQ = Depression score of the General Health Questionnaire; HAM-D = Hamilton Rating Scale for Depression; Hb-MB = hemoglobin-methylene blue; LPH = lipid hydroperoxides; M = males; Meth = N-methylamphetamine; POMS = Profile of Mood States; RIA = radioimmunoassay; SCID = depression module of the Structured Clinical Interview for DSM Axis I Disorders; SLE = systemic lupus erythematosus; T = total; 4-HNE = 4-hydroxy-2-nonenal; 8-ISO = 8-isoprostane.

Confounders adjusted for in some but not all studies: sociodemographics (age, sex, ethnicity, education, income); cardiovascular risk factors (smoking, alcohol consumption, BMI, physical activity); presence of chronic somatic disease (e.g., cerebrovascular disease, diabetes) that may influence oxidative stress parameters; use of antidepressant therapies or anti-oxidant supplements

+ indicates positive (significant) findings; - indicates negative (non-significant) findings; + / - indicates equivocal findings (i.e., trending association or finding only significant in a certain demographic sub-group)
1.5 Summary of Background

Oxidative stress and its resultant damage to lipids represent an emerging mechanism implicated in the pathophysiology of depression and CAD, two frequently co-morbid conditions thought to share common etiopathological ground. Indeed, oxidative stress may interact bi-directionally and synergistically with several aberrant mechanisms associated with depression in CAD including serotonergic imbalance, HPA axis dysregulation, impaired neurogenesis and neuroplasticity, mitochondrial dysfunction, chronic low-grade inflammation, and PAF hyperactivity.

As the presently available antidepressant treatment options for CAD patients are limited, this high-risk population could benefit from reliable biomarkers that can correlate with disease severity and help to better characterize the pathways underlying depression in CAD. Lipid peroxidation is promising in this regard and may be an important correlate of depressive symptoms featuring clinically useful biomarkers in CAD.
2 Materials and Methods

2.1 Study Design

This cross-sectional, observational study investigated the association between depressive symptoms and serum lipid peroxidation markers of CAD. Accordingly, participants were recruited and assessed upon entry into their 6-month cardiac rehabilitation (CR) program, prior to any exercise intervention. Study participants were recruited from two rehabilitation centres: Toronto Rehabilitation Institute (TRI) and Trillium Cardiac Centre (THC). The research ethics boards of both participating institutions approved this study [Appendix A].

2.2 Eligibility Criteria

Patients were eligible for enrolment in the study if they met the following inclusion criteria:

- Age 45-80
- Language (spoke and understood English)
- Diagnosis of CAD based on at least one of the following:
  - angiographic evidence of ≥ 50% stenosis in ≥ 1 major coronary artery
  - previous hospitalization for acute MI
  - prior revascularization procedure (i.e., CABG or PTCA)
- Stable CAD based on no recent (last 4 weeks) hospitalization for cardiac events such as acute MI, unstable angina, congestive heart failure, ventricular arrhythmias, coronary revascularization, or Canadian Cardiovascular Society Class IV angina
- Statin medication use (due to its effects on mood and cognition (Kim et al. 2015, Power et al. 2015) and its widespread use in our patient population (Swardfager et al. 2010))
- Written, informed consent [Appendix B]

Potential participants were excluded from the study if they met any of the following criteria:
Significant acute medical illness (active cancer; anemia; autoimmune condition; drug overdose; hypothyroidism; sepsis; severely impaired kidney, liver, or lung function; uncontrolled diabetes mellitus)

Clinically significant cognitive impairment (MMSE < 24) (Perry et al. 2000)

Other neurologic conditions (birth trauma; brain tumour; clinical stroke; history of epilepsy; Huntington’s chorea; multiple sclerosis; Parkinson’s disease; progressive supranuclear paralysis; subdural hematoma; traumatic brain injury)

Premorbid or current psychiatric diagnosis of any condition other than depression, anxiety, or nicotine dependence

Killip Class III or IV states (indicating high risk of mortality in post-MI patients)

Antidepressant medication use for < 3 months at stable dose

2.3 Demographics and Medical History

After informed consent was obtained from eligible study participants, demographics and clinical characteristics including age, gender, marital status, employment status, socioeconomic status, level of education, smoking history, personal and family psychiatric history, and relevant medical history including co-morbidities independent of CAD were collected from patient interviews. Concomitant medication use and anthropometric, cardiopulmonary, and metabolic data such as heart rate, blood pressure, weight, height, BMI, body fat percentage, VO₂ peak (an indicator of cardiopulmonary fitness) were collected from physical and electronic medical records at the CR centres.

2.4 Assessments

Depressive symptom severity was assessed using the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff 1977). Depression was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria (American
using the depression module of the Structured Clinical Interview for DSM IV Axis I Disorders (SCID) (First et al. 1996). All relevant assessments that were administered to patients are presented in Appendix C.

2.4.1 Center for Epidemiological Studies Depression Scale (CES-D)

The CES-D, used to test the primary hypothesis of the study, is a 20-item, patient-rated questionnaire that measures a variety of symptoms relating to multiple domains of depressive symptomatology (Radloff 1977). It has been established as a reliable tool to assess the severity of depressive symptoms in CAD populations (Dowlati et al. 2010b, McManus et al. 2005, Rudisch and Nemeroff 2003, Swardfager et al. 2010, Swardfager et al. 2011a, Swardfager et al. 2011b), with a score of 16 or greater indicating clinically significant depressive symptoms.

2.4.2 Structured Clinical Interview for Depression (SCID)

The researcher-rated SCID (First et al. 1996), used to test the secondary hypothesis of the study, comprises 9 questions that assess the presence of various symptoms characteristic of a depressive episode according to the Diagnostic and Statistical Manual of Mental Disorders IV (American Psychiatric Association 1994). Diagnostic criteria consist of the following: depressed mood; anhedonia (i.e., inability to experience pleasure); changes in weight and/or appetite; insomnia or hypersomnia; fatigue or loss of energy; feelings of guilt or worthlessness; diminished ability to think or make decisions; and suicidal ideation. Subjects who experience depressed mood and/or anhedonia as well as 5 out of the 9 above criteria for the majority (i.e., for at least 2 weeks) of the past month are considered to have experienced a major depressive episode. Those who meet 3-4 of the 9 overall criteria, including depressed mood and/or anhedonia, over the same time period are deemed to have experienced a minor depressive episode.
2.5 Blood Collection and Assays

Four millilitres of blood were collected by antecubital venipuncture from each study participant following 12 hours of fasting. Most assessments and blood draws were scheduled for 0900 h ± 1 h in order to ensure minimal diurnal or dietary variation that may influence marker concentrations. Blood was drawn into EDTA-containing vacutainer tubes and centrifuged (The Drucker Company; Model 614B) at 1000 x g for 10 minutes. Serum was then isolated and frozen at -80°C. Stored samples were batched for analysis with spectrophotometric assays by Alex Adibfar under the supervision of Dr. Ana Andreazza, an oxidative stress biomarker expert and collaborator in the study. The inter- and intra-assay coefficients of variation, as well as the R² value for each standard, were calculated and are presented in Section 3.3.

2.5.1 Lipid Hydroperoxides (LPH) Assay

Early-stage oxidative damage to lipids was evaluated by measuring the levels of LPH (Cayman Chemical; Item No. 705002) according to manufacturer’s instructions. LPH was extracted from samples by addition of 1 unit of deoxygenated methanol saturated with LPH Assay Extract R and 2 units of deoxygenated cold chloroform per unit of sample. Assay tubes were centrifuged at 1500 x g for 5 minutes at 0°C to isolate the bottom chloroform extract layer to be used in the assay. The chloroform layer was then extracted by carefully inserting a syringe needle along the side of the test tube. Particular attention was paid to avoid collecting the middle protein layer or upper water layer, as any water carried over to the assay tube can interfere with colour development. Following this extraction step, the assay was performed by adding 0.9 unit of 2:1 chloroform-methanol solvent mixture and 0.1 unit of chromogen mixture per unit of chloroform extract. Samples were kept at room temperature for 5 minutes, loaded into 96-well glass plates, and absorbance was then read at 500 nm using a microplate reader.
As the final solvents that were loaded are highly volatile, samples were measured in quadruplicate rather than in duplicate in order to optimize data reliability. Standard hydroperoxide curves were generated for each plate and background absorbance was subtracted from all values before comparing the corrected average absorbance of each sample to determine LPH concentrations, expressed in µM, by interpolation. The sensitivity of this assay kit is 2.5-5 nm of LPH.

2.5.2 4-Hydroxy-2-nonenal (4-HNE) Assay

Late-stage lipid peroxidation was in part assessed by measuring 4-HNE (Cell Biolabs, Inc.; STA-338), which was quantified by standard sandwich enzyme-linked immunosorbent assay (ELISA) designed to detect protein adducts formed via 4-HNE Michael Addition to lysine, histidine, or cysteine residues. Samples were loaded in triplicate, and results were expressed in fmol/µg of protein after comparing average corrected absorbance of each sample with standard curves generated for each plate. The sensitivity of this assay is 0.5-10 µg/mL of 4-HNE.

2.5.3 8-Isoprostone (8-ISO) Assay

Late-stage oxidative damage to lipids was also evaluated by measuring 8-ISO (Cayman Chemical; Item No. 516351), which was quantified with a standard competitive sandwich ELISA using an 8-ISO-acetylcholinesterase conjugate as a tracer and 8-ISO-specific rabbit antiserum. As 8-ISO and the tracer compete for limited antiserum binding, the colour intensity, which reflects tracer binding to the well, is proportional to the amount of tracer. Thus, unlike the LPH and 4-HNE assays, colour development is inversely proportional to the amount of 8-ISO in the well. Samples were loaded in duplicate, and results were expressed in pg/mL after comparing average corrected absorbance of each sample with standard curves generated for each plate. The sensitivity of this assay is 0.8-500 pg/mL of 8-ISO.
2.6 Statistical Analyses

As lipid peroxidation marker levels are positively skewed, the LPH, 4-HNE, and 8-ISO concentrations were log-transformed (base of 10) in order to reduce between-patient variability and normalize the data for subsequent analyses. The following log-transformed ratios were also computed to reflect lipid peroxidation progression: LPH / 4-HNE; LPH / 8-ISO; and LPH / (4-HNE + 8-ISO). In order to avoid negative values that would disrupt the meaning of the ratio, marker concentrations were multiplied by 10 before being log-transformed.

In the primary analysis, a linear regression model was used to assess the association between log-transformed 8-ISO concentration and depressive symptom severity, as measured by CES-D scores, while adjusting for a priori covariates. Variables that were significantly associated with either log-transformed LPH concentrations or CES-D scores were also added to the linear regression model in post-hoc analyses. In the secondary analysis, an analysis of covariance (ANCOVA) model was used to assess mean differences in log-transformed 8-ISO concentration between depressed and non-depressed CAD patients, according to the SCID, while adjusting for the same a priori covariates. Exploratory analyses assessing log-transformed 4-HNE and LPH concentrations, as well as the computed lipid peroxidation ratios, in relation to depressive symptom severity also used linear regression models. For the linear regression models, normality of residuals was confirmed by visual inspection of normal probability plots, and multicollinearity between predictors was assessed and reported as tolerance statistics. Statistical analyses were performed using IBM SPSS version 20 (Chicago, IL, USA). All analyses were 2-tailed, with p value significance at ≤ 0.05.

2.6.1 Covariates

2.6.1.1 Age
Our group has previously found that younger age was associated with greater depressive symptoms in CAD patients (Swardfager et al. 2008). Oxidative stress and its resultant damage to lipids have been thought to contribute to aging and chronic, degenerative disease (Davalli et al. 2016, Maurya et al. 2016, Wolkowitz et al. 2011b). Indeed, concentrations of lipid peroxidation markers are consistently associated with age [Table 1].

2.6.1.2 Gender

Women are more likely than men to experience depression with an earlier onset, longer duration, and greater severity (Naqvi et al. 2005). Accordingly, our group has previously found that female gender was associated with greater depressive symptoms in CAD patients (Swardfager et al. 2008). Sex differences in oxidative stress parameters, while poorly understood, have been observed in clinical studies including those reviewed in Table 1.

2.6.1.3 BMI

BMI has been found to share a U-shaped association with depression, in which underweight (BMI < 18.5) and obese (BMI ≥ 30) individuals feature the greatest prevalence of depression (de Wit et al. 2009, Martin-Rodriguez et al. 2016). Oxidative stress, as measured by various markers, has also been reported to increase continuously with BMI (Block et al. 2002, Keaney et al. 2003, Ramos et al. 2008).

2.6.1.4 Smoking Status

Cigarette smoking and nicotine dependence are associated with depression (Nunes et al. 2013). Smoking promotes oxidative stress, causing tissue damage and resulting in the degradation of lipids and extracellular matrix proteins (Church and Pryor 1985). Additionally, even acute smoke exposure can influence oxidative stress parameters as indicated by a number of a human and animal studies (van der Vaart et al. 2004).

2.6.1.5 Antidepressant Medication Use
While antidepressants are most directly associated with depression, they have been shown to normalize oxidative stress markers in a number of studies including a recent meta-analysis of clinical studies (Jimenez-Fernandez et al. 2015, Liu et al. 2015, Mazereeuw et al. 2015a).

2.6.1.6 Use of Anti-Oxidant Supplements

As the objective of this study is to assess pro-oxidant markers in relation to depressive symptom severity, it was important to account for compounds with definitive anti-oxidant properties. Based on the clinical literature assessing their ability to modulate markers of oxidative stress in vivo, zinc, coenzyme Q10, and vitamins C, D, and E were defined as anti-oxidants for the purpose of this study (Bray and Bettger 1990, Gautam et al. 2012, Ghanwat et al. 2016, Ide et al. 2002, Khajehnasiri et al. 2016, Littarru and Tiano 2007, Sepehrmanesh et al. 2016).

2.7 Sample Size Calculation

Sample size was determined based on effect size estimates from prior reports assessing depressive symptoms in relation to 8-ISO concentration, which ranged from 0.05 to 0.43 [Table 1]. A medium effect size was chosen for this study to reflect the average effect size reported in the literature. Using IBM SPSS SamplePower 3.0 (Chicago, IL, USA), it was found that a linear regression model with 7 pre-determined predictors would require a sample of 180 participants in order to achieve 82% power \(1 - \beta\) and detect a medium effect size of \(r = 0.3\) with a two-tailed significance level \(\alpha\) of 0.05. A recruitment target of 210 participants was set in order to account for lost data arising from incomplete assessments and/or compromised samples.
3 Results

3.1 Participant Recruitment

Between 2010 and 2015, 1607 CAD patients entering a CR program were approached to be contacted for participation in the study; 964 accepted to be contacted by study personnel; and 530 provided informed consent to participate in the study. Of those, 204 met eligibility criteria and were enrolled into the study. Reasons for exclusion are presented in Figure 1.

Figure 1. Participant flow through each stage of study recruitment. Potential participants were approached at local CR centres.
3.2 Participant Characteristics

Demographics and clinical characteristics of the 204 study completers are shown below [Table 2]. Continuous data are presented as mean ± standard deviation, and categorical data are presented as number (and percentage) of participants. Associations with log-transformed 8-ISO concentrations or CES-D scores were determined with one-way analyses of variance (ANOVAs) or bivariate Pearson correlations and are reported as F or r values for categorical or continuous data, respectively.

| Table 2. Demographic and clinical characteristics of study participants (n = 204). |
|-----------------------------------------------|-------------------|--------------------------|
| | Mean ± SD or n (%) | Association with log-transformed [8-ISO] (p ≤ 0.05)* | Association with CES-D (p ≤ 0.05)* |
| **Sociodemographics** | | | |
| Age (years) | 62.7 ± 7.4 | r = -0.13, p = 0.06 | r = -0.18, p = 0.01* |
| Gender (# of males) | 165 (80.9) | F = 1.27, p = 0.26 | F = 6.2, p = 0.01* |
| Ethnicity (# of Caucasians) | 161 (78.9) | F = 0.58, p = 0.68 | F = 0.85, p = 0.50 |
| Total education (years) | 15.8 ± 3.5 | r < 0.01, p = 0.99 | r = -0.19, p = 0.01* |
| Marital status (# married) | 106 (52.0) | F = 2.94, p = 0.02* | F = 4.86, p < 0.01* |
| Employment (# employed) | 117 (57.1) | F = 0.01, p = 0.93 | F = 1.91, p = 0.17 |
| Annual income (SK Cdn) | 103.6 ± 124.7 | r = 0.04, p = 0.59 | r = -0.17, p = 0.02* |
| **Body Composition** | | | |
| Weight (kg) | 84.9 ± 16.6 | r < 0.01, p = 0.95 | r = -0.03, p = 0.63 |
| BMI (kg/m²) | 28.9 ± 5.1 | r = 0.05, p = 0.46 | r = 0.08, p = 0.23 |
| Waist circumference (cm) | 98.9 ± 12.3 | r = 0.06, p = 0.36 | r = 0.09, p = 0.22 |
| Body fat (%) | 31.3 ± 10.7 | r = 0.10, p = 0.17 | r = 0.07, p = 0.35 |
| **Resting Physiology** | | | |
| Resting heart rate (bpm) | 69.9 ± 12.7 | r = 0.16, p = 0.03* | r = 0.19, p = 0.01* |
| Resting systolic blood pressure (mm Hg) | 124.4 ± 18.4 | r = -0.12, p = 0.09 | r = -0.13, p = 0.86 |
| Resting diastolic blood pressure (mm Hg) | 75.8 ± 10.1 | r = -0.05, p = 0.52 | r = -0.02, p = 0.79 |
| **Cardiopulmonary Fitness Parameters** | | | |
| Maximum heart rate (bpm) | 120.6 ± 20.0 | r = -0.11, p = 0.13 | r = -0.01, p = 0.85 |
| Maximum systolic blood pressure (mm Hg) | 168.9 ± 27.5 | r = -0.08, p = 0.27 | r < 0.01, p = 0.98 |
| Maximum diastolic blood pressure (mm Hg) | 78.7 ± 11.4 | r < 0.00, p = 0.97 | r = 0.04, p = 0.62 |
| VO₂ peak (mL/kg/min) | 20.1 ± 5.6 | r = -0.21, p < 0.01* | r = -0.17, p = 0.01* |

Vascular Risk Factors and Medical Co-morbidities
| Smoking status (# of past / current smokers) | 124 (60.8) | F = 2.0, p = 0.13 | F = 4.9, p = 0.01* |
| Alcohol consumption (drinks per week) | 3.4 ± 5.1 | r = 0.03, p = 0.66 | r = -0.02, p = 0.80 |
| COPD | 7 (3.4) | F = 0.49, p = 0.49 | F = 0.72, p = 0.40 |
| Diabetes | 41 (20.1) | F = 2.52, p = 0.11 | F = 1.43, p = 0.23 |
| Hypercholesterolemia | 181 (88.7) | F = 15.24, p < 0.01* | F = 1.89, p = 0.17 |
| Hyperlipidemia | 203 (99.5) | F = 1.41, p = 0.24 | F = 0.64, p = 0.43 |
| Hypertension | 128 (62.7) | F = 0.02, p = 0.90 | F = 0.82, p = 0.37 |

### Cardiac History (# with History)

| Angina | 15 (7.4) | F = 0.16, p = 0.69 | F = 1.59, p = 0.21 |
| MI | 101 (49.5) | F = 0.89, p = 0.35 | F = 1.47, p = 0.23 |
| CABG | 66 (32.4) | F = 0.30, p = 0.59 | F = 0.78, p = 0.38 |
| PTCA | 133 (65.2) | F = 0.36, p = 0.55 | F = 1.24, p = 0.27 |

### CAD Severity

| Cumulative stenosis (%) | 166.7 ± 80.0 | r = 0.18, p = 0.04* | r = 0.05, p = 0.60 |
| Number of stenosed vessels | 2.2 ± 1.0 | r = 0.06, p = 0.42 | r < 0.01, p > 0.99 |
| Time since last ACS (weeks) | 39.1 ± 107.5 | r = 0.17, p = 0.02* | r = 0.10, p = 0.17 |

### Concomitant Medications (%)

| Anti-arrhythmic | 7 (3.4) | F = 1.72, p = 0.19 | F = 0.02, p = 0.90 |
| Anti-hypertensive | 150 (73.5) | F = 0.20, p = 0.66 | F = 0.17, p = 0.68 |
| Anti-oxidants | 48 (23.5) | F = 4.31, p = 0.04* | F = 2.26, p = 0.13 |
| Coenzyme Q | 9 (4.4) | F = 1.67, p = 0.20 | F = 0.47, p = 0.49 |
| Zinc | 3 (1.5) | F = 0.01, p = 0.91 | F = 0.18, p = 0.67 |
| Vitamin C | 19 (9.3) | F = 3.50, p = 0.06 | F = 0.70, p = 0.40 |
| Vitamin D | 34 (16.7) | F = 1.52, p = 0.22 | F = 1.77, p = 0.19 |
| Vitamin E | 9 (4.4) | F = 6.05, p = 0.02* | F = 1.84, p = 0.18 |
| Beta blocker | 154 (75.5) | F = 1.25, p = 0.26 | F = 4.42, p = 0.04* |
| Calcium channel blocker | 35 (17.2) | F = 9.72, p < 0.01* | F = 9.21, p < 0.01* |
| Diuretic | 31 (15.2) | F = 0.01, p = 0.91 | F = 0.07, p = 0.79 |
| Platelet inhibitor (incl. ASA) | 196 (96.1) | F = 0.12, p = 0.73 | F = 0.42, p = 0.52 |
| Anti-inflammatory (NSAID) | 5 (2.5) | F = 0.07, p = 0.79 | F = 0.23, p = 0.63 |
| Statin | 204 (100) | N/A | N/A |

Abbreviations: ACS = acute coronary syndrome; ASA = acetylsalicylic acid; BMI = body mass index; CABG = coronary artery bypass grafting; CES-D = Center for Epidemiological Studies Depression Scale; COPD = chronic obstructive pulmonary disease; MI = myocardial infarction; N/A = not applicable; NSAID = non-steroidal anti-inflammatory drug; PTCA = percutaneous coronary angioplasty; SD = standard deviation; VO₂ peak = peak oxygen uptake; 8-ISO = 8-isoprostane; $K Cdn = thousand dollars Canadian

Additionally, depression characteristics and use of psychotropic medications are summarized and presented in relation to log-transformed 8-ISO concentrations in Table 3.

Patients with major depression, minor depression, or overall SCID-diagnosed depression, a
variable encompassing patients with major and minor depression, had significantly greater log-transformed 8-ISO concentration than their non-depressed counterparts.

Table 3. Depression characteristics of study participants (n = 204).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD or n (%)</th>
<th>Association with log-transformed [8-ISO] (p ≤ 0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CES-D score</td>
<td>10.2 ± 10.3</td>
<td>r = 0.33, p &lt; 0.01*</td>
</tr>
<tr>
<td>SCID-diagnosed depression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major depression</td>
<td>53 (26.0)</td>
<td>F = 17.54, p &lt; 0.01*</td>
</tr>
<tr>
<td>Minor depression</td>
<td>26 (12.7)</td>
<td>F = 17.01, p &lt; 0.01*</td>
</tr>
<tr>
<td>History of depression</td>
<td>52 (25.5)</td>
<td>F = 5.06, p = 0.03*</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>12 (5.9)</td>
<td>F = 12.79, p &lt; 0.01*</td>
</tr>
<tr>
<td>Anxiolytic use</td>
<td>10 (4.9)</td>
<td>F = 0.01, p = 0.92</td>
</tr>
</tbody>
</table>

Table 4. Overview of serum lipid peroxidation marker concentrations (n = 204).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>SD</th>
<th>Range (Min – Max)</th>
<th>Association with CES-D (p ≤ 0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPH (µM)**</td>
<td>30.36</td>
<td>27.15</td>
<td>0.62 – 144.38</td>
<td>β = -0.234, p = 0.001*</td>
</tr>
<tr>
<td>4-HNE (fmol/µg)</td>
<td>43.02</td>
<td>12.67</td>
<td>18.63 – 96.22</td>
<td>β = 0.051, p = 0.468</td>
</tr>
<tr>
<td>8-ISO (pg/mL)</td>
<td>56.18</td>
<td>47.15</td>
<td>4.60 – 294.53</td>
<td>β = 0.348, p &lt; 0.001*</td>
</tr>
<tr>
<td>log-transformed LPH</td>
<td>1.30</td>
<td>0.43</td>
<td>-0.21 – 2.16</td>
<td>β = -0.222, p = 0.002*</td>
</tr>
<tr>
<td>log-transformed 4-HNE</td>
<td>1.62</td>
<td>0.12</td>
<td>1.27 – 1.98</td>
<td>β = 0.075, p = 0.288</td>
</tr>
<tr>
<td>log-transformed 8-ISO</td>
<td>1.61</td>
<td>0.36</td>
<td>0.59 – 2.47</td>
<td>β = 0.332, p &lt; 0.001*</td>
</tr>
</tbody>
</table>

**n = 196, as 8 patients had undetectable concentrations of serum LPH
3.4 Addressing the Hypotheses

3.4.1 Primary Hypothesis

Using a linear regression model, log-transformed 8-ISO concentration was found to be a significant predictor of depressive symptom severity as measured by the CES-D (β = 0.265, p < 0.001). Higher log-transformed 8-ISO concentration was associated with greater CES-D score [Figure 3]. Table 5 shows a summary of the significant model, which was adjusted for the following covariates: age, gender, BMI, smoking status, antidepressant use, and antioxidant use. As shown in Table 6, younger age (β = -0.154, p = 0.020), female gender (β =
0.159, p = 0.015), past or current smoking (β = 0.211, p = 0.001), and antidepressant medication use (β = 0.130, p = 0.052) were also significant predictors of depressive symptom severity in this model, while BMI (β = -0.261, p = 0.794) and use of anti-oxidant supplements (β = -0.050, p = 0.447) were not significantly associated with CES-D score. Multicollinearity was not a problem in this model, as all tolerance values were greater than 0.400 [Table 6].

Figure 3. The association between log-transformed 8-ISO concentration and depressive symptoms measured by the CES-D in CAD patients (n = 204).
Table 5. Summary of the linear regression model examining the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204).

<table>
<thead>
<tr>
<th>Model</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>0.187</td>
<td>7.692</td>
<td>203</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Table 6. Final model in the linear regression analysis examining the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204).

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>t</th>
<th>p value (≤ 0.05)*</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>1.114</td>
<td>0.267*</td>
<td>-</td>
</tr>
<tr>
<td>log-transformed 8-ISO</td>
<td>0.265</td>
<td>3.991</td>
<td>&lt; 0.001*</td>
<td>0.900</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.154</td>
<td>-2.341</td>
<td>0.020*</td>
<td>0.934</td>
</tr>
<tr>
<td>Gender</td>
<td>0.159</td>
<td>2.461</td>
<td>0.015*</td>
<td>0.961</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.018</td>
<td>-0.272</td>
<td>0.786</td>
<td>0.920</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.211</td>
<td>3.286</td>
<td>0.001*</td>
<td>0.973</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>0.130</td>
<td>1.956</td>
<td>0.052*</td>
<td>0.883</td>
</tr>
<tr>
<td>Anti-oxidant use</td>
<td>-0.050</td>
<td>-0.762</td>
<td>0.447</td>
<td>0.926</td>
</tr>
</tbody>
</table>

Furthermore, residuals were normally distributed in this model [Figure 4], as well as in all subsequent linear regression analyses, as assessed by normal probability plots.
3.4.2 Secondary Hypothesis

Using an ANCOVA model, it was found that depressed CAD patients (n = 53) featured significantly greater mean log-transformed 8-ISO concentrations than their non-depressed counterparts (n = 150) according to the SCID (F_{1,194} = 8.860, p = 0.003) [Table 7] [Table 8] [Figure 5]. The same a priori covariates used to test the primary hypothesis were included in the ANCOVA. Antidepressant medication use (F_{1,202} = 7.354, p = 0.007) was also found to be significantly associated with depression as classified by the SCID, whereas age (F_{1,194} = 0.734, p = 0.393), gender (F_{1,194} = 0.601, p = 0.439), BMI (F_{1,194} = 0.109, p = 0.742), smoking status (F_{1,194} = 1.120, p = 0.328), and use of anti-oxidant supplements (F_{1,194} = 1.351, p = 0.247) were
not [Table 8]. The sample size for this analysis was 203 participants, as one patient did not complete the SCID.

Table 7. log-transformed 8-ISO concentrations in the depressed and non-depressed groups of CAD patients according to the depression module of the SCID (n = 203).

<table>
<thead>
<tr>
<th></th>
<th>Depressed (major or minor) (n = 53)</th>
<th>Non-depressed (n = 150)</th>
<th>Significance (p ≤ 0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-transformed 8-ISO concentration</td>
<td>1.78 ± 0.32</td>
<td>1.55 ± 0.35</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

Figure 5. Boxplot illustrating differences in log-transformed 8-ISO concentrations of CAD patients divided into SCID-classified depression groups (n = 203).
Table 8. Coefficients of an ANCOVA analysis detecting differences in mean log-transformed 8-ISO concentrations between depressed and non-depressed CAD patients according to the SCID (n = 203).

<table>
<thead>
<tr>
<th>Source</th>
<th>df error</th>
<th>F</th>
<th>p value (≤ 0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>194</td>
<td>4.077</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>194</td>
<td>53.314</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SCID-diagnosed depression</td>
<td>194</td>
<td>8.860</td>
<td>0.003*</td>
</tr>
<tr>
<td>Age</td>
<td>194</td>
<td>0.734</td>
<td>0.393</td>
</tr>
<tr>
<td>Gender</td>
<td>194</td>
<td>0.601</td>
<td>0.439</td>
</tr>
<tr>
<td>BMI</td>
<td>194</td>
<td>0.109</td>
<td>0.742</td>
</tr>
<tr>
<td>Smoking status</td>
<td>194</td>
<td>1.120</td>
<td>0.328</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>194</td>
<td>8.130</td>
<td>0.005*</td>
</tr>
<tr>
<td>Anti-oxidant use</td>
<td>194</td>
<td>1.351</td>
<td>0.247</td>
</tr>
</tbody>
</table>

Adjusted $R^2 = 0.109$

3.4.3 Exploratory Analyses

3.4.3.1 Exploratory Hypothesis 1: 4-HNE

Using a linear regression model, it was found that log-transformed 4-HNE concentration was not a significant predictor of depressive symptom severity as measured by the CES-D ($\beta = 0.044$, $p = 0.512$) [Figure 6]. Table 9 shows a summary of the significant model, which was adjusted for the following covariates: age, gender, BMI, smoking status, antidepressant use, and anti-oxidant use. As shown in Table 10, younger age ($\beta = -0.183$, $p = 0.008$), female gender ($\beta = 0.184$, $p = 0.007$), current or past smoking ($\beta = 0.206$, $p = 0.002$), and antidepressant use ($\beta = 0.187$, $p = 0.006$) were significant predictors of depressive symptom severity in this model, while BMI ($\beta = -0.023$, $p = 0.741$) and use of anti-oxidant supplements ($\beta = -0.075$, $p = 0.270$) were not significantly associated with CES-D score.

Multicollinearity was not a problem in this model, as all tolerance values were greater than 0.400 [Table 10].
Figure 6. The association between log-transformed 4-HNE concentration and depressive symptoms measured by the CES-D in CAD patients (n = 204). $R^2 = 0.006$

Table 9. Summary of the linear regression model examining the association between log-transformed 4-HNE concentration and CES-D score in CAD patients (n = 204).

<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>$F$</th>
<th>df</th>
<th>p value ($\leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.123</td>
<td>5.083</td>
<td>203</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Table 10. Final model in the linear regression analysis examining the association between log-transformed 4-HNE concentration and CES-D score in CAD patients (n = 204).

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$</th>
<th>$t$</th>
<th>p value ($\leq 0.05$)*</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>1.519</td>
<td>0.130</td>
<td>-</td>
</tr>
<tr>
<td>log-transformed 4-HNE concentration</td>
<td>0.044</td>
<td>0.656</td>
<td>0.512</td>
<td>0.973</td>
</tr>
</tbody>
</table>
Exploratory Hypothesis 2: LPH

Using a linear regression model, a significant inverse association was found between log-transformed LPH concentration and depressive symptom severity as measured by the CES-D (β = -0.150, p = 0.030), in which higher log-transformed LPH concentration predicted lower CES-D score [Figure 7]. Table 11 shows a summary of the significant model, which was adjusted for the following covariates: age, gender, BMI, smoking status, antidepressant use, and use of anti-oxidant compounds. As shown in Table 12, younger age (β = -0.173, p = 0.013), female gender (β = 0.158, p = 0.022), current or past smoking (β = 0.194, p = 0.005), and antidepressant use (β = 0.167, p = 0.016) were significant predictors of depressive symptom severity in this model, whereas BMI (β = -0.009, p = 0.892) and use of anti-oxidant supplements (β = -0.086, p = 0.219) were not. The sample size for this analysis was 196 participants, as 8 patients had undetectable LPH concentrations. Multicollinearity was not a problem in this model, as all tolerance values were greater than 0.400 [Table 12].

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>t</th>
<th>p</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.183</td>
<td>-2.698</td>
<td>0.008*</td>
<td>0.937</td>
</tr>
<tr>
<td>Gender</td>
<td>0.184</td>
<td>2.744</td>
<td>0.007*</td>
<td>0.962</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.023</td>
<td>-0.331</td>
<td>0.741</td>
<td>0.920</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.206</td>
<td>3.071</td>
<td>0.002*</td>
<td>0.963</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>0.187</td>
<td>2.771</td>
<td>0.006*</td>
<td>0.948</td>
</tr>
<tr>
<td>Anti-oxidant use</td>
<td>-0.075</td>
<td>-0.075</td>
<td>0.270</td>
<td>0.932</td>
</tr>
</tbody>
</table>
Figure 7. The association between log-transformed LPH concentration and depressive symptoms measured by the CES-D in CAD patients (n = 196). $R^2 = 0.049$

Table 11. Summary of the linear regression model examining the association between log-transformed LPH concentration and CES-D score in CAD patients (n = 196).

<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>F</th>
<th>df</th>
<th>p value $(\leq 0.05)^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.143</td>
<td>5.634</td>
<td>195</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Table 12. Final model in the linear regression analysis examining the association between log-transformed LPH concentration and CES-D score in CAD patients (n = 196).

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$</th>
<th>t</th>
<th>p value $(\leq 0.05)^*$</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>3.562</td>
<td>&lt; 0.001*</td>
<td>-</td>
</tr>
<tr>
<td>log-transformed LPH concentration</td>
<td>-0.150</td>
<td>-2.190</td>
<td>0.030*</td>
<td>0.934</td>
</tr>
<tr>
<td>Age</td>
<td>-0.173</td>
<td>-2.515</td>
<td>0.013*</td>
<td>0.931</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----</td>
<td>-----</td>
<td>----------</td>
<td>-----</td>
</tr>
<tr>
<td>Gender</td>
<td>0.158</td>
<td>2.310</td>
<td>0.022*</td>
<td>0.942</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.009</td>
<td>-0.136</td>
<td>0.892</td>
<td>0.910</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.194</td>
<td>2.871</td>
<td>0.005*</td>
<td>0.959</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>0.167</td>
<td>2.426</td>
<td>0.016*</td>
<td>0.930</td>
</tr>
<tr>
<td>Anti-oxidant use</td>
<td>-0.086</td>
<td>-1.235</td>
<td>0.219</td>
<td>0.916</td>
</tr>
</tbody>
</table>

3.4.3.3 Exploratory Hypothesis 3: Ratios

Ratios \([\text{LPH} / (4\text{-HNE} + 8\text{-ISO})]\) were computed and assessed to reflect lipid peroxidation progression i.e., the relative abundance of early-stage in relation to late-stage lipid peroxidation markers. A lower value indicates a greater degree of progression, as LPH is an early-stage marker of oxidative damage to lipids, while 4-HNE and 8-ISO are late-stage products (Esterbauer et al. 1991, Morrow et al. 1990, Porter et al. 1995). Since the value of a few serum marker concentrations were between 0 and 1, data were processed by multiplying the concentration of each marked by 10 before being log-transforming them and entering them into the ratio quotient in order to both ensure normal distribution and avoid negative log-transformed values that would perturb the meaning of the ratio [Figure 8].
Figure 8. Distribution of processed early- to late-stage lipid peroxidation marker ratio. Each marker was multiplied by 10 and log-transformed to normalize the data, and the ratio was then computed to reflect lipid peroxidation progression.

Using a linear regression model, the processed lipid peroxidation ratio was found to be a significant predictor of depressive symptom severity as measured by the CES-D ($\beta = -0.236$, $p = 0.001$). A lower ratio, indicating greater lipid peroxidation progression, was associated with greater CES-D score [Figure 9]. Table 13 shows a summary of the significant model, which was adjusted for the following covariates: age, gender, BMI, smoking status, antidepressant use, and use of anti-oxidant compounds. As shown in Table 14, younger age ($\beta = -0.160$, $p = 0.019$), female gender ($\beta = 0.148$, $p = 0.029$), past or current smoking ($\beta = 0.188$, $p = 0.005$), and antidepressant use ($\beta = 0.142$, $p = 0.038$) were also significant predictors of...
depressive symptom severity in this model, whereas BMI (β = -0.012, p = 0.860) and use of anti-oxidant supplements (β = -0.080, p = 0.239) were not. Multicollinearity was not a problem in this model, as all tolerance values were greater than 0.400 [Table 14].

Figure 9. The association between processed early- to late-stage lipid peroxidation ratio and depressive symptoms measured by the CES-D in CAD patients (n = 196). R² = 0.100

Table 13. Summary of the linear regression model examining the association between early- to late-stage lipid peroxidation ratio and CES-D score in CAD patients (n = 196).

<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>0.174</td>
<td>6.850</td>
<td>195</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Table 14. Final model in the linear regression analysis examining the association between early- to late-stage lipid peroxidation ratio and CES-D score in CAD patients (n = 196).

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>t</th>
<th>p value (≤ 0.05)*</th>
<th>Tolerance</th>
</tr>
</thead>
</table>

53
Additionally, more specific ratios of LPH to 4-HNE or 8-ISO, respectively, were computed and analyzed using linear regression models in order to further explore the association between lipid peroxidation progression and depressive symptom severity. Both the processed LPH / 4-HNE ratio ($\beta = -0.159$, $p = 0.021$) and the processed LPH / 8-ISO ratio ($\beta = -0.280$, $p < 0.001$) were significant predictors of depressive symptom severity in their respective models when adjusting for the same covariates. As with previous analyses, younger age, female gender, past or current smoking, and antidepressant use were also predictors of depressive symptom severity in each of these two models, whereas BMI and use of anti-oxidant supplements were not.

### 3.5 Post-hoc Analyses

Any variables found to be significantly associated with either log-transformed 8-ISO concentration or depressive symptom severity as measured by the CES-D were assessed in post-hoc analyses in order to account for any potential confounding of the observed findings. Using bivariate correlational analyses or one-way ANOVAs, it was found that the following 10 variables needed to be accounted for in post-hoc analyses: marital status, annual income, resting heart rate, VO2 peak, hypercholesterolemia, beta blocker use, calcium channel blocker use, personal history of depression, cumulative stenosis, and time since last ACS. Since data pertaining to CAD severity were only obtained for 135 patients, a single linear regression
analysis including all 6 *a priori* and 10 *post hoc* predictors could not be run. Instead, each potential confounder was individually added to the original model to assess its significance as an independent predictor and its influence on the overall model [Table 15].

Using separate linear regression models, it was found that marital status (not married i.e., single, divorced, or widowed) ($\beta = 0.157, p = 0.017$), lower annual income ($\beta = -0.173, p = 0.009$), and personal history of depression ($\beta = 0.321, p < 0.001$) were significant predictors of depressive symptom severity as measured by total CES-D score, whereas resting heart rate ($\beta = 0.080, p = 0.240$), VO$_2$ peak ($\beta = -0.040, p = 0.579$), hypercholesterolemia ($\beta = -0.006, p = 0.932$), beta blocker use ($\beta = -0.070, p = 0.290$), calcium channel blocker use ($\beta = 0.090, p = 0.204$), cumulative stenosis ($\beta = 0.016, p = 0.854$), and time since last ACS ($\beta = 0.030, p = 0.651$) were not [Table 15]. Importantly, none of these predictors eliminated the significant association between log-transformed 8-ISO concentration and CES-D score when included in the original linear regression model that additionally adjusted for age, gender, BMI, smoking status, antidepressant use, and use of anti-oxidant supplements.

**Table 15. Summary of the post hoc linear regression models including potential confounders of the association between log-transformed 8-ISO concentration and CES-D score in CAD patients (n = 204).**

<table>
<thead>
<tr>
<th>Added predictor</th>
<th>Overall model statistics ($\beta_{\text{predictors, adjusted } R^2, \text{ p value}}$)</th>
<th>df</th>
<th>log-transformed [8-ISO] statistics ($\beta_{\text{8-ISO, p value}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td>$\beta = 0.157, R^2 = 0.207, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.234, p = 0.001$</td>
</tr>
<tr>
<td>Annual income</td>
<td>$\beta = -0.173, R^2 = 0.225, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.304, p &lt; 0.001$</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td>$\beta = 0.080, R^2 = 0.186, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.245, p &lt; 0.001$</td>
</tr>
<tr>
<td>VO$_2$ peak</td>
<td>$\beta = -0.040, R^2 = 0.185, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.255, p &lt; 0.001$</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>$\beta = -0.006, R^2 = 0.183, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.264, p &lt; 0.001$</td>
</tr>
<tr>
<td>Beta blocker use</td>
<td>$\beta = -0.070, R^2 = 0.188, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.260, p &lt; 0.001$</td>
</tr>
<tr>
<td>Calcium channel blocker use</td>
<td>$\beta = 0.090, R^2 = 0.190, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.249, p &lt; 0.001$</td>
</tr>
<tr>
<td>Personal history of depression</td>
<td>$\beta = 0.321, R^2 = 0.280, p &lt; 0.001$</td>
<td>203</td>
<td>$\beta = 0.215, p = 0.001$</td>
</tr>
<tr>
<td>Cumulative stenosis</td>
<td>$\beta = 0.016, R^2 = 0.121, p &lt; 0.001$</td>
<td>134</td>
<td>$\beta = 0.246, p = 0.004$</td>
</tr>
<tr>
<td>Time since last ACS</td>
<td>$\beta = 0.030, R^2 = 0.201, p &lt; 0.001$</td>
<td>134</td>
<td>$\beta = 0.273, p &lt; 0.001$</td>
</tr>
</tbody>
</table>
4 Discussion and Conclusion

4.1 Summary of Findings

Oxidative stress, and ensuing lipid peroxidation, have been implicated in etiopathological processes associated with both CAD and depression (Bonomini et al. 2008, Maes et al. 2011a, Vogiatzi et al. 2009). Moreover, the high prevalence of depression in CAD (Burg and Abrams 2001, Carney et al. 1987, Frasure-Smith et al. 1993, 1995a, Ladwig et al. 1991, Lesperance and Frasure-Smith 2003, Patten et al. 2006, Schleifer et al. 1989) renders the study cohort ideal for evaluating the role of lipid peroxidation in depressive symptoms. Based on a number of clinical studies assessing lipid peroxidation markers in relation to depression [Table 1], it was hypothesized that higher log-transformed concentrations of 8-ISO would predict greater depressive symptom severity in individuals with CAD. 8-ISO was selected as the candidate marker for the primary hypothesis for several reasons: 1) it is a late-stage marker of lipid peroxidation (Moore and Roberts 1998, Porter et al. 1995) ideal for an older population with several CVRFs and medical co-morbidities that predispose to high oxidative stress parameters (Young et al. 2016); 2) it is widely considered to be the gold standard for quantifying oxidative stress in vivo due to its chemical stability and reliable assays (Milne et al. 2011, Moore and Roberts 1998, Niki 2008, Roberts et al. 2005); and 3) out of the selected markers, it was the most extensively investigated in the clinical literature [Table 1]. In this study, log-transformed 8-ISO concentration significantly predicted depressive symptom severity as measured by the CES-D, with greater marker levels correlating to higher CES-D scores. This association survived correction for each potential confounding variable. Moreover, the secondary analysis found that patients with depression diagnosed by the SCID featured significantly higher mean concentrations of log-transformed 8-ISO compared to those without depression.
Exploratory analyses revealed surprising and intriguing findings. 4-HNE was not a significant predictor of depressive symptom severity in our sample. LPH was found to be so but in the opposite direction of the anticipated association. Lower log-transformed LPH concentration predicted greater depressive symptom severity after adjusting for age, gender, BMI, smoking status, antidepressant use, and use of anti-oxidant supplements. Accordingly, the ratios \([LPH / (4\text{-HNE} + 8\text{-ISO})]\) computed and analyzed to assess lipid peroxidation staging also predicted greater depressive symptom severity, with lower ratios – indicating greater lipid peroxidation progression – being associated with greater CES-D scores.

### 4.2 Interpretation of Results

The present study is the first to investigate lipid peroxidation markers as predictors of depressive symptom severity in CAD patients. When assessing a CAD population with medical co-morbidities such as depression, the biochemical mediators common to both conditions should be a primary treatment focus. Due to its well-established association with CAD and depression (Bonomini et al. 2008, Maes et al. 2011a, Vogiatzi et al. 2009), lipid peroxidation is ideally suited for the exploration of CAD-relevant biomarkers for depressive symptoms. To this end, it was found that greater depressive symptoms were significantly associated with higher 8-ISO and lower LPH. At first glance, these results seem discordant, as it was hypothesized that LPH would be positively correlated with depressive symptom severity based on their status as a lipid peroxidation marker and the two clinical studies finding them to be positively associated with depression [Table 1]. Upon further inspection, however, it is conceivable that patients with greater depressive symptoms have a biomarker profile characterized by conversion of LPH into late-stage markers such as 4-HNE and 8-ISO. Indeed, the findings obtained in our exploratory analyses appear to be consistent with this notion and
suggest that lipid peroxidation may be an important correlate of depression with clinically useful biomarkers in CAD.

Elevated 8-ISO levels may also reflect an abundance of its parent compound, the ω-6 AA, which can also be broken down via enzymatic oxidation catalyzed by COX enzymes to yield a pro-inflammatory leukotriene, prostaglandin, and thromboxane compounds (Funk 2001). Moreover, 8-ISO itself can activate thromboxane A₂, thereby enhancing platelet aggregation and impairing vascular tone (Basu 2008). Increased AA metabolism may in part arise from deficits in the ω-3 PUFAs, EPA and docosahexaenoic acid (DHA), which are also vulnerable to oxidation due to their many double bonds and therefore act as competitors in regard to AA breakdown (Gao et al. 2006, Nourooz-Zadeh et al. 1997, Nourooz-Zadeh et al. 1998, Roberts et al. 1998). However, products of enzymatic ω-3 PUFA metabolism, such as the EPA-derived resolvin eicosanoids, are thought to be largely anti-inflammatory mediators (Yates et al. 2014). Thus, excess 8-ISO may primarily indicate an altered PUFA metabolic pathway featuring a shift from anti-inflammatory ω-3 to pro-inflammatory ω-6 metabolites, which may arise from the differential availabilities of ω-3 versus ω-6 PUFAs in the periphery.

There is evidence to support this proposed metabolic state, as lower ω-3 PUFAs have been associated with depressive symptoms in a number of studies (Adams et al. 1996, Lotrich et al. 2013, Maes et al. 1999a, Peet et al. 1998, Tiemeier et al. 2003), and a lower ratio of (EPA + DHA) / AA in particular has been recently been associated with greater depressive symptom severity by our group (Mazereeuw et al. 2016). Furthermore, ω-3 supplementation has been shown to reduce depressive-like behaviour while restoring serotonin deficits in pre-clinical studies (Pudell et al. 2014, Vancassel et al. 2008) and to abrogate the onset of depressive symptoms in a clinical study in which it was administered prior to IFN-α treatment (Lotrich et al. 2013). In regard to oxidative stress parameters, ω-3 PUFA supplementation has been shown
to demonstrate anti-oxidant effects in pre-clinical models (Mori and Beilin 2004) and to lower lipid peroxidation marker levels, including F_2-isoprostane concentrations, in a number of clinical studies (Baek and Park 2013, Lee et al. 2013, Mas et al. 2010, Mori et al. 2000).

Therefore, it is possible that in relation to depressive symptoms, 8-ISO is an indirect marker of parent PUFA imbalance.

4-HNE also arises from the AA-derived non-enzymatic metabolism of LPH (Porter et al. 1995). It was selected as the candidate reactive aldehyde over MDA due to its involved role in cellular signalling (Csala et al. 2015, Dalleau et al. 2013) and because its immunoassay is more reliable than the thiobarbituric acid reactive substances (TBARS) assay, which is frequently used to measure MDA but induces artefactual aldehyde production (Niki 2014). In addition to its use as an indicator of lipid peroxidation, 4-HNE is highly bioactive. It can modulate various signalling pathways due to its tendency to form protein adducts, promoting the expression of pro-inflammatory transcription factors such as NF-κB and AP-1, and is accordingly associated with CAD and neurodegeneration when present in excess (Csala et al. 2015, Dalleau et al. 2013). Paradoxically, however, it has also been suggested to possess pleiotropic effects, inducing the expression of atheroprotective anti-oxidant genes such as heme oxygenase-1 (HO-1), leading to its own detoxification through this self-regulatory mechanism (Siow et al. 2007). This may, in part, account for the lack of any notable findings in this population and suggest that perhaps 4-HNE may be more useful as biomarker of disease severity in conditions of extreme oxidative stress such as unstable CAD or end-stage neurodegenerative diseases.

Finally, the mean concentration of 30 µM of LPH in this sample, which was similar to that of a previous study measuring serum LPH in stable CAD patients (Walter et al. 2008), was significantly greater than that of studies assessing participants free of CAD, which featured
mean serum LPH concentrations of < 1 µM (Tsuboi et al. 2004, Vargas et al. 2013). While LPH was elevated in relation to depression in such somatically healthy patients [Table 1], it is possible that in a CAD population with high baseline LPH, the inverse association between depressive symptom severity and LPH may in part reflect a relatively intact anti-oxidant system in those with greater LPH levels, which prevents further degradation into cytotoxic metabolites, as well as a potential cytoprotective signalling role of LPH in up-regulating expression of antioxidants such as GPx (Girotti 1998).

4.3 Limitations

4.3.1 Methodological

While the study was adequately powered for its primary analysis, measures of CAD severity, which were found to be significantly associated with the primary outcome variables, were only assessed in 135 patients. As such, the post-hoc analyses did not feature a sufficiently large sample to allow for the inclusion of all covariates in a single, all-encompassing model [Table 15]. Furthermore, the secondary analysis was hindered by its relatively low proportion (26%) of depressed participants, which is not ideal for group-based statistical tests. While this figure is fairly representative of the CAD population at large, the low preponderance of depressive symptoms may have arisen in part from selection bias, as patients were recruited at entry into a CR program. It is possible that a wealthy, highly educated cohort with the motivation and socioeconomic means to both enroll in CR and volunteer to participate in a research study may be less depressed than and not entirely representative of the general CAD population. Extrapolations from these findings should therefore be made with caution. Other potential limitations regarding the study design were the absence of a somatically healthy control group and the low percentage (19%) of female participants. This gender discrepancy may in part arise from a CR referral bias as suggested by a recent meta-analysis indicating that
CR referral is significantly lower for women than men (Colella et al. 2015). However, as CAD is nonetheless more prevalent in men (Maas and Appelman 2010) and oxidative stress is associated with several chronic age-associated diseases (Davalli et al. 2016, Maurya et al. 2016, Wolkowitz et al. 2011b), the study deliberately intended to capture a group of aging individuals at high risk for depression and other medical co-morbidities, while excluding the most glaring potential confounders such as other psychiatric conditions.

Measurement errors present another set of methodological limitations of the present study. Depression and depressive symptomatology are complex phenomena that are often temporally dynamic and difficult to capture, particularly with self-reported measures. The CES-D, though validated in the CAD literature (Dowlati et al. 2010b, McManus et al. 2005, Rudisch and Nemeroff 2003, Swardfager et al. 2010, Swardfager et al. 2011a, Swardfager et al. 2011b) and possessing high sensitivity and specificity (Beekman et al. 1997, Roberts and Vernon 1983), only assesses items relating to depressive symptoms that were experienced during the week preceding the assessment [Appendix C]. It is therefore subject to large fluctuations in response to stressful events that might influence self-reported mood transiently but not permanently. The SCID covers a longer time period of one month, which represents an improvement but nonetheless might not yield as accurate results as an assessment of a longer timespan such as a year [Appendix C]. Additionally, the CES-D does not possess all the ideal characteristics of a dependent variable in a linear regression analysis. In reality, it is a scalar rather than a continuous measure, as depressive symptoms are quantified with integer-scored items. Moreover, another consequence of the low rates of depression and depressive symptoms in our sample was the positively skewed distribution of the total CES-D score. Nonetheless, a linear regression model should be robust enough to overcome those shortcomings, particularly given that marker concentrations were successfully normalized [Figure 2].
In regard to assay methodology, 8-ISO is presently considered to be the gold standard for measuring oxidative stress in vivo as previously discussed (Milne et al. 2011, Moore and Roberts 1998, Niki 2008, Roberts et al. 2005). However, as with any colorimetric assay, the competitive sandwich ELISA ultimately provides indirect 8-ISO quantification based on colour development. Compounding this inherent shortcoming is the fact that results from immunoassays have not always been found to correlate with those from chromatography-based techniques (Proudfoot et al. 1999). This discrepancy may partly arise from cross-reactivity between the antibodies used to bind 8-ISO and structurally related compounds such as COX-derived prostaglandins, which can artificially alter results. While the Cayman LPH Assay circumvents the problem of over-estimating the LPH concentration due to endogenous ferric ions, which are abundant in blood samples, by extracting LPH into chloroform, this derivation of the FOX assay still possesses numerous drawbacks. Since the reaction that produces the assay’s ferric thiocyanate chromophore involves the generation of alkoxy radicals, which participate in the aforementioned propagation step of lipid peroxidation (Porter et al. 1995), the LPH assay inevitably induces artefactual lipid peroxidation and thus might unduly influence the observed findings (Niki 2014). Moreover, the samples were extracted into highly volatile solvents before being loaded into the glass plate and read by the microplate reader. As such, running the samples in quadruplicate and loading only a small portion of the 96-well plate per readout to minimize evaporation prevented some, but not all, loss of colour development. Another potential limitation affecting all the measured peripheral markers is the artefactual generation or loss of lipid peroxidation products during sampling or storage (Niki 2009, Yoshida et al. 2013). Indeed, significant ex vivo auto-oxidation has even been demonstrated in plasma 8-ISO, the most stable lipid peroxidation marker, stored on ice for 36 hours (Wu et al. 2004). Despite their widespread use and our storage at -80°C, use of appropriate internal
standards, and avoidance of freezing-thawing cycles, little is known regarding the long-term ex vivo stability of these biomarkers when samples are stored for periods greater than 8 months (Janicka et al. 2010).

Additionally, this study is cross-sectional in nature and accordingly precludes any assessment of causality between lipid peroxidation and depressive symptoms. Thus, longitudinal studies are needed to ascertain whether a greater amount and progression of lipid peroxidation induces or results from depressive pathophysiology. Finally, findings from peripheral measurements intended to reflect aberrant CNS processes should be interpreted with caution, particularly with respect to incompletely understood mechanisms such as oxidative stress. However, given that peripheral and central indices of lipid peroxidation have each been independently associated with depression [Table 1] and that our collaborators have recently demonstrated a relationship between serum lipid peroxidation measured by LPH and white matter alterations in adults with bipolar disorder (Versace et al. 2014), it appears that peripherally-measured lipid peroxidation products may be clinically relevant biomarkers for mood disorders.

4.3.2 Mechanistic Considerations

With respect to the lipid peroxidation markers themselves, 4-HNE (Esterbauer et al. 1991) and 8-ISO (Morrow et al. 1990) are selectively formed from the free radical-mediated, non-enzymatic breakdown of AA, a pro-inflammatory ω-6 PUFA. LPH, however, are more ubiquitous markers. For example, they can arise from DHA and then be further processed to 4-hydroxy-2-hexenal (4-HHE), a distinct reactive aldehyde (Long et al. 2008), or a family of isoprostane-like compounds termed neuroprostanes (Nourooz-Zadeh et al. 1998, Roberts et al. 1998). They can also be intermediary products of ω-3 PUFA metabolism, being formed from EPA and subsequently converted to another group of compounds called the F3-isoprostanes.
As such, it cannot be inferred with certainty that the profile seen with the lipid peroxidation ratios results entirely from a conversion of LPH into 4-HNE and 8-ISO via AA-derived peroxidation. Nonetheless, \( \frac{\text{LPH}}{4\text{-HNE} + 8\text{-ISO}} \) can provide an approximation of lipid peroxidation progression and has recently been used in a clinical study conducted by our colleagues (Scola et al. 2016).

While the measurement of three distinct products of lipid peroxidation provided a more reliable and comprehensive set of findings than those from previous clinical studies [Table 1], which mostly measured only one marker, this study assessed only one side of the redox balance. Anti-oxidants constantly act to quench ROS and neutralize LPH back to their parent lipids (Forman et al. 2014). Importantly, recent meta-analyses have shown that depressed patients may have impaired anti-oxidant activity as reflected by lower levels of endogenous anti-oxidants (Black et al. 2015, Jimenez-Fernandez et al. 2015, Liu et al. 2015). Thus, we were unable to determine the degree to which the association between greater depressive symptom severity and lipid peroxidation progression was mediated by impaired anti-oxidant capacity. Furthermore, we did not assess lipid peroxidation in conjunction with related aberrant mechanisms associated with depression such as pro-inflammatory cytokine expression, which can potentiate oxidative stress (Maes et al. 2012a).

Additionally, it appears that oxidative stress and ensuing lipid peroxidation may be an important correlate of depression in CAD, but redox dysregulation is not unique to depression and has been linked to many other psychiatric disorders such as bipolar disorder and schizophrenia (Joshi and Pratico 2014). While the study design accounted for potentially-confounding conditions, it is unlikely that lipid peroxidation presents a biomarker profile that is highly specific to depression. The results of this study must therefore be interpreted cautiously when evaluating the potential clinical utility of these markers. Further compounding
the issue of non-specificity is the reality that lipid peroxidation proceeds largely via non-
enzymatic, free-radical mediated pathways (Porter et al. 1995), rendering it difficult to develop
targeted therapies such as small-molecule inhibitors. Nonetheless, anti-oxidants that mitigate
the deleterious effects of pro-oxidant compounds including lipid peroxidation products may
have clinical potential as antidepressant agents, particularly for treatment-resistant individuals
with elevated oxidative stress such as CAD patients. Indeed, NAC (Berk et al. 2008, Berk et al.
2014, Magalhaes et al. 2011), zinc (Nowak et al. 2003, Siwek et al. 2010, Swardfager et al.
2013), and vitamins C and E (Brody 2002, Cocchi et al. 1980) have all shown some clinical
promise, but most of the evidence supporting their antidepressant benefits remains limited to
pre-clinical studies. Phytochemicals with anti-oxidant properties, such as curcumin and the
aforementioned naringenin and sulforaphane, have also been found to confer antidepressant-
like and neuroprotective effects in experimental models, but their safety and efficacy as
potential adjuncts to antidepressant pharmacotherapy have yet to be thoroughly characterized
in clinical studies (Bahramsoltani et al. 2015).

4.4 Future Directions

High levels of the selected end-stage lipid peroxidation markers might reflect a
preponderance of AA, an established pro-inflammatory and pro-oxidant mediator associated
with increased peripheral concentrations of IL-6 and CRP (Dinan et al. 2009, Lotrich et al.
2013, Schmitz and Ecker 2008). As such, it would be interesting to associate the observed
findings in conjunction with ω-3 / ω-6 fatty acid ratio as measured by (EPA + DHA) / AA,
which has recently been implicated as a potential marker of depressive symptom severity in
CAD patients by our group (Mazereeuw et al. 2016). Furthermore, it would be interesting to
complement these data with concomitant measurements of the F₃-isoprostanes and F₄-
neuroprostanes, the structurally analogous end-products of EPA (Gao et al. 2006, Nourooz-
Zadeh et al. 1997) and DHA (Nourooz-Zadeh et al. 1998, Roberts et al. 1998) metabolism, respectively. While F₄ neuroprostanes (derived from DHA) are thought to be markers of neuronal injury like the F₂-isoprostanes (derived from AA) (Miller et al. 2014), emerging evidence suggests that F₃-isoprostanes (derived from EPA) might confer a cardioprotective effect by reducing F₂-isoprostane production (Alkazemi et al. 2016). Thus, measuring a more complete array of PUFAs together with their metabolites might reveal a more informative biomarker profile as well as provide useful mechanistic insights into the importance of PUFA metabolite distribution in regard to neurodegeneration and depression in CAD.

In keeping with this notion, the complex clinical presentation of depression may in part reflect a diverse network of inter-related central and peripheral processes with genetic and environmental contributions. Indeed, a recent systematic review conducted by our group found that no single thoroughly-studied marker has been able to consistently predict depression in CAD (Adibfar et al. 2016). Thus, defining a profile of changes in a number of important mechanistic pathways may be the key to uncovering diagnostically and prognostically useful markers and, eventually, biomarker-guided therapies. Ideally, future studies should assess several markers relating to multiple inter-dependent processes. For example, longitudinally measuring the IL-6 / IL-10 ratio as an indicator of the balance between pro- and anti-inflammatory cytokine activity in addition to anti-oxidant capacity as well as the aforementioned PUFA metabolism biomarker profile may reveal insights into the complex interplay between the two associated processes.

Additionally, different depressive symptom clusters may be associated with varying clinical outcomes. For example, cognitive depressive symptoms are associated with increased risk of coronary artery calcification (Stewart et al. 2012), while somatic depressive symptoms have been linked to an elevated inflammatory profile (Liu et al. 2016) and increased risk of
acute ischemic events (Davidson et al. 2005, Deverts et al. 2010, Stewart et al. 2007, Stewart et al. 2009). Future research efforts intending to replicate and expand upon the findings of this study should explore the differential association between lipid peroxidation and distinct clusters of depressive symptoms to further elucidate the parameters of biomarker utility.

Future studies should also aim to identify a distinct sub-type of depressed individuals characterized by excess pro-oxidant and impaired anti-oxidant activity. Research into inflammation-associated depression, a closely related process that has been more thoroughly studied and is particularly relevant to CAD, has revealed certain depressed patients with excessive pro-inflammatory activity are resistant to conventional antidepressant pharmacotherapy but may experience improved mood when given anti-inflammatory agents (Liu et al. 2016). As such, assessing oxidative stress in addition to inflammatory parameters in this treatment-resistant sub-group might not only better characterize the pathways underlying depression in CAD but also lead to targeted adjunctive antidepressant therapies that may be efficacious in this population.

4.5 Conclusion

In this cross-sectional study of 204 CAD patients, higher log-transformed concentrations of 8-ISO, a late-stage marker of lipid peroxidation, predicted greater depressive symptom severity. This association remained significant when adjusting for age, gender, BMI, smoking status, antidepressant use, anti-oxidant use, and a number of other potential confounders. Another late-stage marker, 4-HNE, did not exhibit any significant association in relation to depression, while the early-stage marker, LPH, was found to be inversely associated with depressive symptom severity. A lower ratio of early- to late-stage lipid peroxidation markers [LPH / (4-HNE + 8-ISO)], indicating greater lipid peroxidation progression, significantly predicted greater depressive symptoms. Collectively, these results suggest that
oxidative stress and ensuing lipid peroxidation may be an important correlate of depression in CAD. Future studies investigating the association between depression and a greater number and variety of related markers, particularly longitudinally, are warranted.
5 References


Alkazemi D, Jackson RL, Man Chan H, Kubow S. Increased F3-Isoprostanes in the Canadian Inuit Population Could be Cardioprotective by Limiting F2-Isoprostane Production. J Clin Endocrinol Metab 2016; jc20154096.


Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1990; 87(4): 1620-4.


Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci U S A 1987; 84(21): 7725-9.


Gocmen AY, Sahin E, Semiz E, Gumuslu S. Is elevated serum ceruloplasmin level associated with increased risk of coronary artery disease? Can J Cardiol 2008b; 24(3): 209-12.

Goldstein LE, Leopold MC, Huang X, Atwood CS, Saunders AJ, Hartshorn M, Lim JT, Faget KY, Muffat JA, Scarpa RC, Chylack LT, Jr., Bowden EF, Tanzi RE, Bush AI. 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen peroxide and


Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142(2): 231-55.


Koga M, Serritella AV, Sawa A, Sedlak TW. Implications for reactive oxygen species in schizophrenia pathogenesis. Schizophr Res 2015.


Lenaz G. The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. IUBMB Life 2001; 52(3-5): 159-64.


Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. J Affect Disord 2012; 139(3): 230-9.


Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011b; 35(3): 702-21.


Martins JG, Bentsen H, Puri BK. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry 2012; 17(12): 1144-9; discussion 63-7.


Patten SB, Williams JV, Lavorato DH, Bulloch AG, MacQueen G. Depressive episode characteristics and subsequent recurrence risk. J Affect Disord 2012; 140(3): 277-84.


Selley ML. Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. J Affect Disord 2004; 80(2-3): 249-56.


Wu CL, Chen SD, Yin JH, Hwang CS, Yang DI. Nuclear Factor-kappaB-Dependent Sestrin2 Induction Mediates the Antioxidant Effects of BDNF Against Mitochondrial Inhibition in Rat Cortical Neurons. Mol Neurobiol 2015.


List of Publications and Abstracts

Peer-Reviewed Publications


Published Abstracts


Appendices

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

From: Dr. Philip Hébert  

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. Hebert, MD PhD FCFPC  OR  Miriam Shuchman, MD
Chair, Research Ethics Board  Vice-Chair, Research Ethics Board
January 30, 2012

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

Dear Dr. Lancot:

**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 *PHIPA*.

Toronto Rehab is a teaching and research hospital fully affiliated with the University of Toronto.
Page 2
January 30, 2012
Dr. Krista Lanctot

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,

[签名]

[ ] Paul Oh MD, MSc, FRCP, 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 PHIPA
Appendix B – Informed Consent Form
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 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 Science 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 medical treatment.

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. Philip C. Hébert, Chair of the Sunnybrook Research Ethics Board at (416) 480-4276.

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

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 25 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 Co-ordinator, Maisha Khan (416-480-6100 x3185), Dr. Krista Lanctôt (416-480-6100 x2241) or Dr. Paul Oh (416-597-3422 x5263).

Should you have any questions about your rights as a research subject, you may 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

<table>
<thead>
<tr>
<th>Name of participant/Substitute decision-maker (print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Name of Person obtaining consent (print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Name of Investigator (print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C – Assessments
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 | 1-2 Days | 3-4 Days | 5-7 Days
--- | --- | --- | ---
Rarely or Never | Some or Little of the Time | Occasionally or a Moderate amount of Time | Most or All of the Time

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

Total CES-D Score

---

104
## Structured Clinical Interview for the DSM-IV—Depression Module (SCID)

The following two questions relate to the patient’s mood over the last month.

<table>
<thead>
<tr>
<th>Question</th>
<th>Response Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has there been a period of time when you were feeling depressed or</td>
<td>Yes</td>
</tr>
<tr>
<td>down most of the time nearly every day? What was that like?</td>
<td></td>
</tr>
<tr>
<td>If yes: How long did it last?</td>
<td></td>
</tr>
<tr>
<td>Rate if depression lasts most of the day, nearly every day</td>
<td></td>
</tr>
<tr>
<td>2. What about losing interest or pleasure in things you usually</td>
<td>Yes</td>
</tr>
<tr>
<td>enjoyed?</td>
<td></td>
</tr>
<tr>
<td>If yes: Nearly every day? How long did it last?</td>
<td></td>
</tr>
<tr>
<td>Rate if markedly diminished pleasure in all or almost all activities</td>
<td></td>
</tr>
<tr>
<td>most of every day, based on patient’s responses or on observations of</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td></td>
</tr>
</tbody>
</table>

The following seven questions focus on the worst two weeks in the past month.

<table>
<thead>
<tr>
<th>Question</th>
<th>Response Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Did you lose or gain any weight? How was your appetite?</td>
<td>Yes</td>
</tr>
<tr>
<td>Rate if significant weight lose when dieting, weight gain, or change in</td>
<td></td>
</tr>
<tr>
<td>appetite nearly every day</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Weight Loss/Decreased Appetite</td>
<td></td>
</tr>
<tr>
<td>□ Weight Gain/Increased Appetite</td>
<td></td>
</tr>
<tr>
<td>4. How were you sleeping?</td>
<td>Yes</td>
</tr>
<tr>
<td>Rate if insomnia or hypersomnia nearly every day</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Insomnia</td>
<td></td>
</tr>
<tr>
<td>□ Hypersomnia</td>
<td></td>
</tr>
<tr>
<td>5. Were you so fidgety or restless that you were unable to sit still?</td>
<td>Yes</td>
</tr>
<tr>
<td>What about the opposite—talking or moving more slowly than normal for</td>
<td></td>
</tr>
<tr>
<td>you?</td>
<td></td>
</tr>
<tr>
<td>Rate if evening agitation or retardation nearly every day, observable by</td>
<td></td>
</tr>
<tr>
<td>others or according to behaviour during interview</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Agitation</td>
<td></td>
</tr>
<tr>
<td>□ Retardation</td>
<td></td>
</tr>
<tr>
<td>6. What was your energy like? Were you tired all the time? Nearly</td>
<td>Yes</td>
</tr>
<tr>
<td>every day?</td>
<td></td>
</tr>
<tr>
<td>Rate if fatigue or loss of energy nearly every day</td>
<td></td>
</tr>
<tr>
<td>had or had not done?</td>
<td></td>
</tr>
<tr>
<td>Do not rate if merely self-reproach or guilt about being ill. Code as</td>
<td></td>
</tr>
<tr>
<td>absent or equivocal if only low self-esteem</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Worthlessness</td>
<td></td>
</tr>
<tr>
<td>□ Guilt</td>
<td></td>
</tr>
<tr>
<td>8. Did you have trouble thinking or concentrating? Was it hard to</td>
<td>Yes</td>
</tr>
<tr>
<td>make decisions about everyday things?</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Diminished ability to think</td>
<td></td>
</tr>
<tr>
<td>□ Indecisiveness</td>
<td></td>
</tr>
<tr>
<td>9. Were things so bad that you were thinking a lot about death or that</td>
<td>Yes</td>
</tr>
<tr>
<td>you would be better off dead?</td>
<td></td>
</tr>
<tr>
<td>What about thinking about hurting yourself?</td>
<td></td>
</tr>
<tr>
<td>Do not rate if only fear of dying</td>
<td></td>
</tr>
<tr>
<td>Check if: □ Thoughts of own death □ Specific plan</td>
<td></td>
</tr>
<tr>
<td>□ Suicidal ideation</td>
<td></td>
</tr>
<tr>
<td>□ Suicide attempt</td>
<td></td>
</tr>
</tbody>
</table>

**Classify:** □ Depressed (at least 5 of above including #1 or #2) OR □ Non-Depressed

**Inquire:** □ Family or □ personal Hx depression or □ Antidepress. Use/discont.__________