Platelet Activating Factors and Depressive Symptoms in Coronary Artery Disease Patients

Graham Mazereeuw, 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 Graham Mazereeuw (2013)
Platelet Activating Factors and Depressive Symptoms in Coronary Artery Disease Patients

Graham Mazereeuw, BSc
Graduate Department of Pharmacology and Toxicology
University of Toronto
2013

Abstract

Depression is highly prevalent in coronary artery disease (CAD) and confers an increased risk of morbidity and mortality, yet mechanisms are unknown. Platelet activating factor (PAF) lipids are associated not only with CAD but also with inflammation, oxidative/nitrosative stress, vascular endothelial dysfunction and platelet reactivity which are proposed etiopathological mechanisms for depression. This study investigated the relationship between PAF species and depressive symptoms in 20 CAD patients. Plasma analyses were performed using electrospray ionization mass spectrometry (precursor ion scan). Primary analysis revealed no association between the potent pro-inflammatory PAF PC(O-16:0/2:0) and depressive symptoms measured by the Hamilton Depression Rating Scale [HAM-D] (F=0.405, p=0.533) or Beck Depression Inventory [BDI]-II (F=0.120, p=0.733) in a linear regression. Exploratory analyses revealed potential associations between greater PC(O-18:1/0:0) and greater HAM-D score and greater PC(O-22:6/2:0) concentrations with a greater BDI-II score. This study suggests that specific PAFs might be biomarkers for depressive symptoms in CAD patients.
Acknowledgements

The experience that I have gained from my time with the Neuropsychopharmacology Research Group at Sunnybrook Health Sciences Centre has been immeasurable. I have made great strides as a critical thinker and scientist and I feel well prepared for the next steps in my development due to this experience. I sincerely thank my supervisors, the directors of the Neuropsychopharmacology Research Group, Dr. Krista Lanctôt and Dr. Nathan Herrmann for their insight and guidance during this time. Dr. Lanctôt, your expertise in clinical research and ability to continually generate novel and meaningful contributions to your field has set a standard that I aspire to in my career. I truly appreciate the time and dedication that you have given me as your student. Dr. Herrmann, I admire your dedication to your profession and the passion that you show for research. Your emphasis on being direct and thorough has instilled these important qualities into my mentality when considering my contributions to the literature. I am sure that the skills you both have passed on to me will serve me well in my career.

I also thank and Dr. Paul Oh, Susan Marzolini, and the staff at the Toronto Rehabilitation Institute - Cardiac Rehabilitation Centre for their help with participant recruitment and enthusiasm for this project. Drs. Steffany Bennett and Hongbin Xu of the CIHR Training Program in Neurodegenerative Lipidomics have been fantastic mentors for my education in lipidomic techniques and research and their contributions to this project cannot be overstated. Finally, I thank the members of the Neuropsychopharmacology Research Group, past and present, for their help with this project; particularly Dr. Walter Swardfager for his guidance and support with project ideas and papers, the CAD team for helping me juggle assessments and recruitment, and Abby Li for keeping things light with her merciless sarcasm.
# Table of Contents

Acknowledgements .......................................................................................................................... iii

Table of Contents .............................................................................................................................. iv

List of Tables ...................................................................................................................................... vi

List of Figures ...................................................................................................................................... vii

List of Appendices ............................................................................................................................ viii

List of Abbreviations ......................................................................................................................... ix

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 ........................................... 3

1.3.1 Primary Hypothesis .............................................................................................................. 3

1.3.2 Secondary Hypothesis ......................................................................................................... 4

1.3.3 Exploratory Analyses ............................................................................................................ 4

1.4 Review of the literature .............................................................................................................. 6

1.4.1 Coronary Artery Disease ...................................................................................................... 6

1.4.2 Depression and CAD ........................................................................................................... 6

1.4.3 Current Interventions for Depression in CAD .................................................................... 7

1.4.4 Proposed Mechanisms for Depression in CAD ................................................................. 10

1.4.5 Platelet Activating Factors .................................................................................................. 14

1.5 Summary of background ........................................................................................................... 21

Materials and Methods ................................................................................................................... 24

2.1 Study Design ............................................................................................................................... 24

2.2 Schedule of Assessments ......................................................................................................... 26

2.3 Plasma Collection and Lipidomics Analyses .......................................................................... 28

2.4 Statistical Methods ................................................................................................................... 29

2.4.1 Covariates .......................................................................................................................... 30

2.5 Sample Size Calculation ........................................................................................................... 31

Results ................................................................................................................................................ 32

3.1 Participant Recruitment ............................................................................................................. 32

3.2 Normalization of PC (O-16:0/2:0) Abundance ........................................................................ 34
3.3 Addressing the Hypotheses........................................................................................................................................34
  3.3.1 Primary Hypothesis ........................................................................................................................................34
  3.3.2 Secondary Hypothesis .......................................................................................................................................35
3.4 Exploratory Analyses..................................................................................................................................................36
  3.4.1 The PAF AAGPC Profile and Depressive Symptoms Measured by the HAM-D .......................36
  3.4.2 Covariate-Adjusted Linear Regression Analyses .........................................................................................40
  3.4.3 Depressive Symptoms Measured by the BDI-II ..........................................................................................43
  3.4.4 Depressive Symptom Clusters (HAM-D) .................................................................................................44
3.5 Post-hoc Analyses......................................................................................................................................................45
  3.5.1 Variations in HAM-D and BDI-II Associated with Participant Characteristics .........................45
  3.5.2 Variations in PAF Abundance Associated with Participant Characteristics ..............................45
  3.5.3 The Association Between HAM-D score and BDI-II score .................................................................47
  3.5.4 Post-hoc Power Calculation for Selected PAFs ....................................................................................47
Discussion, Conclusions and Recommendations ..................................................................................................48
  4.1 Study Findings and Interpretation ......................................................................................................................48
  4.2 Limitations .........................................................................................................................................................53
  4.3 Recommendations for Future Studies ...............................................................................................................56
  4.4 Conclusions ......................................................................................................................................................58
References .................................................................................................................................................................60
List of Publications and Abstracts ..........................................................................................................................79
List of Awards and Sources of Funding ..................................................................................................................81
Appendices ...............................................................................................................................................................82
List of Tables

Table 1. Shared Inflammatory and Physiological Characteristics in CAD and Depression...... 22

Table 2. Sample sizes needed to detect the association between PC (O-16:0/2:0) abundance and HAM-D score. ................................................................................................................................. 31

Table 3. Participant characteristics (N = 20). .................................................................................. 33

Table 4. Linear regression correlations between PAF species and depressive symptoms, N = 20. ............................................................................................................................................ 39

Table 5. Selected PAF species and the severity of depression measured by the HAM-D in a linear regression, N = 20.......................................................... 40

Table 6. Selected PAF species and the severity of depression measured by the HAM-D in an age-adjusted linear regression, N = 20. ................................................................. 42

Table 7. Selected PAF species and the severity of depression measured by the HAM-D in a gender-adjusted linear regression, N = 20. ................................................................. 42

Table 8. Selected PAF species and the severity of depression measured by the HAM-D in an fVO2 peak-adjusted linear regression, N = 20. ............................................................... 42

Table 9. PAF species and depressive symptom clusters measured by the HAM-D in an unadjusted linear regression, N = 20. ........................................................................................... 44

Table 10. Post-hoc investigation of the association between participant characteristics and depressive symptoms measured by the HAM-D and BDI-II, N = 20. ...................................................... 46

Table 11. Post-hoc investigation of the association between participant characteristics and abundance of PC (O-18:1/0:0) and PC (O-16:0/2:0), N = 20...................................................... 46
List of Figures

Figure I. The Alkylacylglycerophosphocholine remodeling pathway. .................................................. 16

Figure II. Schematic for PAFs and associated inflammatory mechanisms. ........................................... 23

Figure III. A representation of the plasma lipidome using Analyst software and electrospray ionization mass spectrometry (precursor ion scan) . ................................................................. 29

Figure IV. Participant flow through each stage of study recruitment. .................................................... 32

Figure V. The distribution of PC (O-16:0/2:0) abundance in plasma from depressed CAD patients. ........................................................................................................................................................................ 34

Figure VI. The association between baseline plasma abundance of PC (O-16:0/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients [primary hypothesis]. ........................................................................................................... 35

Figure VII. The association between baseline plasma abundance of PC (O-16:0/2:0) and depressive symptoms measured by the BDI-II in 20 depressed CAD patients [secondary hypothesis]. ............................................................................................................... 36

Figure VIII. The association between baseline plasma abundance of PC (O-12:0/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. ........................................... 37

Figure IX. The association between baseline plasma abundance of PC (O-18:1/0:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. ........................................... 38

Figure X. The association between baseline plasma abundance of PC (O-18:3/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. ........................................... 38

Figure XI. The association between baseline plasma abundance of PC (O-20:6/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. ........................................... 39

Figure XII. The association between baseline plasma abundance of PC (O-22:6/2:0) and depressive symptoms measured by the BDI-II in 20 depressed CAD patients. ........................................... 43
List of Appendices

Appendix A: Letters of Research Ethics Board (REB) approval…………………………………………………………..82

Appendix B: Study participant Informed Consent Form (ICF)…………………………………………………………………………87

Appendix C: Depression module of Structured Clinical Interview for DSM-IV Axis I (SCID-I) disorders………………………………………………………………………………………………………………98

Appendix D: The 17-Item Hamilton Rating Scale for Depression (HAM-D)………………………………………..100

Appendix E: The 21-Item Beck Depression Inventory II (BDI-II)……………………………………………………………107

Appendix F: The modified Bligh and Dyer lipid extraction protocol…………………………………………………………110

Appendix G: Letter of personal communication from Dr. Steffany A.L. Bennett……………………………………114
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAGPC</td>
<td>Alkylacylglycerophosphocholine</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetylsalicylic acid</td>
</tr>
<tr>
<td>BDI-II</td>
<td>Beck depression inventory II</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CREATE</td>
<td>Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CTPNL</td>
<td>CIHR Training Program in Neurodegenerative Lipidomics</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ENRICHD</td>
<td>ENhancing Recovery in Coronary Heart Disease</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>fVO$_2$</td>
<td>Fraction of age and gender expected VO$_2$ peak (fractional VO$_2$)</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton rating scale for depression</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl-coenzyme A</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal (axis)</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LPCAT</td>
<td>Lysophosphatidylcholine acyltransferase</td>
</tr>
<tr>
<td>Lp-PLA$_2$</td>
<td>Lipoprotein phospholipase A$_2$</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</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>MMSE</td>
<td>Mini-Mental State Examination</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PAF</td>
<td>Platelet activating factor</td>
</tr>
<tr>
<td>PAF-AH</td>
<td>Platelet activating factor acetylhydrolase</td>
</tr>
<tr>
<td>PAFR</td>
<td>Platelet activating factor receptor</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>PTCA</td>
<td>Percutaneous transluminal coronary angioplasty</td>
</tr>
<tr>
<td>PLA₂</td>
<td>Phospholipase A₂</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>SADHART</td>
<td>Sertraline AntiDepressant Heart Attack Randomized Trial</td>
</tr>
<tr>
<td>SCID-I</td>
<td>Structured Clinical Interview for DSM-IV Axis I Disorders – Depression Module</td>
</tr>
<tr>
<td>SIGH-D</td>
<td>Structured interview guide for the Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>Th1</td>
<td>Type 1 helper T cells</td>
</tr>
<tr>
<td>THC</td>
<td>Trillium Health Centre</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TRI</td>
<td>Toronto Rehabilitation Institute</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule -1</td>
</tr>
<tr>
<td>VO₂</td>
<td>Volume of oxygen</td>
</tr>
</tbody>
</table>
Introduction

1.1 Statement of Problem

Depression is highly prevalent in patients with coronary artery disease (CAD) (Carney et al., 1987; Schleifer et al., 1989; Ladwig et al., 1991; Frasure-Smith et al., 1993, 1995a; Lesperance et al., 1996; Burg and Abrams, 2001; Lesperance and Frasure-Smith, 2003; Strik et al., 2004; van Melle et al., 2004; Patten et al., 2006). Persistent depression is clinically important in CAD. It is associated with an elevated risk of recurrent acute coronary syndrome (ACS) and related mortality independently of traditional cardiac risk factors (Januzzi et al., 2000b, a; Lesperance and Frasure-Smith, 2000; Penninx et al., 2001b; Friedmann et al., 2006). It is also associated with accelerated cognitive decline (Freiheit et al., 2012) and an increased rate of transition to dementia (Saczenski et al., 2010; Barnes et al., 2012). Response to antidepressant therapies, including pharmacotherapies, psychotherapies, and exercise interventions, in CAD patients is modest and unpredictable (Mendes de Leon et al., 2006; Lesperance et al., 2007; Dowlati et al., 2010b; Pizzi et al., 2011; Blumenthal et al., 2012). There is a clinical need for novel therapeutic targets and/or biomarkers that suggest probable response to specific therapies. Presently, the underlying mechanisms mediating the association between depression and CAD are unclear, therefore limiting our ability to identify these novel targets. However, emerging evidence implicates elevated inflammatory activity (as indicated by peripheral plasma biomarkers), oxidative and nitrosative stress pathways, vascular endothelial dysfunction, and elevated platelet reactivity as pathophysiological processes shared by depression and CAD (de Jonge et al., 2010; Khan et al., 2010; Stapelberg et al., 2011; Baune et al., 2012). Persistence of these pathways contributes to the progression of neurodegenerative processes and the susceptibility to depressive future episodes (Moylan et al., 2012). Platelet activating factors (PAFs) are a family
of potent inflammatory phospholipids that are associated with CAD (Nemcsik et al., 2004; Chen et al., 2010; Penna et al., 2011; Zheng et al., 2012) and neurodegeneration (Bazan, 2006; Farooqui et al., 2007; Frisardi et al., 2011). PAFs are also associated with the activity and progression of inflammation (Rola-Pleszcynski et al., 1992; Thivierge and Rola-Pleszcynski, 1992; Im et al., 1996; Farooqui et al., 2007; Adibhatla et al., 2008), oxidative and nitrosative stress markers (Kubes et al., 1991; Schmidt et al., 1992; Schwappach et al., 1995; Kurose et al., 1996; Klabunde and Anderson, 2002), vascular endothelial dysfunction (Zimmerman et al., 1990; Uhl et al., 1999a; Uhl et al., 1999b; Klabunde and Anderson, 2002; Farooqui et al., 2007; Yang et al., 2010), and platelet reactivity (Im et al., 1996; Gabbasov et al., 1998; Farooqui et al., 2007); proposed mechanisms of depression in CAD. As such, PAFs may be useful biomarkers of depressive symptoms and related pathophysiology in depressed CAD patients.

1.2 Purpose of the Study and Objective

The purpose of this study is to evaluate the potential cross-sectional association between the plasma abundance of various PAF species and depressive symptoms in depressed CAD patients. This study will also explore the associations between PAFs and specific clusters of depressive symptoms in these patients. This study represents an initial step in the investigation of PAFs as potential biomarkers for depression in CAD. Should PAFs be associated with total depressive symptoms, and/or particular symptom clusters in CAD patients, then the future investigation of PAFs as biomarkers may be warranted.
1.3 Statement of Research Hypotheses and Rationale for Hypotheses

1.3.1 Primary Hypothesis

**Hypothesis 1:** A greater plasma abundance of PC (O-16:0/2:0) \(^{A1}\) will be associated with a greater severity of depressive symptoms as measured by the 17-item HAM-D (researcher-rated) in depressed CAD patients.

*Rationale:* PC (O-16:0/2:0) is a particularly potent PAF species and is the most common species in studies that have reported species identity (Saeki et al., 1983; Rouis et al., 1988; Carolan and Casale, 1990; Handa et al., 1991). For example, PC (O-16:0/2:0) has been reported as the most potent PAF in recruiting neutrophils (Carolan and Casale, 1990) and was the first PAF shown to activate reactive oxygen species (Rouis et al., 1988). As such, PC (O-16:0/2:0) is a rational species to target in our investigation of PAFs as inflammatory lipid biomarkers of depressive symptoms in CAD.

The 17-item Hamilton Rating Scale for Depression (HAM-D) is considered to be the gold standard clinician/researcher-rated measure of depressive symptoms and has been used as the primary outcome measure in several major studies of depression in CAD (Glassman et al., 2002; Honig et al., 2007; Lesperance et al., 2007). The HAM-D is further described in Section 2.2, Schedule of Assessments. For these reasons, the HAM-D was used for the primary outcome.

\(^{A1}\) PC(O-16:0/2:0) \([C16PAF]\) defines a PAF with a phosphocholine polar head group (PC), an ether linkage at the \(sn-1\) position (\(O\>-), carbon chain lengths at the \(sn-1\) and \(sn-2\) positions of 16 and 2 carbons respectively, with the \(sn-1\) chain and the \(sn-2\) chain fully saturated (i.e. :0; the number of double bonds would indicated by :1, :2 etc.).
1.3.2 Secondary Hypothesis

_Hypothesis 2:_ A greater plasma abundance of PC (O-16:0/2:0) will be associated with a greater severity of depressive symptoms as measured by the BDI-II (patient-rated) in depressed CAD patients.

_Reason:_ The Beck Depression Inventory (BDI) II is an established patient-rated measure of depressive symptoms and has been used by several studies of depression in CAD (Glassman et al., 2002; Kaptein et al., 2006; Honig et al., 2007; Lesperance et al., 2007). Using the patient-rated BDI-II in addition to the clinician-rated HAM-D allowed for a comprehensive evaluation of depressive symptoms in our sample population. The BDI-II also assesses particular symptoms which are not assessed by the HAM-D. For example, the HAM-D evaluates only a reduction in appetite, whereas the BDI-II evaluates a reduction or an increase in appetite, both demonstrated to be symptoms of depression. The BDI-II also assesses the severity of depression over the most recent two weeks, whereas the HAM-D considers only the most recent week. Therefore, it is appropriate to investigate the relationship between PC (O-16:0/2:0) and depressive symptoms as measured by the patient-rated BDI-II in addition to the clinician-rated HAM-D.

1.3.3 Exploratory Analyses

_Exploratory Analysis 1:_ This study will investigate the profile of PAF species and their association with depressive symptoms as measured by the HAM-D and the BDI-II.

_Hypothesis:_ Different PAF species will demonstrate variable associations with HAM-D and BDI-II scores in depressed CAD patients.

_Reason:_ Much of the literature investigating PAFs as mediators within the vascular system and elsewhere has failed to define species specificity. As leading-edge techniques in mass spectrometry have allowed for species differentiation, it has become clear that individual PAF species have differential effects. Therefore, we will explore the profile of PAFs detected by our
plasma analyses and their relationship with depressive symptoms. This exploration may elucidate the differential utility of PAF species as biomarkers for depression.

**Exploratory Analysis 2:** This study will investigate the association between PC (O-16:0/2:0) and depressive symptom clusters as measured by the HAM-D. PAF species identified in Exploratory Analysis 1 that demonstrate consistent associations (significant association or a trend) with HAM-D score when adjusting for all relevant covariates will also be examined for variable associations with depressive symptom clusters.

**Hypothesis:** PAF species will demonstrate variable associations with depressive symptom clusters measured by the HAM-D.

**Rationale:** Investigating the relationship between PAFs and different clusters of depressive symptoms can explore their utility as biomarkers beyond the total HAM-D score. It is possible for two participants to have the same total score on the HAM-D but have different degrees of severity for certain symptom clusters. Recent studies have suggested differential effects of certain symptom clusters for depressive and cardiac outcomes. For example, the affective/mood cluster has been associated with future coronary artery calcification (Stewart et al., 2012), whereas the somatic cluster has been associated with an increased risk of acute ischaemic events (Davidson et al., 2005; Stewart et al., 2007; Stewart et al., 2009; Deverts et al., 2010). Symptom clusters may differentially respond to antidepressant pharmacotherapy and predict non-response (Trivedi et al., 2005).

We will explore the association between PC (O-16:0/2:0) and depressive symptom clusters as PC (O-16:0/2:0) was our primary PAF species of interest. We will also require that any exploratory PAF species meet the criterion of a consistent association with HAM-D score after adjusting for all relevant covariates in order to add rigor to the study design. Exploring the association between PAF species and depressive symptom clusters measured by the HAM-D may clarify their biomarker utility and potential mechanisms.
1.4 Review of the literature

1.4.1 Coronary Artery Disease

CAD is the leading cause of mortality in the developed world (WHO, 2011). CAD is defined as a narrowing of one or more of the coronary arteries (at least 50% of vessel diameter) due to the development of inflammation within the perivascular space and the formation of atherosclerotic plaques. Narrowing of the coronary arteries can reduce oxygen supply to the myocardial tissue and revascularization interventions, such as coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA, stent), may be required to reduce the risk of myocardial infarction. Circulating inflammatory mediators, in conjunction with vascular risk factors such as hypertension, dyslipidemia, and diabetes, are associated with the development of vascular endothelial dysfunction, atherogenesis in the coronary and peripheral vessels, and an elevated risk of thrombosis (Mizuno et al., 2011). These lead to progressive narrowing of the coronary arteries and CAD-related morbidity, increasing the risk of acute ischemic events such as myocardial infarction or stroke (Mizuno et al., 2011).

1.4.2 Depression and CAD

Depression, a term encompassing both major and minor depression, is a clinically important mental illness affecting 7.9% to 8.6% of Canadians over 18 years of age, with between 4% and 5% of the population experiencing major depression over 12 months (Bland et al., 1988; Parikh and Lam, 2001; Patten et al., 2006). Minor depression shares diagnostic criteria with major depression but satisfies fewer criterion symptoms (American Psychiatric Association). Both major and minor depression are associated with a large socioeconomic burden (Judd et al., 1996), poorer quality of life (Hofer et al., 2005; Stafford et al., 2007), and an increased risk of suicide (Lester, 1993; Bostwick and Pankratz, 2000). In patients with CAD, the prevalence of major depression in the first year following an ACS is approximately 20%, at least four-fold
higher than the annual rate in the general adult population (Patten et al., 2006), with a further 27% of CAD patients suffering from minor depression (Carney et al., 1987; Schleifer et al., 1989; Ladwig et al., 1991; Frasure-Smith et al., 1993, 1995a; Lesperance et al., 1996; Burg and Abrams, 2001; Lesperance and Frasure-Smith, 2003; Strik et al., 2004; van Melle et al., 2004). Transient or sub-clinical depressive symptoms are a risk factor for future major depressive episodes (Patten et al., 2012). As 1 in 3 persons are likely to experience CAD before the age of 70 (1998; WHO, 2011), a considerable percentage of the population is likely to experience depression co-morbid with CAD.

Both major and minor depression significantly and substantially increase the risk of mortality or myocardial infarction (MI) independently of traditional cardiac risk factors (Januzzi et al., 2000b; Lesperance and Frasure-Smith, 2000; Penninx et al., 2001a; Friedmann et al., 2006) in those with stable CAD (Stern et al., 1977; Ladwig et al., 1991; Frasure-Smith et al., 1993, 1995a; Wassertheil-Smoller et al., 1996; Murray and Lopez, 1997; Lesperance and Frasure-Smith, 2000), CABG (Blumenthal et al., 2003), or post-MI (Ahern et al., 1990; Ladwig et al., 1991; Frasure-Smith et al., 1993, 1995a, b). The negative impact of depression increases with symptom severity (Lesperance et al., 2000; Penninx et al., 2001b), increasing, for example, the risk of hospitalization due to complications of CAD dose-dependently (Rutledge et al., 2006). In the context of secondary prevention, depression negatively interacts with outcomes such as psychosocial rehabilitation (Stern et al., 1977; Mayou et al., 1978), adherence to cardiac medications (Blumenthal et al., 1982) and cardiopulmonary fitness (Lavoie et al., 2004).

1.4.3 Current Interventions for Depression in CAD

Antidepressant therapies demonstrate efficacy in reducing depressive symptoms in CAD patients; however, response rates (defined as a 50% reduction in depressive symptoms over the course of treatment) are modest and heterogeneous (Dowlati et al., 2010b; Pizzi et al., 2011).
Two large placebo-controlled randomized controlled trials (RCTs), the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy (CREATE) and the Sertraline AntiDepressant Heart Attack Randomized Trial (SADHART) reported treatment response rates of 52.8% and 67% to the selective serotonin reuptake inhibitors (SSRIs) citalopram and sertraline respectively (Glassman et al., 2002; Lesperance et al., 2007). Furthermore, 64.1% of participants in the CREATE trial continued to experience depressive symptoms after 12 weeks despite treatment with citalopram (Lesperance et al., 2007). The efficacy of SSRIs in treatment symptoms of minor depression is unclear as the previously mentioned trials only included CAD patients experiencing a major depressive episode. One trial, the Myocardial Infarction and Depression – Intervention Trial (MIND-IT), evaluated the efficacy of mirtazapine, a tetracyclic antidepressant, in CAD patients experiencing both major and minor depression (Honig et al., 2007). That trial found a modest benefit of mirtazapine for CAD patients, similar to the effect of SSRIs; however, the response rate was only 47%.

SSRIs are considered to be first-line therapy for depression in CAD due to the cardiac side effects of other classes (Lichtman et al., 2008). However, SSRIs are not innocuous in CAD patients. For example, Citalopram use is associated with a dose-dependent prolongation of the QT interval (Cooke and Waring, 2012). SSRIs such as fluoxetine and paroxetine can inhibit the cytochrome (CYP) P450-2D6 isozyme, leading to increased plasma concentrations of beta-blocker class medications such as metoprolol or propranolol, commonly used in CAD (Spina et al., 2008). Other notable side-effects include nausea, diarrhea, weight loss, increased sweating, and/or sexual dysfunction (Chemali et al., 2009). SSRI use has also been associated with reduced bone marrow density and, paradoxically, an increased risk of suicide (Chemali et al., 2009). Thus, the clinical benefit of antidepressant pharmacotherapy in CAD patients has yet to be optimized.
Omega-3 fatty acids have also been investigated as potential antidepressants in CAD. Recent meta-analyses suggest efficacy for omega-3 fatty acids when preparations containing at least 60% eicosapentaenoic acid (EPA) are used (Sublette et al., 2011; Martins et al., 2012). However, in the three largest RCTs using high percentage EPA formulations to date, the response rate ranged between 45-62%, similar to that of SSRIs (Peet and Horrobin, 2002; Su et al., 2008; Mischoulon et al., 2009). Thus, the antidepressant effects of omega-3 fatty acids remain modest.

The antidepressant effects of psychotherapy in CAD patients have been evaluated by the ENhancing Recovery in Coronary Heart Disease (ENRICHD) and CREATE trials which found a mild effect and no effect compared to usual care respectively (Mendes de Leon et al., 2006; Lesperance et al., 2007). Thus, psychotherapies have also failed to demonstrate a benefit in many CAD patients with depression.

Recent meta-analyses have confirmed the antidepressant effects of exercise interventions both in the general population (Rimer et al., 2012) and in CAD patients (Gellis and Kang-Yi, 2012). However, not all depressed patients with CAD benefit from these interventions. For example, our pilot data (n=366) showed that depression persisted after 6 months of cardiac rehabilitation in 44% of CAD patients with depression at baseline.

Non-response to antidepressant therapy has important clinical implications. For example, in the MIND-IT trial, non-responders to mirtazapine experienced a considerably higher incidence of secondary ACS (25.4% in non-responders vs 7.4% in responders) (de Jonge et al., 2007). In the ENRICHD trial, patients who were refractory to sertraline and cognitive behavioural therapy experienced increased mortality compared to those who responded to treatment (Carney et al., 2004).
1.4.4 Proposed Mechanisms for Depression in CAD

1.4.4.1 Inflammation

It is now well established that elevated pro-inflammatory activity is an important process in CAD (Ridker, 2007). Pro-inflammatory mediators, such as the cytokines and eicosanoids, can induce the expression of endothelial and leukocyte-based cellular adhesion molecules (e.g. vascular cell adhesion molecule; VCAM-1) which facilitate the recruitment of monocytes to the vascular endothelium (Weber and Noels, 2011). Once recruited, monocytes become activated and participate in the development of atherosclerotic plaques by releasing inflammatory mediators such as matrix metalloproteinases, nitric oxide (NO), and the cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α (Weber and Noels, 2011). Type 1 helper T (Th1) cells also infiltrate the developing plaque and are activated by oxidized low density lipoprotein (Weber and Noels, 2011). Active Th1 cells also release pro-inflammatory cytokines such as IFN-γ and TNF-α and these are found abundantly in atherosclerotic lesions and can exacerbate atherogenesis (Weber and Noels, 2011). Elevated plasma concentrations of pro-inflammatory mediators such as C reactive protein (CRP) (Kaptoge et al., 2010), VCAM-1 (Blankenberg et al., 2001), IL-6 (Lindmark et al., 2001) and TNF-α (Bruunsgaard et al., 2000) confer an increased risk of mortality in CAD patients.

Depression is also characterized by elevated inflammatory activity. Heightened peripheral blood concentrations of TNF-α (Howren et al., 2009; Dowlati et al., 2010a; Liu et al., 2011), IL-6 (Howren et al., 2009; Dowlati et al., 2010a; Liu et al., 2011) IL-1β (Howren et al., 2009), CRP (Howren et al., 2009) and the soluble IL-2 receptor (Liu et al., 2011) have been observed in depressed patients. Elevated concentrations of IL-6 have been detected in the cerebrospinal fluid (CSF) of suicide attempters when compared to controls and these concentrations were related to the severity of depressive symptoms (Lindqvist et al., 2009). Elevated gene expression of pro-
inflammatory cytokines in the brains of depressed patients (Shelton et al., 2011) and suicide victims (Tonelli et al., 2008; Pandey et al., 2012) has also been observed. Collectively, these findings implicate inflammation in the periphery and the central nervous system (CNS) in the pathophysiology of depression. Although the directionality of these associations is likely heterogeneous in depressed CAD patients, inflammatory activity has demonstrable effects on the persistence/induction of depressive symptoms (Moylan et al., 2012).

While the mechanism underlying the association between peripheral and central inflammation in depression is unclear, these findings support a role for inflammatory activity as a clinical marker for depression and a possible mechanistic link between depression and CAD. As such, novel biomarkers for depression in CAD may be derived from other shared pathophysiological processes that are associated with inflammatory activity.

1.4.4.2 Oxidative and Nitrosative Stress

The induction of oxidative and nitrosative stress pathways has also been implicated in the pathophysiology and progression of depression (Maes, 2008; Maes et al., 2009; Miller et al., 2009) and CAD (Maes et al., 2011; Rajesh et al., 2011). Reactive oxygen species (ROS), such as the superoxide anion, can combine with NO to form the powerful oxidant, peroxynitrite, a reactive nitrogen species (RNS) (Beckman et al., 1990). Elevated activity of ROS and RNS mediators can generate oxidative and nitrosative damage to proteins and lipids, which not only potentially modify their signalling capacity but also perpetuate the inflammatory response in depression (Maes et al., 2010; Leonard and Maes, 2012; Stefanescu and Ciobica, 2012). Peroxynitrite and other oxidative/nitrosative agents in the vascular endothelium adjacent to brain tissue may induce neurotoxic cascades in brain tissue leading to depressive symptoms (reviewed (Maes et al., 2009)).
1.4.4.3 Vascular Endothelial Dysfunction

Vascular endothelial dysfunction describes a state of impaired vasodilatory response that is characterized by the reduced bioavailability of NO due to either decreased formation, increased utilization, or both (Hinderliter and Caughey, 2003). Inflammatory mediators or vascular risk factors such as hypertension can lead to elevated production of superoxide which can combine with NO, reducing its bioavailability (Hinderliter and Caughey, 2003; Forstermann and Munzel, 2006) and instead forming the longer-lived peroxynitrite. Peroxynitrite can exacerbate damage to the vascular endothelium leading to further impairment of vascular endothelial function (Forstermann and Munzel, 2006). Vascular endothelial dysfunction has been strongly linked to the pathophysiology of depression and CAD (Celano and Huffman, 2011). Impaired flow-mediated vessel dilation (FMD), an indicator of vascular endothelial dysfunction, is consistently present in depressed patients. In a recent meta-analysis, the degree of endothelial dysfunction, as measured by FMD, was positively associated with the severity of depressive symptoms, with particularly strong correlations in CAD patients (Cooper et al., 2011). It has also been observed that small artery dilation to acetylcholine, a measure of endothelial function, is impaired in depressed patients compared to controls (Paranthaman et al., 2012). That study also found that endothelial function worsened in a gradient from controls to depressed antidepressant responders to depressed non-responders, although the difference between responders and non-responders did not reach significance. The vascular endothelial-protective strategy of ischemic post-conditioning, used to minimize endothelial dysfunction following ischemic-reperfusion injury, was shown to be ineffective in depressed patients when compared to non-depressed controls (Zhuo et al., 2011). That study noted that greater endothelial impairment was associated with greater depression severity and lower NO bioavailability. Collectively, these studies implicate endothelial dysfunction as a factor in at least part of the association between depression and CAD. It may also be important in depression-related CAD morbidity,
particularly since vascular endothelial dysfunction is an independent risk factor for ACS in CAD patients (Hinderliter and Caughey, 2003), and is associated with coronary artery restenosis after PTCA (Munk et al., 2011).

1.4.4 Platelet Reactivity
Platelet reactivity, the degree to which platelets become activated and release inflammatory and thrombotic mediators in response to an agonist, is associated with the presence of CAD and depression. Platelet reactivity is also linked with endothelial function through these inflammatory and thrombotic pathways. For example, platelet-released serotonin can stimulate the endothelial release of NO which leads to vasodilation in healthy endothelium. Serotonin also increases platelet reactivity in co-operation with other, more potent platelet agonists such as adrenaline or arachidonic acid (Ziegelstein et al., 2009). NO is an inhibitor of platelet reactivity and serves to regulate the serotonin release from platelets, thus maintaining platelet-endothelial homeostasis (Gkaliagkousi and Ferro, 2011). NO bioavailability is reduced in dysfunctional vessels and therefore the NO response to serotonin released by activated platelets is suppressed (Celano and Huffman, 2011). The suppression of NO, and therefore the persistence of platelet activation, can lead to the development of pro-thrombotic platelet-monocyte interactions, the increased release of inflammatory mediators (Passacquale et al., 2011), and the exacerbation of vascular endothelial dysfunction (Gkaliagkousi and Ferro, 2011).

Elevated platelet reactivity is associated with depression and with an increased risk of ischemic events in those with CAD (Musselman et al., 1996; Celano and Huffman, 2011). In a large community-based study (Canan et al., 2011), mean platelet volume, an indicator of platelet activity, was significantly greater in depressed patients when compared to controls. Platelet reactivity markers such as β-thromboglobulin, platelet factor 4, and P-selectin are also elevated in depressed CAD patients when compared to their non-depressed CAD counterparts (Laghrissi-
Thode et al., 1997). This was also observed in depressed patients without CAD compared to non-depressed controls without CAD (Piletz et al., 2000). The increased presence of platelet-monocyte aggregates have been observed in CAD patients and contributes to the increased risk of ACS when compared to controls (Wang et al., 2007). Platelet reactivity is also associated with the secretion of pro-inflammatory mediators through the phospholipase A2 (PLA2) and cyclooxygenase (COX) pathways (Aukrust et al., 2010), as well as elevated production of adrenaline, due to overactivity of the hypothalamic-pituitary-adrenal (HPA) axis, or high concentrations of arachidonic acid - both elevated in depression (Gold et al., 2005; Dinan et al., 2009).

In summary, platelet reactivity and vascular endothelial dysfunction are strongly linked and are both consistently observed in depressed patients and those with CAD. Emerging evidence suggests that inflammation, oxidative and nitrosative stress mediators, vascular endothelial dysfunction, and platelet reactivity may collectively contribute to depression in CAD. The persistence of these processes may be associated with the persistence of depressive symptoms and the increased risk of ACS in depressed CAD patients. Identifying therapeutic targets associated with these processes is likely important for elucidating disease etiopathology and potential future interventions.

1.4.5 Platelet Activating Factors

PAFs are a family of potent pro-inflammatory phospholipids that are released by several cell types such as leukocytes and endothelial cells that participate in vascular and immune processes (Farooqui et al., 2007). PAFs were the first ether-linked lipid family identified by their biological activity and were shown to be released from histamine-stimulated rabbit basophils eliciting platelet aggregation (Benveniste et al., 1972). PAF lipids are members of the 1-alkyl-2-acylglycerophosphocholine subclass (GP0102) of glycerophosphocholines (GP01)
Family members are structurally defined by an alkyl ether linkage at the sn-1 position, an acetyl group at the sn-2 position, and a phosphocholine at the sn-3 position. For example, the C16 PAF species is referred to as PC (O-16:0/2:0). Related PAF-like lipids include the phosphatidylcholines with a short chain acetyl group at the sn-2 position, ethanolamine-PAFs (GP0202) (LipidMAPS, 2012), inositol-PAFs (GP0602) (Fischer et al., 2006; LipidMAPS, 2012), acyl-PAFs, and oxidized alkylacyl- and phosphatidyl glycerophosphocholines (Watson et al., 1995; Chen et al., 2007). PAF lipids participate in many diverse physiological processes, such as bronchoconstriction (Kasperska-Zajac et al., 2008), vasodilation (Kuebler et al., 2010), and in the CNS, facilitation of long-term potentiation (Arai and Lynch, 1992; Wieraszko et al., 1993; Chen et al., 2001).

### 1.4.5.1 The PAF Synthesis Pathway

PAF lipids can be produced by two different pathways; the de novo synthesis pathway and the remodelling pathway. The de novo synthesis pathway is a minor pathway for PAF synthesis that regulates homeostatic levels of PAFs, whereas the remodelling pathway is the primary pathway for alkyl- and acyl-PAF production in response to cellular activation. In this two-step pathway, the acyl moiety at the sn-2 position of either alkylacylglycerophosphocholines (AAGPC) or phosphatidylcholine (PC) is cleaved by cytosolic PLA₂ (cPLA₂), resulting in a lyso-PAF or lyso-PC intermediate respectively and free arachidonic acid (Snyder et al., 1996). Arachidonic acid can then be metabolized to inflammatory eicosanoid species by COX₂ (Minghetti, 2004). Lyso-PAF and lyso-PC are then acetylated to PAF or to acyl-PAF respectively by lysophosphatidylcholine acyltransferase (LPCAT) 1 or 2 (LPCAT1 is constitutively expressed whereas LPCAT2 is inducible) with acetyl-coA as a donor (Snyder et al., 1996; Shindou et al., 2009). If acyl-CoA is the donor, then the LPCATs regenerate a precursor PC or AAGPC parent lipid from the lyso-intermediates. Thus, proceeding to PAF synthesis from a lyso-intermediate is
dependent on the microenvironment. The production of alkyl-PAFs over acyl-PAFs is favoured during periods of inflammation as the lyso-PAF acyltransferase activity of LPCATs is enhanced whereas the lyso-PC acyltransferase activity is not (Shindou et al., 2009). Therefore, alkyl-PAFs will be referred to as PAFs from here onward.

Plasma PAF-acetylhydrolase (PAF-AH), also known as lipoprotein-associated phospholipase A$_2$ (lp-PLA$_2$) (one of three PAF-AH subtypes) is secreted into circulation by both endothelial cells and leukocytes (Asano et al., 1999) and can inactivate both PAFs and acyl-PAFs through a deacetylation mechanism at their sn-2 position to reform precursor lyso-intermediates (Arai et al., 2002). Lyso-PAFs (or lyso-PCs) may then be converted back to parent AAGPCs or PCs via the LPCATs (Shindou et al., 2009) [Figure I].

**Figure I. The Alkylacylglycerophosphocholine remodeling pathway.** Also known as Lands’ cycle, this two-step pathway is the primary PAF synthesis pathway during an inflammatory response. In this example, arachidonic acid is cleaved from a membrane AAGPC, PC (O-16:0/20:4), by cPLA$_2$ at the inner plasma membrane. The lyso-PAF intermediate, PC (O-16:0/0:0), is remodelled to a PAF, PC (O-16:0/2:0), using acetyl-coA as a donor. Synthesized PAFs can then flip to the outer plasma membrane where they are released into the extracellular space. PAFs that are not released may be remodelled back to the parent AAGPC through a PAF-AH mediated deacetylation, followed by the addition of arachidonic acid to the lyso-PAF intermediate via the LPCATs. Abbreviations: AAGPC, alkylacylglycerophosphocholine; cPLA$_2$, cytosolic phospholipase A$_2$; LPCAT, lysophosphatidylcholine acyltransferase; PAF, platelet activating factor; PAF-AH, PAF acetylhydrolase. Figure courtesy of the CIHR Training Program in Neurodegenerative Lipidomics (Dr. SAL Bennett).
In the periphery, PAF lipids are predominantly synthesized by endothelial cells, where they are then displayed at the cell surface for intercellular communication (McIntyre et al., 1985; Balestrieri et al., 2003). They can also be released from monocytes/macrophages, basophils, and activated leukocytes to activate the inflammatory response or to signal platelet aggregation (Elstad et al., 1988; Cluzel et al., 1989). Both PAF receptor (PAFR)-dependent and independent signalling pathways have been identified in the CNS (Izquierdo et al., 1995; Chen et al., 2001; Brewer et al., 2002; Ryan et al., 2007); however, in the periphery, PAFs and related lipids typically signal through the G-protein coupled PAFR (Ishii and Shimizu, 2000).

Heterogeneity in linkage, degree of unsaturation, and carbon chain length of the alkyl or acyl chains at the sn-1 position dictates, in part, signalling specificity eliciting various pro- or anti-apoptotic signal transduction pathways following PAFR activation (Satouchi et al., 1981; Tokumura et al., 1989; Handa et al., 1991; Erger and Casale, 1996; Ryan et al., 2008). PAFR is abundantly expressed by cells within the immune and cardiovascular systems (McIntyre, 2012), suggesting that PAFs act as pleiotropic communicators in plasma (Montrucchio et al., 2000). PAF concentrations are tightly regulated under normal circumstances; however, they can become elevated during extended periods of immune activation (Callea et al., 1999), and this has been observed in CAD (Zheng et al., 2012) and in the CNS (Farooqui et al., 2007).

### 1.4.5.3 PAFs and CAD

PAFs have been documented as early mediators in the onset of myocardial ischemia in animal studies (Nemcsik et al., 2004; Penna et al., 2011). In clinical samples, heightened concentrations of PAFs in the plasma have been associated with a greater risk of developing CAD (Zheng et al., 2012) and with the presence of CAD when compared to healthy controls (Chen et al., 2010). Elevated plasma activity levels of the enzyme PAF-AH in unstable CAD patients have also been observed and these studies suggest that PAF-AH activity was independently associated with the
presence of CAD when compared to controls (Samsamshariat et al., 2011; Zheng et al., 2012). PAF-AH has also been suggested as a potential risk marker (Winkler et al., 2007) and therapeutic target for CAD (Carlquist et al., 2007). In keeping with the known role of PAF-AH, it is possible that higher PAF-AH activity is a compensatory response to elevated PAF concentrations. Collectively, these findings support the investigation of the PAF metabolism pathway in the etiopathology of CAD.

1.4.5.4 PAFs in the CNS
In the CNS, PAFs can facilitate long-term potentiation at physiological concentrations (Arai and Lynch, 1992; Wieraszko et al., 1993; Chen et al., 2001). It is thought that PAFs accomplish this by acting as retrograde messengers, diffusing from the post-synaptic site where they are synthesized to the pre-synaptic terminal where they promote the vesicular release of glutamate (Kato et al., 1994; Kato and Zorumski, 1996; Kornecki et al., 1996). Accordingly, during an inflammatory response, elevated PAF production can promote glutamate excitotoxicity (Bennett et al., 1998; Xu and Tao, 2004) and this is one mechanism of their neurodegenerative effects at elevated concentrations (Bazan, 2006; Farooqui et al., 2007; Frisardi et al., 2011). PAFs are also neurodegenerative through their pro-inflammatory effects and interactions with ROS and RNS mediators, as well as their pro-apoptotic effects on neurons via PAFR-dependent and independent mechanisms (Ryan et al., 2007; Ryan et al., 2008). These effects implicate PAFs as potent participants in the neuroinflammatory cascades implicated in depression and neurodegeneration. The relationship between PAFs and depression has not yet been directly investigated. However, PAFs are independently associated with several leading mechanisms thought to mediate depression in CAD.
1.4.5.5 PAFs and Proposed Mechanisms for Depression in CAD

1.4.5.5.1 Inflammation

During an immune response, pro-inflammatory mediators such as cytokines may initiate the PAF synthesis cascade by activating several isoforms of PLA$_2$ (Adibhatla et al., 2008). cPLA$_2$ is primarily responsible for the liberation of lyso-PAF, lyso-PC, and arachidonic acid from membrane phospholipids and it plays an important role in potentiating the inflammatory eicosanoid cascade (Kita et al., 2006). Other isoforms, such as secreted PLA$_2$ (sPLA$_2$) and Lp-PLA$_2$, are also associated with PAF activity and have been implicated in the development of endothelial dysfunction and atherosclerosis (Farooqui et al., 2007; Adibhatla et al., 2008).

PAFs promote local pro-inflammatory cascades by inducing the expression of Ca$^{2+}$-independent PLA$_2$ (iPLA$_2$) and COX$_2$ which release and metabolize free arachidonic acid into eicosanoid metabolites (Bazan et al., 1997; Farooqui et al., 2007). In fact, it has been shown that PAFs can preferentially mediate COX$_2$ expression over IL-1$\beta$ (Serou et al., 1999). PAFs can also induce the release of cytokines such as monocyte chemotactic protein-1 (Verouti et al., 2011), IL-1$\beta$ (Rola-Pleszczynski et al., 1992), IL-6 (Thivierge and Rola-Pleszczynski, 1992) and TNF-\(\alpha\) (Rola-Pleszczynski et al., 1992) from leukocytes upon binding to PAFR (Farooqui et al., 2007).

1.4.5.5.2 Oxidative and Nitrosative Stress

PAFs are well known stimulators of ROS, such as the superoxide anion and hydroxyl radical, as RNS mediators (Kubes et al., 1991; Schmidt et al., 1992; Schwappach et al., 1995; Kurose et al., 1996; Klabunde and Anderson, 2002). These pathways are implicated in the onset of neuroinflammation in depressed patients (Leonard and Maes, 2012). RNS metabolites induced by PAFs, such as peroxynitrite, can also induce vascular endothelial permeability (Klabunde and
Anderson, 2002). Under oxidative stress conditions, peroxynitrite can deactivate PAF-AH and therefore impede PAF inactivation (MacRitchie et al., 2007).

1.4.5.5.3 Vascular Endothelial Dysfunction

PAF activity is associated with leukocyte adhesion and infiltration across the vascular endothelium by promoting the surface expression of endothelial cellular adhesion molecules such as VCAM-1 (Kubes et al., 1990; Farooqui et al., 2007). PAF stimulation can contribute to pro-inflammatory effects on vascular smooth muscle cells through the induction of prostaglandin E2 synthesis (Vadas and Perelman, 2012). Elevated PAF signalling is also associated with reduced NO bioavailability within the vascular endothelium, a core feature of vascular endothelial dysfunction (Yang et al., 2010). Animal models have shown that PAFs are associated with the development of endothelial dysfunction during cerebral ischemia and can induce leukocyte-endothelial interactions in cerebral vessels (Uhl et al., 1999a; Uhl et al., 1999b). PAFs are also thought to mediate increased endothelial permeability, in part via peroxynitrite (Klabunde and Anderson, 2002), contributing to the extravasation of leukocytes into the perivascular spaces and the possible induction of neuroinflammatory cascades implicated in the etiology of depression (Montrucchio et al., 2000).

1.4.5.5.4 Platelet Reactivity

PAFs are potent stimulators of platelet reactivity and lead to increased platelet aggregation and the release of inflammatory and platelet activity mediators (Farooqui et al., 2007). These mediators can then promote the secretion of TNF-alpha and inflammatory lipids (such as eicosanoids) from endothelial cells and leukocytes, contributing to a feed-forward mechanism that ultimately generates greater PAF release and therefore greater platelet activation (Montrucchio et al., 1994; Im et al., 1996). PAF-mediated platelet activation has been shown to
be elevated in hypertensive patients compared to normotensive controls (Gabbasov et al., 1998), suggesting that PAFs may interact with vascular risk factors to increase the risk of ACS.

1.5 Summary of background

Inflammation, oxidative and nitrosative stress, vascular endothelial dysfunction, and platelet reactivity are individually associated with both depression and CAD and may be connected through common mechanisms. These processes may contribute to the increased prevalence and/or progression of depression in CAD patients, and associated risk of mortality and cognitive decline.

While the efficacy of current antidepressant medications appears to be limited in CAD patients, the optimization of such pharmacotherapies may benefit from reliable biomarkers that identify novel mechanistic pathways or treatment-responsive patient subgroups. Inflammatory lipids are promising in this regard. In particular, PAFs are potent pro-inflammatory lipid mediators that are associated with CAD and neurodegeneration when elevated in the CNS. PAFs are also associated with progressive inflammation, oxidative and nitrosative stress pathways, vascular endothelial dysfunction, and platelet reactivity, leading proposed mechanisms of depression in CAD [Table 1] [Figure II]. Therefore, PAFs may be markers of depressive symptoms in CAD patients.
Table 1. Shared Inflammatory and Physiological Characteristics in CAD and Depression.

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>CAD</th>
<th>Depression</th>
<th>PAFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ↑ CRP, IL-6, and TNF-α associated with increased risk of mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• TNF-α, IFN-γ, MMPs secreted by immune cells during atherosclerotic plaque development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Higher Lp-PLA₂ (PAF-AH) activity is associated with risk of CAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ TNF-α, IL-6, IL-1β, CRP, sIL-2R in the serum of depressed patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ IL-6 in CSF of suicide attempters associated with depressive symptom severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ pro-inflammatory cytokine expression in the brains of depressed patients and suicide victims</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pro-inflammatory cytokines activate PLA₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• PLA₂ activation releases lyso-PAF and arachidonic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• PAFs activate iPLA₂ and COX₂ which metabolize arachidonic acid into inflammatory eicosanoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• PAFs induce IL-6 and TNF-α release from leukocytes and platelets</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular Endothelial Dysfunction</th>
<th>CAD</th>
<th>Depression</th>
<th>PAFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Independent risk factor for ACS in CAD patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Increased risk of coronary artery restenosis after PTCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Vascular risk factors associated with endothelial damage and dysfunction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Impaired FMD is associated with severity of depressive symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Impaired small artery dilatation in depressed patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Ineffective ischemic postconditioning in depressed patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Elevated sPLA₂ and Lp-PLA₂ activity associated with endothelial dysfunction and atherosclerosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• PAFs induce surface expression of ICAM-1 and VCAM-1, facilitating leukocyte adhesion to endothelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Associated with reduced NO bioavailability and enhanced peroxynitrite formation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelet Reactivity</th>
<th>CAD</th>
<th>Depression</th>
<th>PAFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Associated with increased risk of ACS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ platelet-monocyte aggregates in CAD patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ β-thromboglobulin, platelet factor 4, P-selectin, and mean platelet volume in depression with and without CAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• ↑ plasma serotonin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Potent activators of platelet aggregation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• PAFs induce further PAF production through feed-forward TNF-α release, causing greater platelet activation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; MMP, matrix metalloproteinase; CAD, coronary artery disease; Lp-PLA₂, lipoprotein phospholipase A₂; PAF-AH, platelet activating factor acetylhydrolase; CSF, cerebrospinal fluid; ACS, acute coronary syndrome; PTCA, percutaneous transluminal coronary angioplasty; FMD, flow-mediated dilation; sIL-2R, soluble IL-2 receptor; COX₂, cyclooxygenase 2; iPLA₂, Ca²⁺-independent phospholipase A₂; sPLA₂, secreted phospholipase A₂; PAF, platelet activating factor; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; NO, nitric oxide.
Figure II: Schematic for PAFs and associated inflammatory mechanisms. Mechanisms linking PAFs to inflammation, platelet activation, and the production of reactive oxygen and nitrogen species such as peroxynitrite (ONOO-). PAFs can be released by vascular endothelial cells and leukocytes and they can activate the PAF receptor (PAFR) present on endothelial cells, leukocytes, and platelets. PAFR activation leads to greater PAF release and the release of pro-inflammatory cytokines (TNF-α, IL-6) and eicosanoids (not shown). PAFs in the blood can be degraded by PAF acetylhydrolase (PAF-AH). Collectively, PAFs and other pro-inflammatory mediators, including peroxynitrite, can activate neuroinflammatory cascades in the central nervous system (CNS), implicated in the progression of depression and other neurodegenerative diseases. Abbreviations: LPCAT, lysophosphatidylcholine acyltransferase; TNF-α, tumor necrosis factor α; TNFR1, TNF receptor 1; IL-6, interleukin 6; PLA₂, phospholipase A₂; eNOS, endothelial nitric oxide synthase; iNOS, inducible NOS; NADPH oxidase; nicotinamide adenine dinucleotide phosphate-oxidase.
II

Materials and Methods

2.1 Study Design

This study was a cross-sectional investigation of the association between the plasma abundance of PAF species and depressive symptoms in depressed CAD patients. This study was ancillary to a longitudinal, placebo-controlled RCT of omega-3 fatty acids in depressed patients with CAD who were participating in an exercise-based cardiac rehabilitation program (the CAROTID omega-3 trial). As participants in this study were also participating in the CAROTID trial, the informed consent processes for both of these studies were addressed as one entity and this document was approved by the research ethics boards of the participating institutions [Appendix A]. Participant recruitment, assessment, data management, and analyses both for this study and for the CAROTID trial were performed primarily by Graham Mazereeuw. This study involved two visits: a screening visit and, two weeks later, a baseline visit, at which time the severity of depressive symptoms was analyzed and fasting blood was collected for plasma analyses. Study participants were CAD patients recruited from the Toronto Rehabilitation Institute (TRI) and Trillium Health Centre (THC) cardiac rehabilitation centres. At the first class of the cardiac rehabilitation program ("intake"), participants were approached by their rehabilitation program supervisor to determine their interest in being contacted for our study. Participants who were interested were then interviewed by study staff after their intake class. Those participants who indicated interest in the study but who could not be interviewed after their intake class were contacted by study staff to schedule an interview appointment. As exercise and cardiac rehabilitation programs have demonstrated antidepressant effects, recruiting CAD patients from rehabilitation centres allows for the future examination of the
longitudinal effects of exercise in the recruited participants. Participants were deemed eligible for this study based on the following inclusion criteria:

- Age 45-80
- Diagnosis of CAD (history of MI, PTCA, CABG, Ischemic heart disease, or at least 50% stenosis in one or more coronary artery based on angiographic documentation)
- Stable CAD (based on no prior hospitalizations for at least 7 weeks)
- Speaks and understands English
- Meets DSM-IV criteria for a major depressive episode or minor depression as assessed by the SCID-I (First et al., 1996)
- Written, informed consent [Refer to Appendix B]

Potential participants were excluded from the study if they met any of the following exclusion criteria:

- Significant acute medical illness (sepsis, autoimmune condition, drug overdose, uncontrolled diabetes mellitus, severely disturbed liver, kidney or lung function, anemia, hypothyroidism)
- Clinically significant cognitive impairment (MMSE<24) (Perry et al., 2000)
- Other neurologic conditions (Parkinson's disease, Huntington's chorea, history of epilepsy, birth trauma, significant traumatic brain injury, clinical stroke, progressive supranuclear paralysis, brain tumour, subdural hematoma, multiple sclerosis)
- Canadian Cardiovascular Society Class 4 (indicating unstable angina)
- Killip class greater than II (indicates high risk of mortality in post-MI group)
- Premorbid or concurrent psychiatric diagnoses of schizophreniform or bipolar depressive disorders, current ethanol or substance abuse or any premorbid psychiatric condition requiring hospitalization
- History of electroconvulsive therapy.
- Suicidal ideation or a history of suicidal ideation/attempts (determined during SCID-I at screening/baseline visits)
- Current or history of psychotic episode or personality disorder.

Potential participants who have been using antidepressants (including St. John’s Wort) or psychotherapy for depression were considered for inclusion providing that they continued to meet the study criteria for depression. Concomitant use of antidepressants was allowed providing that a stable dose was used for 3 months or greater. Participants using omega-3 fatty
acid supplements were excluded as these have been shown to affect PAF metabolism (Mayer et al., 2002; Simopoulos, 2002).

2.2 Schedule of Assessments

Eligible study participants were invited to a screening visit where investigators discussed the study in detail and obtained informed consent from the participant. At screening, participant demographic and anthropomorphic data (including blood pressure, body mass index [BMI], and VO₂ peak) were noted, as well as concomitant medications and medical history. The Mini-Mental State Examination (MMSE) (Folstein et al., 1975) was used to identify and exclude participants with poor cognitive status. The presence of a depressive episode was assessed using the Structured Clinical Interview for DSM IV Disorders – Depression scale (SCID-I) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV (First et al., 1996) [Appendix C]. The SCID-I consists of 9 questions that assess the presence of particular symptoms characteristic of a depressive episode. To be classified as depressed, a participant must have experienced a depressed mood and/or anhedonia for the majority of the most recent month with additional changes in weight and/or appetite, sleep schedule (i.e. insomnia or hypersomnia), agitation or retardation, the presence of fatigue, feelings of worthlessness or guilt about personal actions/mistakes, diminished ability to focus or make decisions, or suicidal ideation. Participants were considered to be experiencing a major depressive episode based on the presence of a depressed mood and/or anhedonia in addition to at least 3-4 of the secondary criteria (totalling at least 5 of 9 symptoms present). Participants were considered to be experiencing a minor depressive episode based on the presence of a depressed mood and/or anhedonia in addition to at least 1-2 of the secondary criteria (totalling 3 of 9 symptoms present). These classifications were based on standard DSM-IV criteria for a depressive episode. If identified as depressed, participants were scheduled for the baseline visit two weeks later. Those
who did not meet criteria for depression at screening were excluded from the study. Additionally, the medical and psychiatric histories of each participant were thoroughly discussed in order to rule out the presence of other Axis I psychiatric disorders.

At the baseline visit, participants were re-evaluated for the presence of a depressive episode using the above criteria. The 2-week interval between the screening and baseline visits was designed to exclude participants who experienced spontaneous remission of depressive symptoms over that time. Participants were again assessed using the MMSE to exclude those presenting with poor cognitive status. The presence or absence of the medical inclusion and exclusion criteria was determined by the study physician at TRI (co-investigator Dr. Paul I. Oh) and THC (co-investigator Dr. Cheng Tao Wang). The severity of depressive symptoms was assessed at the baseline visit using the investigator-rated 17-item HAM-D (Davidson et al., 2006) [Appendix D]. Participants who did not meet the 17-item HAM-D cut-off score for depression (a score ≥ 7 (Frank et al., 1991)) were excluded from the study. The HAM-D is highly reliable and sensitive (Strik et al., 2001), and was chosen as the primary outcome measure. The HAM-D is the gold standard for assessing antidepressant efficacy as recommended by expert consensus (Davidson et al., 2006) in trials with CAD patients. This has been the primary outcome variable in the SADHART (Glassman et al., 2002), CREATE (Lesperance et al., 2007), and MIND-IT (Honig et al., 2007) trials, as well as in clinical trials assessing the antidepressant efficacy of omega-3 fatty acids (Stoll et al., 1999; Nemets et al., 2002; Su et al., 2003). Furthermore, HAM-D scores have been inversely correlated with health-related quality of life in patients with heart disease demonstrating the meaningfulness of this scale (Sullivan et al., 1999; Jamieson et al., 2002; Sullivan et al., 2004; de Jonge et al., 2006).

The HAM-D was conducted in a structured manner (SIGH-D) (Williams, 1988), under the training and supervision of Dr. Nathan Herrmann (co-investigator and MSc Advisor), an
experienced geriatric psychiatrist.

As a complementary secondary outcome, depressive symptoms were also measured using the self-report BDI-II (Beck et al., 1961; Davidson et al., 2006) [Appendix E], which is appropriately sensitive in antidepressant trials in CAD patients (Glassman et al., 2002; Kaptein et al., 2006; Honig et al., 2007; Lesperance et al., 2007) and of potential utility in this population as a self-report instrument.

2.3 Plasma Collection and Lipidomics Analyses

Four millilitres of blood was obtained from the antecubital vein at the baseline visit after 12 hours of fasting. Blood was then centrifuged (Model 614B, The Drucker Company) at 3,150 ± 100 RPM for 10 minutes and plasma was collected and frozen at -80°C. We performed plasma analyses on all detectible PAF species to generate exploratory analyses. Lipidomic analyses of plasma were performed by Drs. Hongbin Xu and Steffany Bennett (CIHR Training Program in Neurodegenerative Lipidomics (CTPNL), University of Ottawa, Ottawa, ON) using precursor ion scan electrospray ionisation mass spectrometry (450-600 amu) using a diagnostic fragment of 184.2 amu in positive precursor mode as described (Hou et al., 2008; Smith et al., 2008; Wislet-Gendebien et al., 2008; Hou et al., 2011) [see Appendix F for the lipid extraction protocol]. Spectra for each participant were analysed by Graham Mazereeuw and the Neuropsychopharmacology Research Group, Sunnybrook Health Sciences Centre, using Analyst1.4 Software (Applied Biosystems™, Life Technologies™) and CTPNL protocols [refer to Figure III for methodology]. PAF species and their associated lyso-PAF precursors were identified based on the mass-to-charge ratio and retention time coordinates for each species, as established by the CTPNL. The peak intensity for each species was noted and adjusted using the peak intensity of a known internal-standard [PC (13:0/0:0)].
Figure III. A representation of the plasma lipidome using Analyst software and electrospray ionization mass spectrometry (precursor ion scan). A) In this example, PC (O-16:0/2:0) is identified by its mass-to-charge ratio (as established through the CTPNL protocols) of 524.8 amu. For all PAF species, we systematically selected a mass-to-charge boundary of +/-1.5 amu from the established mass-to-charge ratio for the specific species. B) After selecting the mass-to-charge ratio boundaries for a PAF, the correct peak is selected using established retention time boundaries (CTPNL protocols and internal deuterated standards). Once the correct peak is identified and isolated (grey peak in this example), the peak intensity is noted and adjusted as described in this section.

2.4 Statistical Methods

To adjust for small peak intensities that lead to negative log values, each standard-adjusted peak intensity was multiplied by $10^6$. The logarithm (base of 2) of this value was then obtained for each PAF species to reduce between-patient variability and normalize the data for statistical analyses. This value is henceforth referred to as the abundance of a given PAF species. The association between the abundance of each PAF species and depressive symptoms at baseline
(HAM-D and BDI-II) was analyzed using linear regression, adjusting for relevant covariates (SPSS statistical software, version 13.0, Chicago, IL, USA).

Depressive symptom clusters as measured by the HAM-D were organized as follows: Mood (items 1, 2, 3, 7, 8, 13, 14), sleep and psychic anxiety (items 4, 5, 6, 9, 10), reduced appetite (item 12), and somatic symptoms (items 11, 15, 17) (O'Brien and Glaudin, 1988; Gullion and Rush, 1998). Scores for items in each cluster were summed to provide a total score for that cluster. The association between PAF species and individual symptom clusters was explored using linear regression as described above.

2.4.1 Covariates

2.4.1.1 Age
We have previously found that a younger age was associated with greater depressive symptoms in CAD patients (Swardfager et al., 2008). We will therefore include age as a covariate in our analyses.

2.4.1.2 Gender
We have previously found that female gender was associated with greater depressive symptoms in CAD patients (Swardfager et al., 2008). We will therefore include gender as a covariate in our analyses.

2.4.1.3 Cardiopulmonary Fitness
The peak volume of oxygen consumption (VO$_2$) during exercise is the most reliable and reproducible indicator of cardiopulmonary fitness (Milani et al., 2006). We have previously reported that lower VO$_2$ peak is independently associated with greater depressive symptoms in CAD patients after controlling for age and gender (Swardfager et al., 2008). We will control for this association in our analyses by calculating a value representative of the ratio of the VO$_2$ peak for each participant relative to their age and gender expected norm (fractional VO$_2$ [fVO$_2$]).
2.5 Sample Size Calculation

Previous studies investigating the linear correlation between HAM-D score and inflammatory/oxidative stress markers in plasma have revealed Pearson correlation coefficients between 0.30 – 0.58 (Abbasi et al., 2012; Karlović et al., 2012; Zhang et al., 2012), with a mean of 0.41. Based on those findings, we estimate that the lower limit, mean, and upper limit $R^2$ values for the association between PC (O-16:0/2:0) and HAM-D score would be approximately 0.09, 0.16, and 0.34, respectively. To achieve 80% power to detect an $R^2 = 0.34$ for our primary outcome using a two-tailed significance level ($\alpha$) of 0.05 requires 18 participants [Table 2]. In order to allow for the inclusion of 1 covariate, we increased this to 20 participants.

<table>
<thead>
<tr>
<th>Power Table</th>
<th>$R^2$ =0.09</th>
<th>$R^2$ =0.16</th>
<th>$R^2$ =0.34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size for 80% power</td>
<td>82</td>
<td>44</td>
<td>18</td>
</tr>
<tr>
<td>Power of n=20</td>
<td>0.27</td>
<td>0.46</td>
<td>0.86</td>
</tr>
</tbody>
</table>

If the covariates account for 0.34 of the variance, there is still 80% power to detect a significant association with PC (O-16:0/2:0) if the increment to $R^2$ is at least 0.21. After analyzing this preliminary sample of 20, we will re-evaluate sample power and adjust the recruitment target accordingly.
III

Results

3.1 Participant Recruitment

Between September 2010 and May 2012, 220 CAD patients were invited to participate in this study. Of these, 125 were excluded and 95 were assessed for study eligibility at the screening visit, yielding 38 participants who met inclusion criteria. These 38 patients were reassessed at the baseline visit (2 weeks later) where 18 were excluded and 20 participants remained (for participant characteristics, see Table 3). Reasons for exclusion are presented in Figure IV.

Figure IV. Participant flow through each stage of study recruitment. Potential participants were approached at local cardiac rehabilitation centres.
Table 3. Participant characteristics (N = 20).

<table>
<thead>
<tr>
<th>Sociodemographic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>59.9 (9.6)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>65.0</td>
</tr>
<tr>
<td>Living alone (%)</td>
<td>35.0</td>
</tr>
<tr>
<td>Employed (%)</td>
<td>35.0</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>60.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular risk factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension (%)</td>
<td>70.0</td>
</tr>
<tr>
<td>Smoking history (%)</td>
<td>75.0</td>
</tr>
<tr>
<td>Years smoked (mean ± SD)</td>
<td>26.7 (14.1)</td>
</tr>
<tr>
<td>Cigarettes per day (mean ± SD)</td>
<td>15.9 (11.4)</td>
</tr>
<tr>
<td>Diabetic (%)</td>
<td>40.0</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>29.4 (5.4)</td>
</tr>
<tr>
<td>BMI &gt; 30 (%)</td>
<td>45.0</td>
</tr>
<tr>
<td>Waist circumference (mean ± SD) M/F</td>
<td>100.6 (13.4)/104.3 (19.3)</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>80.0</td>
</tr>
<tr>
<td>Number of vascular risk factors (mean ± SD)</td>
<td>3.5 (1.5)</td>
</tr>
<tr>
<td>VO2 Peak (ml/kg/min)</td>
<td>18.5 (4.7)</td>
</tr>
<tr>
<td>Resting systolic blood pressure (mm Hg)</td>
<td>123.9 (18.4)</td>
</tr>
<tr>
<td>Resting diastolic blood pressure (mm Hg)</td>
<td>75.8 (11.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cardiac history</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PTCA (%)</td>
<td>20.0</td>
</tr>
<tr>
<td>MI/Ischaemic heart disease (%)</td>
<td>45.0</td>
</tr>
<tr>
<td>CABG (%)</td>
<td>35.0</td>
</tr>
<tr>
<td>Time since event [weeks] (mean ± SD)</td>
<td>87.5 (182.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Psychometric (mean ± SD)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>28.9 (1.2)</td>
</tr>
<tr>
<td>SCID major/minor (%)</td>
<td>90.0/10.0</td>
</tr>
<tr>
<td>History of depression (%)</td>
<td>45.0</td>
</tr>
<tr>
<td>HAM-D</td>
<td>13.9 (4.1)</td>
</tr>
<tr>
<td>BDI-II</td>
<td>23.6 (10.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concomitant medications (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidepressants</td>
<td>5.0</td>
</tr>
<tr>
<td>ASA</td>
<td>75.0</td>
</tr>
<tr>
<td>Platelet inhibitor</td>
<td>60.0</td>
</tr>
<tr>
<td>Statin</td>
<td>95.0</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>5.0</td>
</tr>
<tr>
<td>Anti-diabetic agents</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; BMI, body mass index; M/F, male/female; VO2, volume of oxygen; PTCA, percutaneous transluminal coronary angioplasty; MI, myocardial infarction; CABG, coronary artery bypass graft; MMSE, Mini Mental State Examination; SCID, Structure clinical interview for DSM-IV axis I disorders – depression; HAM-D, Hamilton rating scale for depression; BDI-II, Beck depression inventory II; ASA, acetylsalicylic acid.
3.2 Normalization of PC (O-16:0/2:0) Abundance

Figure V. The distribution of PC (O-16:0/2:0) abundance in plasma from depressed CAD patients. Peak intensities were normalized to become the abundance value as described in section 2.4.

Plasma PC (O-16:0/2:0) peak intensities were successfully normalized [Figure V]. As the plasma abundance of PC (O-16:0/2:0) fits a normal distribution, the linear regression analyses are less likely to be confounded by between-patients variability.

3.3 Addressing the Hypotheses

3.3.1 Primary Hypothesis

There was no association between the plasma abundance of PC (O-16:0/2:0) and the severity of depression as measured by the HAM-D in a linear regression [Table 4] [Table 5] [Figure VI]. Using three individual models, the linear regression was adjusted for participant age, gender, and fVO₂, none of which revealed an underlying trend [Tables 6-8]. Age (β = -0.158, p = 0.517), gender (β = 0.108, p = 0.665), and fVO₂ (β = 0.282, p = 0.304) were not significant predictors in these models.
Figure VI. The association between baseline plasma abundance of PC (O-16:0/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients [primary hypothesis]. Summary statistics: r = 0.148, R² = 0.02, p = 0.533.

3.3.2 Secondary Hypothesis

There was no association between the plasma abundance of PC (O-16:0/2:0) and the severity of depression as measured by the BDI-II in a linear regression (F = 0.120, p = 0.733) [Table 4] [Figure VII]. Using independent linear regression models to adjust for age (F = 0.530, p = 0.598), gender (F = 0.116, p = 0.891), and fVO₂ (F = 0.281, p = 0.759) did not reveal an underlying association. Age (β = -0.230, p = 0.346), gender (β = 0.085, p = 0.736), and fVO₂ (β = -0.189, p = 0.498) were not significant predictors in these models.
The association between baseline plasma abundance of PC (O-16:0/2:0) and depressive symptoms measured by the BDI-II in 20 depressed CAD patients [secondary hypothesis]. Summary statistics: $r = 0.082$, $R^2 = 0.007$, $p = 0.733$.

3.4 Exploratory Analyses

3.4.1 The PAF AAGPC Profile and Depressive Symptoms Measured by the HAM-D

Ten PAF species [PC (O-12:0/2:0), PC (O-14:0/2:0), PC (O-14:1/2:0), PC (O-16:0/2:0), PC (O-16:1/2:0), PC (O-18:0/2:0), PC (O-18:1/2:0), PC (O-18:3/2:0), PC (O-20:6/2:0), PC (O-22:6/2:0)] and three lyso-PAF species [PC (O-16:0/0:0), PC (O-18:0/0:0), PC (O-18:1/0:0)] were detected in plasma.

The associations between PAF species and HAM-D were individually tested using linear regression [Table 4]. Significant associations between a greater plasma abundance of PC (O-12:0/2:0) [Figure VIII], PC (O-18:1/0:0) [Figure IX], PC (O-18:3/2:0) [Figure X], and PC (O-20:6/2:0) [Figure XI] and a greater HAM-D score were detected [Table 5]. A trend was observed between a greater plasma abundance of PC (O-14:0/2:0), PC (O-14:1/2:0), and PC (O-...
22:6/2:0) and a greater HAM-D score. There was no association between PC (O-16:0/0:0), PC (O-16:1/2:0), PC (O-18:0/0:0), or PC (O-18:1/2:0) and HAM-D score using linear regression.

Figure VIII. The association between baseline plasma abundance of PC (O-12:0/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. Summary statistics: \( r = 0.457, R^2 = 0.209, p = 0.043 \).
Figure IX. The association between baseline plasma abundance of PC (O-18:1/0:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. Summary statistics: $r = 0.549$, $R^2 = 0.302$, $p = 0.012$.

Figure X. The association between baseline plasma abundance of PC (O-18:3/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. Summary statistics: $r = 0.566$, $R^2 = 0.320$, $p = 0.009$. 
Figure XI. The association between baseline plasma abundance of PC (O-20:6/2:0) and depressive symptoms measured by the HAM-D in 20 depressed CAD patients. Summary statistics: $r = 0.481$, $R^2 = 0.231$, $p = 0.032$.

Table 4. Linear regression correlations between PAF species and depressive symptoms, $N = 20$.

<table>
<thead>
<tr>
<th>PAF species</th>
<th>HAM-D total score</th>
<th>BDI-II total score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>p Value</td>
</tr>
<tr>
<td>PC (O-12:0/2:0)</td>
<td>0.457</td>
<td>0.043</td>
</tr>
<tr>
<td>PC (O-14:0/2:0)</td>
<td>0.440</td>
<td>0.052</td>
</tr>
<tr>
<td>PC (O-14:1/2:0)</td>
<td>0.400</td>
<td>0.081</td>
</tr>
<tr>
<td>PC (O-16:0/0:0)</td>
<td>0.066</td>
<td>0.783</td>
</tr>
<tr>
<td>PC (O-16:0/2:0)</td>
<td>0.148</td>
<td>0.533</td>
</tr>
<tr>
<td>PC (O-16:1/2:0)</td>
<td>0.224</td>
<td>0.343</td>
</tr>
<tr>
<td>PC (O-18:0/0:0)</td>
<td>-0.194</td>
<td>0.412</td>
</tr>
<tr>
<td>PC (O-18:0/2:0)</td>
<td>0.054</td>
<td>0.821</td>
</tr>
<tr>
<td>PC (O-18:1/0:0)</td>
<td>0.549</td>
<td>0.012</td>
</tr>
<tr>
<td>PC (O-18:1/2:0)</td>
<td>-0.263</td>
<td>0.262</td>
</tr>
<tr>
<td>PC (O-18:3/2:0)</td>
<td>0.566</td>
<td>0.009</td>
</tr>
<tr>
<td>PC (O-20:6/2:0)</td>
<td>0.481</td>
<td>0.032</td>
</tr>
<tr>
<td>PC (O-22:6/2:0)</td>
<td>0.420</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Note: PAF, platelet activating factor; HAM-D, 17-Item Hamilton Rating Scale for Depression; BDI-II, Beck Depression Inventory II. Trends are highlighted in orange; significant associations are highlighted in yellow. $p$ value significance at $p \leq 0.05$. 
Table 5. Selected PAF species and the severity of depression measured by the HAM-D in a linear regression, N = 20.

<table>
<thead>
<tr>
<th>PAF species</th>
<th>HAM-D total score</th>
<th>F statistic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC (O-12:0/2:0)</td>
<td></td>
<td>4.795</td>
<td>0.043</td>
</tr>
<tr>
<td>PC (O-14:0/2:0)</td>
<td></td>
<td>4.315</td>
<td>0.052</td>
</tr>
<tr>
<td>PC (O-14:1/2:0)</td>
<td></td>
<td>3.424</td>
<td>0.081</td>
</tr>
<tr>
<td>PC (O-16:0/2:0)</td>
<td></td>
<td>0.405</td>
<td>0.533</td>
</tr>
<tr>
<td>PC (O-18:1/0:0)</td>
<td></td>
<td>7.775</td>
<td>0.012</td>
</tr>
<tr>
<td>PC (O-18:3/2:0)</td>
<td></td>
<td>8.482</td>
<td>0.009</td>
</tr>
<tr>
<td>PC (O-20:6/2:0)</td>
<td></td>
<td>5.413</td>
<td>0.032</td>
</tr>
<tr>
<td>PC (O-22:6/2:0)</td>
<td></td>
<td>3.846</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Note: PAF, platelet activating factor; HAM-D, 17-Item Hamilton Rating Scale for Depression; Trends are highlighted in orange; significant associations are highlighted in yellow. p value significance at \( p \leq 0.05 \).

3.4.2 Covariate-Adjusted Linear Regression Analyses

Significant associations between PAF species and HAM-D score in linear regression were tested in covariate-adjusted linear regression analyses using age, gender, and fVO₂ peak as covariates in independent models.

3.4.2.1 PC (O-12:0/2:0)

The association between PC (O-12:0/2:0) and HAM-D score was no longer significant when adjusting for age [Table 6] or gender [Table 7]. A trend remained when adjusting for fVO₂ peak [Table 8]. Age (\( \beta = -0.134, p = 0.538 \)), gender (\( \beta = 0.093, p = 0.673 \)), and fVO₂ (\( \beta = 0.018, p = 0.937 \)) were not significant predictors in these models.

3.4.2.2 PC (O-14:0/2:0)

A trend was observed between PC (O-14:0/2:0) and HAM-D score after adjusting for age [Table 6]. No association remained after adjusting for gender [Table 7] or fVO₂ peak [Table 8]. Age (\( \beta = -0.230, p = 0.210 \)), gender (\( \beta = 0.111 p = 0.613 \)), and fVO₂ (\( \beta = 0.245, p = 0.354 \)) were not significant predictors in these models.
3.4.2.3  PC (O-14:1/2:0)
No association between PC (O-14:1/2:0) and HAM-D score remained after adjusting for age [Table 6], gender [Table 7], or fVO₂ peak [Table 8]. Age (β = -0.264, p = 0.251), gender (β = 0.147, p = 0.513), and fVO₂ (β = -0.189, p = 0.581) were not significant predictors in these models.

3.4.2.4  PC (O-18:1/0:0)
The association between PC (O-18:1/0:0) and HAM-D score remained significant after adjusting for age [Table 6] or gender [Table 7]. A trend remained when adjusting for fVO₂ peak [Table 8]. Age (β = -0.272, p = 0.185), gender (β = 0.093, p = 0.652), and fVO₂ (β = -0.013, p = 0.953) were not significant predictors in these models.

3.4.2.5  PC (O-18:3/2:0)
The association between PC (O-18:3/2:0) and HAM-D score remained significant after adjusting for age [Table 6] or gender [Table 7]. No association remained after adjusting for fVO₂ peak [Table 8]. Age (β = 0.092, p = 0.674), gender (β = 0.206, p = 0.306), and fVO₂ (β = -0.139, p = 0.545) were not significant predictors in these models.

3.4.2.6  PC (O-20:6/2:0)
The association between PC (O-20:6/2:0) and HAM-D score remained significant after adjusting for age [Table 6]. A trend remained after adjusting for gender [Table 7]. No association remained after adjusting for fVO₂ peak [Table 8]. Age (β = -0.403, p = 0.075), gender (β = 0.156, p = 0.465), and fVO₂ (β = -0.017, p = 0.947) were not significant predictors in these models.

3.4.2.7  PC (O-22:6/2:0)
No association between PC (O-22:6/2:0) and HAM-D score remained after adjusting for age [Table 6], gender [Table 7], or fVO₂ peak [Table 8]. Age (β = -0.021, p = 0.927), gender (β =
0.061, \( p = 0.786 \), and \( fVO_2 (\beta = 0.331, p = 0.171) \) were not significant predictors in these models.

### Table 6. Selected PAF species and the severity of depression measured by the HAM-D in an age-adjusted linear regression, \( N = 20 \).

<table>
<thead>
<tr>
<th>PAF species</th>
<th>HAM-D total score</th>
<th>F statistic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC (O-12:0/2:0)</td>
<td></td>
<td>2.497</td>
<td>0.112</td>
</tr>
<tr>
<td>PC (O-14:0/2:0)</td>
<td></td>
<td>3.087</td>
<td>0.072</td>
</tr>
<tr>
<td>PC (O-14:1/2:0)</td>
<td></td>
<td>2.459</td>
<td>0.115</td>
</tr>
<tr>
<td>PC (O-16:0/2:0)</td>
<td></td>
<td>0.415</td>
<td>0.667</td>
</tr>
<tr>
<td>PC (O-18:1/0:0)</td>
<td></td>
<td>5.073</td>
<td>0.019</td>
</tr>
<tr>
<td>PC (O-18:3/2:0)</td>
<td></td>
<td>4.140</td>
<td>0.034</td>
</tr>
<tr>
<td>PC (O-20:6/2:0)</td>
<td></td>
<td>4.898</td>
<td>0.021</td>
</tr>
<tr>
<td>PC (O-22:6/2:0)</td>
<td></td>
<td>1.822</td>
<td>0.192</td>
</tr>
</tbody>
</table>

### Table 7. Selected PAF species and the severity of depression measured by the HAM-D in a gender-adjusted linear regression, \( N = 20 \).

<table>
<thead>
<tr>
<th>PAF species</th>
<th>HAM-D total score</th>
<th>F statistic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC (O-12:0/2:0)</td>
<td></td>
<td>2.364</td>
<td>0.124</td>
</tr>
<tr>
<td>PC (O-14:0/2:0)</td>
<td></td>
<td>2.202</td>
<td>0.141</td>
</tr>
<tr>
<td>PC (O-14:1/2:0)</td>
<td></td>
<td>1.881</td>
<td>0.183</td>
</tr>
<tr>
<td>PC (O-16:0/2:0)</td>
<td></td>
<td>0.290</td>
<td>0.752</td>
</tr>
<tr>
<td>PC (O-18:1/0:0)</td>
<td></td>
<td>3.882</td>
<td>0.043</td>
</tr>
<tr>
<td>PC (O-18:3/2:0)</td>
<td></td>
<td>4.824</td>
<td>0.022</td>
</tr>
<tr>
<td>PC (O-20:6/2:0)</td>
<td></td>
<td>2.919</td>
<td>0.081</td>
</tr>
<tr>
<td>PC (O-22:6/2:0)</td>
<td></td>
<td>1.862</td>
<td>0.186</td>
</tr>
</tbody>
</table>

### Table 8. Selected PAF species and the severity of depression measured by the HAM-D in an \( fVO_2 \) peak-adjusted linear regression, \( N = 20 \).

<table>
<thead>
<tr>
<th>PAF species</th>
<th>HAM-D total score</th>
<th>F statistic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC (O-12:0/2:0)</td>
<td></td>
<td>3.363</td>
<td>0.062</td>
</tr>
<tr>
<td>PC (O-14:0/2:0)</td>
<td></td>
<td>1.904</td>
<td>0.183</td>
</tr>
<tr>
<td>PC (O-14:1/2:0)</td>
<td></td>
<td>1.596</td>
<td>0.235</td>
</tr>
<tr>
<td>PC (O-16:0/2:0)</td>
<td></td>
<td>0.697</td>
<td>0.513</td>
</tr>
<tr>
<td>PC (O-18:1/0:0)</td>
<td></td>
<td>3.568</td>
<td>0.054</td>
</tr>
<tr>
<td>PC (O-18:3/2:0)</td>
<td></td>
<td>2.554</td>
<td>0.111</td>
</tr>
<tr>
<td>PC (O-20:6/2:0)</td>
<td></td>
<td>2.041</td>
<td>0.164</td>
</tr>
<tr>
<td>PC (O-22:6/2:0)</td>
<td></td>
<td>2.671</td>
<td>0.102</td>
</tr>
</tbody>
</table>

**Note:** PAF, platelet activating factor; HAM-D, 17-Item Hamilton Rating Scale for Depression; \( fVO_2 \), fraction of age and gender expected \( VO_2 \) peak. Trends are highlighted in orange; significant associations are highlighted in yellow. Age, gender, and \( fVO_2 \) peak were not significant predictors in these models. \( p \) value significance at \( p \leq 0.05 \).
3.4.3 **Depressive Symptoms Measured by the BDI-II**

There was a significant association between PC (O-22:6/2:0) and a greater BDI-II score using linear regression [Figure XII]. No associations between BDI-II score and the other PAF species were observed [Table 4]. The association between PC (O-22:6/2:0) and BDI-II score remained significant in linear regression after adjusting for age (F = 4.443, p = 0.028), or gender (F = 4.380, p = 0.029) as covariates. A trend remained when adjusting for fVO$_2$ (F = 3.531, p = 0.055). Age ($\beta = -0.059$, p = 0.776), gender ($\beta = -0.006$, p = 0.975), and fVO$_2$ ($\beta = -0.042$, p = 0.850) were not significant predictors in these models.

![Figure XII](image.png)

**Figure XII.** The association between baseline plasma abundance of PC (O-22:6/2:0) and depressive symptoms measured by the BDI-II in 20 depressed CAD patients. Summary statistics: $r = 0.583$, $R^2 = 0.340$, p = 0.007.
3.4.4  Depressive Symptom Clusters (HAM-D)

Our analyses suggest that PC (O-18:1/0:0) is associated with HAM-D score in linear regression. The associations between PC (O-18:1/0:0) and PC (O-16:0/2:0), as it is our primary PAF species of investigation, and depressive symptom clusters measured by the HAM-D were investigated.

3.4.4.1  Mood Cluster

There was no association between the mood cluster score and PC (O-18:1/0:0) or PC (O-16:0/2:0) in linear regression [Table 9].

3.4.4.2  Sleep and Psychic Anxiety Cluster

The association between PC (O-18:1/0:0) and sleep and psychic anxiety score was significant in a linear regression. There was no association between PC (O-16:0/2:0) and this cluster [Table 9].

3.4.4.3  Appetite Cluster

There was no association between the appetite cluster score and PC (O-18:1/0:0) or PC (O-16:0/2:0) in linear regression [Table 9].

3.4.4.4  Somatic Cluster

There was no association between the somatic cluster score and PC (O-18:1/0:0) or PC (O-16:0/2:0) in linear regression [Table 9].

<table>
<thead>
<tr>
<th>PAF Species</th>
<th>PC (O-18:1/0:0)</th>
<th>PC (O-16:0/2:0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F Statistic</td>
<td>p Value</td>
</tr>
<tr>
<td>Mood</td>
<td>1.872</td>
<td>0.188</td>
</tr>
<tr>
<td>Sleep and Psychic Anxiety</td>
<td>5.282</td>
<td>0.034</td>
</tr>
<tr>
<td>Appetite</td>
<td>2.845</td>
<td>0.109</td>
</tr>
<tr>
<td>Somatic</td>
<td>0.278</td>
<td>0.605</td>
</tr>
</tbody>
</table>

Note: PAF, platelet activating factor; HAM-D, 17-Item Hamilton Rating Scale for Depression; Trends are highlighted in orange; significant associations are highlighted in yellow. p value significance at ≤ 0.05.
3.4.4.5 Covariate-Adjusted Linear Regression of Symptom Cluster Associations
The association between PC (O-18:1/0:0) and the sleep and psychic anxiety cluster of the HAM-D was reduced to a trend when adjusting for age in a linear regression (F = 2.873, p = 0.084).
The association was significant when adjusting for gender (F = 3.887, p = 0.041) but not when adjusting for fVO₂ (F = 2.311, p = 0.133). Age (β = 0.165, p = 0.455), gender (β = 0.296, p = 0.161), and fVO₂ (β = 0.080, p = 0.743) were not significant predictors in these models.

3.5 Post-hoc Analyses

3.5.1 Variations in HAM-D and BDI-II Associated with Participant Characteristics
A greater HAM-D score and BDI-II score were both significantly associated with the presence of major depression over minor depression according to DSM-IV criteria. A greater BDI-II score was also associated with SSRI use; however, only one participant used an SSRI therefore conclusions regarding this association are tenuous. No other associations between participant characteristics and HAM-D score or BDI-II score were detected [Table 10].

3.5.2 Variations in PAF Abundance Associated with Participant Characteristics
No interactions were found between plasma abundance of PC (O-18:1/0:0), or PC (O-16:0/2:0) and the presence of diabetes mellitus, obesity (BMI greater than or equal to 30), platelet inhibitor use (clopidogrel or prasugrel), acetylsalicylic acid (ASA) use, major or minor depression, the number of vascular risk factors, or time since most recent ACS. A trend between antidepressant use and greater PC (O-18:1/0:0) abundance was observed. No association between antidepressant use and PC (O-16:0/2:0) was observed [Table 11].
### Table 10. Post-hoc investigation of the association between participant characteristics and depressive symptoms measured by the HAM-D and BDI-II, N = 20.

<table>
<thead>
<tr>
<th>Participant Characteristics</th>
<th>HAM-D</th>
<th></th>
<th>BDI-II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Statistic</td>
<td>p Value</td>
<td>T Statistic</td>
<td>p Value</td>
</tr>
<tr>
<td>DM</td>
<td>0.446</td>
<td>0.647</td>
<td>0.510</td>
<td>0.616</td>
</tr>
<tr>
<td>Obesity</td>
<td>-0.733</td>
<td>0.473</td>
<td>-0.262</td>
<td>0.796</td>
</tr>
<tr>
<td>AD use</td>
<td>1.338</td>
<td>0.197</td>
<td>2.400</td>
<td>0.027</td>
</tr>
<tr>
<td>PI use</td>
<td>-0.418</td>
<td>0.681</td>
<td>-0.226</td>
<td>0.824</td>
</tr>
<tr>
<td>ASA use</td>
<td>0.164</td>
<td>0.871</td>
<td>-0.049</td>
<td>0.961</td>
</tr>
<tr>
<td>Major/Minor Depression</td>
<td>-2.496</td>
<td>0.022</td>
<td>-2.508</td>
<td>0.022</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td>r = 0.221</td>
<td>0.348</td>
<td>r = 0.320</td>
<td>0.169</td>
</tr>
<tr>
<td>Time since event</td>
<td>r = -0.072</td>
<td>0.769</td>
<td>r = -0.303</td>
<td>0.207</td>
</tr>
</tbody>
</table>

### Table 11. Post-hoc investigation of the association between participant characteristics and abundance of PC (O-18:1/0:0) and PC (O-16:0/2:0), N = 20.

<table>
<thead>
<tr>
<th>Participant Characteristics</th>
<th>PC (O-18:1/0:0)</th>
<th></th>
<th>PC (O-16:0/2:0)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Statistic</td>
<td>p Value</td>
<td>T Statistic</td>
<td>p Value</td>
</tr>
<tr>
<td>DM</td>
<td>-0.110</td>
<td>0.913</td>
<td>0.774</td>
<td>0.449</td>
</tr>
<tr>
<td>Obesity</td>
<td>-0.506</td>
<td>0.619</td>
<td>-1.440</td>
<td>0.167</td>
</tr>
<tr>
<td>AD use</td>
<td>1.743</td>
<td>0.098</td>
<td>1.014</td>
<td>0.324</td>
</tr>
<tr>
<td>PI use</td>
<td>0.108</td>
<td>0.915</td>
<td>1.119</td>
<td>0.279</td>
</tr>
<tr>
<td>ASA use</td>
<td>1.473</td>
<td>0.159</td>
<td>-0.225</td>
<td>0.825</td>
</tr>
<tr>
<td>Major/Minor Depression</td>
<td>-1.643</td>
<td>0.118</td>
<td>-0.118</td>
<td>0.907</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td>r = 0.207</td>
<td>0.382</td>
<td>r = -0.208</td>
<td>0.380</td>
</tr>
<tr>
<td>Time since event</td>
<td>r = 0.066</td>
<td>0.789</td>
<td>r = -0.139</td>
<td>0.571</td>
</tr>
</tbody>
</table>

**Note:** The PAF species selected for post-hoc analyses were based on the established study hypotheses and the significant associations with depressive symptoms in the covariate-adjusted linear regressions. In both tables, the relationship between HAM-D and BDI-II score and participant characteristics [Table 10] and PAF abundance and participant characteristics [Table 11] was analysed using an independent samples T-test for all except vascular risk factors and time since event. For these continuous variables, a bivariate Pearson correlation was used. Abbreviations: PAF, platelet activating factor; DM, diabetes mellitus; AD, antidepressant; PI, platelet inhibitor; ASA, acetylsalicylic acid. Trends are highlighted in orange. p value significance at ≤ 0.05.
3.5.3 The Association Between HAM-D score and BDI-II score

HAM-D score and BDI-II score were significantly correlated in this sample (Spearman’s Rho = 0.564, p = 0.010).

3.5.4 Post-hoc Power Calculation for Selected PAFs

3.5.4.1 PC (O-16:0/2:0) and HAM-D

The unadjusted linear regression equation between PC (O-16:0/2:0) and HAM-D score is \( y = -15.68 + 1.04x \), therefore the minimal detectible difference is 1.043. Using a two-tailed significance level (\( \alpha \)) of 0.05 and a sample of 20 participants, we can detect the association between PC (O-16:0/2:0) abundance at baseline and HAM-D score with 9.1% power. We would require a sample size of 349 participants to detect this association with 80% power and prove our primary hypothesis. The \( R^2 \) values for the associations between PC (O-18:1/0:0) and HAM-D score and PC (O-22:6/2:0) and BDI-II score were 0.30 to 0.34, respectively. Our corresponding power to detect those relationships ranged from 80% to 86%. In a future study, a sample size of 50 would be sufficient to detect small relationships between these PAFs and depression severity (\( r^2=0.09 \)), with a set of covariates accounting for an expected 34% of the variance (\( r^2=0.34 \)) with 80% power at a significance level (\( \alpha \)) of 0.05.
IV
Discussion, Conclusions and Recommendations

4.1 Study Findings and Interpretation

As PC (O-16:0/2:0) is a potent pro-inflammatory PAF species associated with CAD, neurodegeneration, and several proposed mechanisms underlying depressive symptoms in CAD patients, we hypothesized that a greater plasma abundance of PC (O-16:0/2:0) would be associated with a greater severity of depressive symptoms in CAD patients. We found no association between PC (O-16:0/2:0) and HAM-D score in the investigation of our primary hypothesis. Similarly, no association between PC (O-16:0/2:0) and BDI-II score was observed in the investigation of our secondary hypothesis. Furthermore, PC (O-16:0/2:0) was not associated with any symptom clusters measured by the HAM-D. Thus, our findings suggest that the plasma abundance of PC (O-16:0/2:0) is unlikely to be a marker of depressive symptom severity in CAD patients.

Exploratory analyses revealed associations between HAM-D score and the PAF species PC (O-12:0/2:0), PC (O-18:3/2:0), PC (O-20:6/2:0), and the lyso-PAF PC (O-18:1/0:0). Trends were observed for the PAFs PC (O-14:0/2:0), PC (O-14:1/2:0), and PC (O-22:6/2:0). After adjusting for the individual effects of the covariates age, gender, and fVO₂ peak in three independent analyses, only PC (O-18:1/0:0) was consistently associated with HAM-D (although it was reduced to a trend, \( p = 0.054 \), when adjusting for fVO₂ peak). PC (O-18:3/2:0) remained significantly associated with HAM-D score after adjusting for age and gender, although this association was no longer present when adjusting for fVO₂ peak. PC (O-22:6/2:0) was significantly associated with BDI-II score even after adjusting for the covariates in independent analyses (although, like PC (O-18:1/0:0), it was reduced to a trend when adjusting for fVO₂.
peak). It is noteworthy that fVO$_2$, like age and gender, was not a significant predictor of depressive symptoms in any of these models.

It is interesting that none of the PAF species investigated were associated with the BDI-II (except for PC (O-22:6/2:0)), despite several being associated with the HAM-D. These differences may be partly due to the fact that the HAM-D is an investigator-rated scale whereas the BDI-II is completed by the participant. As mentioned, these scales are both established measures of depressive symptoms and have been used in several previous studies in CAD. Furthermore, a post-hoc analysis demonstrated that scores on these scales are significantly correlated in our sample, further supporting the use of both scales. However, each scale weighs certain depressive symptoms differently. For example, the HAM-D attributes up to 6 points to sleep disturbances, whereas the BDI-II attributes a maximum of 3 points. While both scales are valid for assessing depression in CAD, previous studies have identified discordance between investigator and participant-rated assessment of depression, despite a correlation between the two (Enns et al., 2000; Carter et al., 2010) suggesting that the different outcomes in this study are not unexpected.

As PC (O-18:1/0:0) was the only exploratory PAF species that was consistently associated with HAM-D score, we investigated its association with depressive symptom clusters. PC (O-18:1/0:0) was significantly associated with the sleep and psychic anxiety scale; however, this association was attenuated when adjusting for age or fVO$_2$. Thus, we cannot make any conclusions about the association between PC (O-18:1/2:0) and this symptom cluster or the others.

In post-hoc analyses, none of the participant characteristics, including selected medications, were associated with depressive symptoms on the HAM-D or BDI-II, except for the variable
presence of major or minor depression (both scales) or antidepressant use (BDI-II). These associations are expected as a greater HAM-D and BDI-II score would logically be associated with the presence of a major depressive episode rather than minor depression. Our results indicated that antidepressant use was associated with greater depressive symptoms; however, only one participant was using an antidepressant, therefore limiting our exploration of this association. Similarly, none of the participant characteristics were associated with the selected PAF species in our depression analysis, except for a trend with antidepressant use, exploration of which was limited. The lack of association between participant characteristics and PC (O-16:0/2:0) or PC (O-18:1/0:0) plasma abundance suggests that the relationship between PAF abundance and depressive symptoms was not an artifact of participant characteristics in our analyses.

Although not identified by our study, previous studies have demonstrated interactions between certain pharmacotherapies and PAF metabolism. For example, while the particular mechanisms are unclear, HMG-CoA reductase inhibitor (statin) treatment has been shown to affect PAF metabolism and PAF-AH activity (Tsantila et al., 2011). As statins have been associated with lower rates of depression in CAD patients (Stafford and Berk, 2011) and their use is also known to reduce cerebrovascular reactivity (Murakami et al., 2008), it is possible that statins exert these effects partly though their effects on PAF metabolism. We were unable to investigate the association between statin use and PAF abundance in our sample as statin treatment was used by nearly all study participants.

As this is the first targeted lipidomic analysis of PAF species in a clinical sample that we are aware of, the variable findings in the correlations between specific PAF species and depressive symptoms are unprecedented. As mentioned, PC (O-16:0/2:0) is the best-described PAF and has been shown to be a highly potent activator of cytokine release and leukocyte recruitment
For these reasons, PC (O-16:0/2:0) was selected as our primary PAF for investigation as a biomarker. However, other PAF species, such as PC (O-18:0/2:0) and PC (O-18:1/2:0) have also been described (Weintraub et al., 1985; Levi et al., 1989; Handa et al., 1991; Erger and Casale, 1996; Ryan et al., 2008). Since the majority of PAF actions in the periphery are thought to result from PAFR activation (Ishii and Shimizu, 2000), it is possible that differential effects of PAF species result from differential binding potential of the PAFR.

Although PAF species differences in PAFR binding affinity have yet to be investigated, a recent study demonstrated that different PAF species may activate different intracellular signalling systems in neurons (Ryan et al., 2008). In that study, both PC (O-16:0/2:0) and PC (O-18:0/2:0) could activate apoptotic pathways independently of the PAFR; however, PC (O-16:0/2:0) activated the caspase-7 pathway whereas the PC (O-18:0/2:0) signal was caspase independent. Furthermore, that study showed that PC (O-16:0/2:0) binding of the PAFR was anti-apoptotic, whereas PC (O-18:0/2:0) binding was pro-apoptotic. Based on those findings, we can infer that differential signalling effects likely extend to other PAF species, beyond PC (O-16:0/2:0) and PC (O-18:0/2:0), both PAFR dependently and independently. Those findings suggest that the lack of association between PC (O-16:0/2:0) and depressive symptoms may be species specific and not characteristic of all PAF species, thus adding perspective to the failure of our primary hypothesis.

That rationale may be further supported by the present findings as exploratory analyses found possible associations between certain PAF species and depressive symptoms. In particular, a positive correlation between the abundance of the lyso-PAF intermediate, PC (O-18:1/0:0), and depressive symptoms (HAM-D) was observed. Interestingly, this was the only lyso-PAF species shown to be associated with depressive symptoms; all other positive associations involved PAF species. Although not statistically significant, the association between the PC18:1 PAF, PC (O-
18:1/2:0), and HAM-D was negative in direction, suggesting either that the PC18:1 PAF pathway might have opposing effects to the other PAFs studied, or that the \textit{lyso}-intermediate is more potent.

Elevated concentrations of PC (O-18:1/0:0) have been detected in the brains of Alzheimer’s disease patients post-mortem (Ryan \textit{et al.}, 2009), implicating this species as a marker of neurodegeneration. The relationship between PAFs in plasma and PAFs in the CNS is unclear. Whether a certain PAF or lyso-PAF species can more easily cross the blood-brain barrier has not been determined. Therefore, we cannot yet suggest that plasma concentrations of PC (O-18:1/0:0) are reflective of elevated CNS concentrations. Furthermore, animal studies indicate that PAF biomarkers in the CNS may be reflected by different PAF biomarkers in plasma. For example, PC (O-16:0/2:0) is neurotoxic when elevated in the CNS but this is not reflected by plasma concentrations (Bennett SAL, personal communication [\textbf{Appendix G}]). Indeed, plasma concentrations of PC (O-18:0/2:0) appear to be representative of neurodegeneration in animals (Bennett SAL, personal communication). Those findings not only supporting the lack of association between plasma PC (O-16:0/2:0) and depressive symptoms in our study, but also suggest that potential PAF biomarkers in plasma ought to be investigated despite incongruence with CNS PAFs.

Accordingly, we hypothesize that PC (O-16:0/2:0) measured in the CSF or in post-mortem brain tissue would be associated with the degree of depression in a sample of CAD patients and that plasma concentrations might consistently fail to reveal this association. Thus, the failure of our primary hypothesis might be due to the medium of PC (O-16:0/2:0) sampling, rather than PC (O-16:0/2:0) lacking association with depressive symptoms. The use of a comprehensive lipidomics approach to investigate the profile of PAF species in plasma would confirm the
relevance of PC (O-18:1/0:0) as a plasma marker for depressive symptoms and explore other related lipids indicating pathophysiological mechanisms.

4.2 Limitations

The small sample size of this study limited the analyses to only one covariate. The small sample was mainly a consequence of eligible participants being excluded at the baseline visit due to a resolution of depressive symptoms over the previous two weeks (between screening and baseline). The requirement that participants meet DSM-IV depression criteria at both the screening visit and the baseline is therefore a major contributor to the small sample size generated from 220 eligible CAD patients. The spontaneous remission observed in these patients could be a result of the transient nature of depressive symptoms and/or the possibility that the depressive symptoms initially observed at screening were reflective of an adjustment syndrome resulting from the stress of a cardiac event, which had naturally resolved. Despite the implications to sample size, requiring that participants meet DSM-IV criteria at both visits generated a more representative sample of depression in CAD and is therefore an appropriate design for this study. Furthermore, as the presence of other Axis I psychiatric disorders was not assessed using structured DSM-IV criteria, it is possible that our sample contained depressed patients experiencing depression due to bipolar depression or a primary anxiety disorder. However, we did establish that none of our participants had a history of a diagnosed Axis I disorder other than (unipolar) depression.

As a result of the small sample size, we were unable to include age, gender, and VO$_2$ peak as covariates in the same linear regression model when analyzing the relationship between PAFs and depressive symptoms. However, we were able to examine the individual effects of these covariates on the association between PAFs and depressive symptoms, thus approximating the
combined effect of these covariates on this model. Furthermore, we limited our symptom cluster and post-hoc analyses to PC (O-18:1/0:0) in addition to PC (O-16:0/2:0), our primary PAF of investigation, as this species was the only one to remain potentially associated with HAM-D score when adjusting for all three covariates. The PAF PC (O-18:3/2:0) demonstrated a significant association with HAM-D when adjusting for age and gender, however, it was no longer associated with HAM-D score when adjusting for $fVO_2$, and was therefore not considered for this exploratory analysis. It is possible that the small sample size was a factor in the differential associations observed between PC (O-18:1/0:0) and PC (O-18:3/2:0) and HAM-D score when adjusting for $fVO_2$, thus limiting our exploration of a potential association between PC (O-18:3/2:0) and symptom clusters on the HAM-D. However, the purpose of this criterion was to add rigor to the study design and, considering the limited sample size, it was more appropriate to remain conservative with the exploration of our findings. A larger sample size would have also allowed for the exploration of depressive symptom factors that are unique to this population of CAD patients and the corresponding relationships with PAF species. Thus, we have acknowledged the limited analytical power of this study due to the small sample size through our conservative conclusions from the exploratory analyses. In addition, we have determined through post-hoc analyses that this study achieved only 9.1% power to detect a significant association between PC (O-16:0/2:0) and HAM-D score, with 349 participants required to achieve 80% power. However, post-hoc analyses suggest that a sample size of 50 participants would provide 80% power to investigate the association between the plasma abundance of PC (O-18:1/0:0) and HAM-D score, as well as PC (O-22:6/2:0) and BDI-II score, including relevant covariates.

The small sample size also limits the investigation of different treatment effects on PAF levels. For example, only one participant used SSRI antidepressants; therefore, the analysis of
differential PAF abundance between SSRI users and non-users was limited by the small sub-
group size of SSRI users. Similarly, only two participants were classified as experiencing minor
depression, thus limiting the interpretation of post-hoc analyses for this outcome. A greater
sample size would generate greater representation of each group, thus allowing for an
appropriate investigation of the interactions between drugs and participant characteristics and
PAF species.

As this study is cross-sectional, we cannot assess causality in the relationship between PAFs and
depressive symptoms. Therefore, we cannot comment on whether a greater abundance of certain
PAF species is a result of depressive pathophysiology or an antecedent to a greater severity of
depression. Another limitation is the lack of a non-depressed CAD control group. We are
therefore unable to compare the plasma abundance of PAFs between depressed and non-
depressed CAD patients. Despite this limitation, we have nevertheless detected associations
between specific PAFs and the severity of depression, suggesting that their abundance in plasma
is related to the degree of depression. Based on these findings, we can hypothesize that the mean
plasma abundance of these PAFs would be higher in depressed CAD patients compared to non-
depressed CAD patients.

While a strength of this study is the relative homogeneity of the sample (all CAD), a limitation
is that we cannot extrapolate our findings to a non-CAD population. Therefore, the association
between PAF species and depressive symptoms in the general depressive population cannot be
determined by this study. Furthermore, as participants were excluded if they were aged 45 or
younger, regardless of a CAD diagnosis, our findings are limited to a middle-late aged
population. Therefore, we cannot suggest an association between PAFs and depressive
symptoms in a younger adult population.
This study was also limited to the investigation of PAFs derived from AAGPCs. As described in the introduction, many PAF lipids exist and their effects have been previously characterized. Our analysis was designed to identify only alkyl-PAFs containing a phosphocholine headgroup as these PAFs are the most abundant in the lipidome and their pro-inflammatory and neurodegenerative effects have been well described. Future studies might use a lipidomics approach across all \( sn-1 \) and \( sn-3 \) variants to capture the spectrum of PAF species in the plasma lipidome. This might lead to a better understanding of the mechanisms underlying bioactive lipids and neurodegenerative diseases, possibly identifying future therapeutic targets.

4.3 Recommendations for Future Studies

The present findings must be replicated in a larger sample in order to determine the appropriate future directions. However, the potential positive associations between the PAFs PC (O-18:1/0:0) and PC (O-22:6/2:0) and depressive symptoms from our exploratory analyses suggest that a higher plasma abundance of these species is associated with a greater severity of depressive symptoms in CAD patients. We can therefore propose that the underlying mechanisms of these PAFs in the plasma warrant exploration. Future studies that investigate the utility of these PAFs in predicting depressive outcomes over time due to antidepressant pharmacotherapies, psychotherapies, and/or exercise interventions may clarify their biomarker capabilities. To this end, we hypothesize that a greater concentration of specific PAFs, potentially PC (O-18:1/0:0) or PC (O-22:6/2:0), may be associated with a benefit from exercise interventions rather than antidepressant pharmacotherapy. This prediction is based on to the anti-inflammatory effects of exercise (Swardfager et al., 2012), the positive association between high concentrations of inflammatory biomarkers and antidepressant effects of exercise (Rethorst et al., 2012), and the poor treatment efficacy of SSRI pharmacotherapies in patients with high
levels of inflammatory biomarkers (Lanquillon et al., 2000; Eller et al., 2008). This hypothesis can be tested by future studies in our cardiac rehabilitation population.

Examining the fluctuations in PAF species abundance over the course of antidepressant interventions and with the corresponding resolution of depressive symptoms or lack thereof may provide insight into their etiopathological mechanisms in depression. In particular, studying the longitudinal effects of exercise on PAF metabolism is warranted as the association between PAF species and HAM-D score was attenuated by the fractional VO₂ value in many cases.

Should the PAF species identified by this study, or others, be consistently associated with depressive symptoms and the course of treatment response, future studies examining selective antagonists of PAF production or PAFR activation might be appropriate. Inhibiting the production of PAFs by selective PLA₂ antagonists, such as pyrrophenone, has been explored in animal models, but has yet to reach the clinic, possibly due to the multitude of PLA₂ mediated pathways (Flamand et al., 2006). Omega-3 fatty acids have also been shown to inhibit PAF production but these have not been linked in the setting of depression (Akisu et al., 2002; Simopoulos, 2002).

The PAF pathway has been more successfully targeted using PAFR antagonists, particularly in animal and clinical models of acute ischemia. Cerebrovascular pathology in depression has been consistently documented and is the basis of the vascular hypothesis of depression in late-life as first described by Alexopoulos and colleagues (Alexopoulos et al., 1997) and since reviewed (Alexopoulos, 2006; Naarding and Beekman, 2011). Cerebrovascular pathology can also be a feature of CAD (Ozeren et al., 1998; Hoshide et al., 2001; Vlek et al., 2009; Geerlings et al., 2010). Animal (Frerichs et al., 1990; Lindsberg et al., 1990) and clinical (Satoh et al., 1992; Adunsky et al., 1999) studies have documented that elevated concentrations of PAFs have been
found in ischemic tissue and that PAF concentrations have been linked to infarct size and severity in stroke. As PAF concentrations become swiftly elevated in the early stages of ischemia, it is suggested that PAFs are instigators of cerebrovascular damage (Lindsberg et al., 1990). In animal models of cerebral ischemia, the use of selective PAFR antagonists, such as LAU-0901 or BN 50739, is associated with greater local cerebral blood flow, reduced microglial infiltration, greater astrocytic and neuronal survival post-ischemia, and reduced infarct size (Frerichs et al., 1990; Liu et al., 2001; Belayev et al., 2008; Belayev et al., 2009; Belayev et al., 2012). Combined with their known neurodegenerative properties, their effects on cerebral vasculature during ischemia, and their associations with inflammation, oxidative and nitrosative stress, and vascular endothelial dysfunction, it is possible that PAFs contribute to vascular damage or pathophysiology during periods of elevated inflammatory activity, a feature of depression and CAD. Investigating the profile of PAFs across subtypes and age-ranges of depression might elucidate the particular population of use should PAFs become important biomarkers. Examining the potential of PAFR antagonists for treatment of depressive symptoms might also prove beneficial.

4.4 Conclusions

In this study of twenty depressed CAD patients, there was no association between the plasma abundance of the potent pro-inflammatory PAF species PC (O-16:0/2:0) and depressive symptoms. However, we identified potential associations between depressive symptoms and the lyso-PAF species PC (O-18:1/0:0) as well as the PAF species PC (O-22:6/2:0). Both species remained associated with depressive symptoms when adjusting for age or gender; however, these associations were reduced to a trend when adjusting for fractional VO2. Although this study was limited by the small sample size, this is the first time that PAF species have been associated with depressive symptoms in CAD or otherwise. Therefore, larger studies
investigating the association between PAF species and depression in CAD, particularly longitudinally, are warranted.
References


Alexopoulos, G.S., 2006. The vascular depression hypothesis: 10 years later. Biological Psychiatry 60, 1304-1305.


Karlović, D., Serretti, A., Vrkić, N., Martinac, M., Marčinko, D., 2012. Serum concentrations of CRP, IL-6, TNF-α and cortisol in major depressive disorder with melancholic or atypical features. Psychiatry Research 198, 74-80.


Liu, Y., Ho, R.C., Mak, A., 2011. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. J Affect Disord Aug 25 [Epub ahead of print].


Maes, M., Ruckoanich, P., Chang, Y.S., Mahanonda, N., Berk, M., 2011. Multiple aberrations in shared inflammatory and oxidative & nitrosative stress (IO&NS) pathways explain the co-association of depression and cardiovascular disorder (CVD), and the increased risk for CVD and due mortality in depressed patients. Progress in Neuro-Psychopharmacology & Biological Psychiatry 35, 769-783.


cells and mouse cortex using liquid chromatography/multi-stage mass spectrometry (LC/MS3). Rapid Communications in Mass Spectrometry 22, 3579-3587.


Stafford, L., Berk, M., 2011. The use of statins after a cardiac intervention is associated with reduced risk of subsequent depression: proof of concept for the inflammatory and oxidative hypotheses of depression? J Clin Psychiatry 72, 1229-1235.


List of Publications and Abstracts

Peer-reviewed publications


Meeting/Conference Abstracts


Mazereeuw G, Herrmann N, Swardfager W, Bennett SAL, Ma D, Oh P, Lanctôt KL (2011) The role of platelet-activating factor in cerebrovascular dysfunction and depression. *Faculty of Medicine and CIHR Training Program in Neurodegenerative Lipidomics Symposia* (Ottawa, Canada) (*Supported by a CTPNL Travel Award*)


List of Awards and Sources of Funding

Scholarships

2011/2012 - Toronto Rehabilitation Institute Student Scholarship - $5,000
2011/2012 - Ontario Graduate Scholarship (OGS) - $15,000
2011/2012 - CIHR Training Program in Neurodegenerative Lipidomics - $5,000
   Graduate Student Supplement Award
2010/2011 - Ontario Graduate Scholarship (OGS) - $15,000
2010/2011 - CIHR Training Program in Neurodegenerative Lipidomics - $5,000
   Graduate Student Supplement Award

Travel Awards

2012 – CIHR Training Program in Neurodegenerative Lipidomics Travel Award
2011 – CIHR Training Program in Neurodegenerative Lipidomics Travel Award

Awards

2011 - Visions in Pharmacology Research Day 2011 Master’s Poster Award – Tied 3rd ($50.00)
Appendices
Appendix A – REB Approval
MEMORANDUM

To: Dr. K. Lanctot
Psychiatry
Room FG05

From: Dr. Philip Hébert

Date: July 28, 2009

Subject: CAROTID: CAD Randomized Omega-3 Trial in Depression

The Research Ethics Board is in receipt of your letter dated July 15, 2009 in response to the comment letters dated June 8 and June 15, 2009.

The Board has given provisional approval to submit your amended documents as follows to Health Canada.

REB approval will be provided upon receipt to this document.

- Information sheet/consent form dated July 14, 2009
- Protocol Version 1.1 dated July 14, 2009

To enable us to complete our review of this study, please provide a response to each comment in a letter to the Chair and forward, along with a copy of all revised documents, to the Research Ethics Office, Room C8 19. We look forward to hearing from you and to approving your study.

Philip G. Hébert MD PhD FCFPC
Chair, Research Ethics Board
May 27, 2010

Dr Paul Oh
TRI - Rumsey Centre (Cardio)
347 Rumsey Road
Toronto, ON., M4G 1R7

Dear Dr. Oh:

RE: TRI REB # 09-014
CAROTID: CAD Randomized Omega-3 Trial in Depression

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 dated April 15, 2010 and the consent form dated May 25, 2010 are 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.

The following documents are also approved for use:

Data Collection Forms received January 20, 2010
Advertisement Poster received January 15, 2010

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,

[Signature]

Yves Hecquebert
Chair, Research Ethics Board
Toronto Rehabilitation Institute

May 27, 2010
Date of Initial REB Approval
June 21, 2010

Dr. Krista Lanctôt
Department of Psychiatry
Sunnybrook Health Sciences Centre
2075 Bayview Avenue, Room FG05
Toronto, Ontario
M4N 3M5

Dear Dr. Lanctôt,

RE: CAROTID: CAD Randomized Omega-3 Trial in Depression (ID#411)

Ethics Approval

Expiry Date: June 17, 2011

This letter is to inform you that the above-named research study has been granted approval by the Medical Advisory Committee and Research Review Team (RRT) with a full-quorum of voting members on June 17, 2010. This study has been granted approval effective June 17, 2010 for a period of one year. The following documents have been approved until the expiry date noted above:

- CAROTID: CAD Randomized Omega-3 Trial in Depression Patient Information and Consent, version dated May 28, 2010
- CAROTID: CAD Randomized Omega-3 Trial in Depression study protocol version 1.4, dated April 15, 2010
- Updated Resource Impact Estimate Form, version received June 7, 2010

Please note that ongoing projects must be renewed prior to the expiry date.

During the course of the research, any significant deviations from the approved protocol (that is, any deviation which would lead to an increase in risk or a decrease in benefit to participants) and/or any unanticipated developments within the research should be brought to the attention of the Research Review Team. In the event of a privacy breach, you are responsible for reporting the breach to the Research Review Team and Trillium Health Centre’s Privacy Officer (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004 (PHIPA)). Additionally, the RRT requires reports of inappropriate/authorized use of information. As the Principal Investigator, you are responsible for the ethical conduct of this study.


Sincerely,

Dianne Godkin, RN PhD
Senior Ethicist, Regional Ethics Program
Acting Chair, Research Review Team
Trillium Health Centre

dob
May 28, 2012

Dr. Krista Lanctôt  
Sunnybrook Health Sciences Centre  
2075 Bayview Avenue, Room FG-05  
Toronto, Ontario  
M4N 3M5

Dear Dr. Lanctôt,

RE: CAROTID - CAD Randomized Omega-3 Trial in Depression (ID#411)

Renewal Approval  
Expiry Date: June 17, 2013

This letter is to inform you that renewal was granted for the above mentioned study by the Trillium Health Centre site Research Ethics Board (REB) with a full-quorum of voting members on May 17, 2012 for a period of one year effective June 17, 2012. The following documents are included as part of this renewal approval:

- CAROTID protocol, version 1.6 dated April 10, 2012
- Patient Information and Consent, version dated April 10, 2012

During the course of the research, any significant deviations from the approved protocol (that is, any deviation which would lead to an increase in risk or a decrease in benefit to participants) and/or any unanticipated developments within the research should be brought to the attention of the Trillium Health Centre site REB. In the event of a privacy breach, you are responsible for reporting the breach to the REB and the Trillium Health Centre site Privacy Officer (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004 (PHIPA)). Additionally, the REB requires reports of inappropriate/unauthorized use of information. As the Principal Investigator, you are responsible for the ethical conduct of this study.

The Trillium Health Centre site REB operates in compliance with the Tri-Council Policy Statement, ICH GCP Guidelines, PHIPA, and Part C, Division 5 of the Health Canada Food and Drug Regulations.

Sincerely,

Dianne Godkin, RN PhD  
Senior Ethicist, Regional Ethics Program  
Co-Chair, Research Ethics Board  
Trillium Health Centre
Appendix B – Informed Consent Form
You are being invited to consider participating in a research study funded by the Ontario Mental Health Foundation (OMHF). 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.

This form explains the purpose of this research study, provides information about the study, the tests and procedures involved, possible risks and benefits, and the rights of participants.

Please read this form carefully and ask any questions you may have. You may take as much time as you wish to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. Please ask the study staff or one of the investigator(s) 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.
PURPOSE OF THE STUDY

You are being invited to take part in this study because you have coronary artery disease, are currently in a cardiac rehabilitation program, have exhibited a high number of mood symptoms, which may indicate that you are depressed, and are not currently already taking omega-3 or fish oil supplements. The goal of our project is to determine whether omega-3 supplements have beneficial effects on mood and thinking, as well as whether they may improve quality-of-life.

Symptoms of depressed mood are sometimes experienced by patients with coronary artery disease (CAD). Patients with CAD are also likely to have lower levels of omega-3 fatty acids in their blood than patients without CAD, and this is especially likely in patients with CAD who experience mood symptoms.

Omega-3 fatty acids are considered essential fatty acids – essential to human health, but not produced by the body. Therefore, they must be obtained from outside sources, such as food or supplements. This study will use omega-3 fatty acid supplements, which have been approved by Health Canada for use to promote brain functions and cardiovascular health. While omega-3 fatty acids have been approved for use by Health Canada, they are not yet approved as a standard treatment in patients with CAD and this study aims to test this possibility.

What is the Usual Treatment?

Currently, patients with depression may be prescribed anti-depressants such as citalopram, fluoxetine and sertraline. Participating in this study will not require you to stop these medications, as long as they have been on a steady dose for at least 3 months.

WHAT OTHER CHOICES ARE THERE?

You do not have to participate in this study to receive treatment for your coronary artery disease or any symptoms of depression you may have. Other medications and treatments are available and can be discussed with your doctor. Resources are already in place at your rehabilitation facility to assist you if you are feeling symptoms of depression.

Why is This Study Being Done?

The purpose of this study is to see whether omega-3 supplements have any effects on mood, as well as whether they may improve quality-of-life.

What Will Happen During This Study?

If you agree to participate in this study, you will be asked to undergo an initial assessment with a trained researcher. This will involve a review of your demographic data (age, gender and diagnoses), medical history, electrocardiogram results and medications. To screen for mood symptoms, we would ask to review a questionnaire that you complete for the rehab staff. If you have a high number of mood symptoms, we will interview you further to assess the severity of these symptoms.
If you are eligible to participate in this study, you will be randomly assigned to receive omega-3 supplements or placebo (inactive substance). You have a 50% chance of receiving omega-3 supplements, and a 50% chance of receiving a placebo, containing soybean and corn oil. Neither you, nor the investigators will know which you are receiving. We would ask that you take three pills each morning, preferably with breakfast, for the duration of the study. We ask that while you are taking part in the study, you do not use any additional omega-3 pills purchased over the counter from health food stores or pharmacies.

During the study you will be monitored and assessed by trained study personnel. There will be 5 assessments (a screening visit, a baseline visit and follow-up visits at 4, 8 and 12 weeks after baseline), each of which will take approximately two hours to complete. The total time in this study from screening to completion will be 14 weeks. The study assessments will include questionnaires about your mood and quality-of-life. At 2 of these visits, we will take approximately 34mL (2⅓ tablespoons) of blood to test the levels of lipids (fats), including omega-3s, and certain signalling molecules related to the inflammatory system.

If you decide to participate in this study, you will be asked to do several activities. All of these activities (with the exception of the 3-day food diary and demographic questions) are exclusive to your participation in this study and would not otherwise be completed as part of your normal cardiac rehab program.

**Screening Visit:**
This visit will take approximately 2 hours.

- **Demographic Questions:** You will be asked to give personal information about yourself, such as your name, date of birth, race, etc.
- **Health and Medication Questions:** You will be asked to answer questions about your health, your medication history and the medications you take.
- **Height, Weight:** We will measure how tall you are and see how much you weigh.
- **Mood Testing:** You will be asked questions to determine your mood.
- **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.
- **Blood Pressure:** Your blood pressure will be checked by putting a band around your arm. This will squeeze your arm for about a minute.
- **Pregnancy Test:** Women of childbearing potential will be asked to provide an 8.5 mL blood sample (less than 2 teaspoons) to confirm that they are not pregnant. NOTE: If the pregnancy test is positive, you will be excluded from the trial.

**Baseline Visit:**
2 weeks after screening, this visit will take approximately 2 hours.

- **Mood Testing:** You will be asked questions to determine your mood.
- **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.
- **Quality of Life Survey:** You will be asked questions regarding your quality of life.
- **Blood Testing:** A 34.0 ml blood draw (2⅓ tablespoons) will be done to do laboratory tests.
• **Food Diary:** You will be asked to keep a 3-day food diary to assess your nutritional intake. This is standard procedure for your cardiac rehabilitation.

• **Capsule Count:** You will be asked to bring in any leftover study supplements you may have.

• In addition, we will review your medical chart for changes in diagnoses, cardiopulmonary fitness, demographics, medication, and recent ECG results.

### 4-Week Follow-up Visit
4 weeks after baseline, this visit will take approximately 1.5 hours.

• **Mood Testing:** You will be asked questions to determine your mood.

• **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.

• **Quality of Life Survey:** You will be asked questions regarding your quality of life.

• **Food Diary:** You will be asked to keep a 3-day food diary to assess your nutritional intake. This is standard procedure for your cardiac rehabilitation.

• **Capsule Count:** You will be asked to bring in any leftover study supplements you may have.

• **Adverse Event Checklist:** We will ask you if you are experiencing any problems with the study medication

• In addition, we will review your medical chart for changes in diagnoses, cardiopulmonary fitness, demographics, medication, and recent ECG results.

### 8-Week Follow-up Visit
8 weeks after baseline, this visit will take approximately 1.5 hours.

• **Mood Testing:** You will be asked questions to determine your mood.

• **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.

• **Quality of Life Survey:** You will be asked questions regarding your quality of life.

• **Food Diary:** You will be asked to keep a 3-day food diary to assess your nutritional intake. This is standard procedure for your cardiac rehabilitation.

• **Capsule Count:** You will be asked to bring in any leftover study supplements you may have.

• **Adverse Event Checklist:** We will ask you if you are experiencing any problems with the study medication

• In addition, we will review your medical chart for changes in diagnoses, cardiopulmonary fitness, demographics, medication, and recent ECG results.

### Termination Visit
12 weeks after baseline, this visit will take approximately 2 hours.

• **Mood Testing:** You will be asked questions to determine your mood.

• **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.

• **Blood Testing:** A 34.0 ml blood draw (2½ tablespoons) will be done to do laboratory tests.

• **Quality of Life Survey:** You will be asked questions regarding your quality of life.

• **Food Diary:** You will be asked to keep a 3-day food diary to assess your nutritional intake. This is standard procedure for your cardiac rehabilitation.

• **Capsule Count:** You will be asked to bring in any leftover study supplements you may have.

• **Adverse Event Checklist:** We will ask you if you are experiencing any problems with the study medication
• In addition, we will review your medical chart for changes in diagnoses, cardiopulmonary fitness, demographics, medication, and recent ECG results.

**HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?**
It is anticipated that about 254 people will participate in this study at 2 centres (Toronto Rehab Institute and Trillium Health Centre) throughout Toronto. The entire study is expected to take approximately 2 years to complete.

**WHAT ARE THE RESPONSIBILITIES OF PARTICIPANTS?**
By participating in this study you agree to be honest with study staff about capsule consumption, reporting of any adverse events and attending any scheduled visits.

If at any point during the study, you change your dose of or begin taking new medications (including natural health products), or if you begin psychotherapy, you must inform study staff.

If you become pregnant during the course of this study, you must stop supplementation immediately and inform study staff.

**WHAT ARE THE RISKS OF PARTICIPATING IN THIS STUDY?**
Side Effects: Omega-3 fatty acids are registered with Health Canada as a safe natural health product (food supplement). Omega-3 fatty acids are found in everyday foods, and omega-3 supplements are already recognized to help maintain good health and to support brain function. These supplements may produce mild side effects. The most common side effect of omega-3 supplements is fishy burps. Nausea, diarrhea, and pain in the middle abdomen also occur in approximately 3.8% of patients. Runny nose (3.3%), upper respiratory tract infection (3.3%), dyspepsia (2.5%) and skin abnormalities (1.7%) also occur.

These side effects are similar for the omega-3 pills and the placebo pills.

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected (30-100%)</td>
<td>Less Likely (10-30%)</td>
<td>Rare (0-1%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Runny nose</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Skin abnormalities</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Fish flavoured burps</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Omega-3 supplements may increase the risk of bleeding, as omega-3s can act as a blood thinner. There is, therefore, a theoretical risk that you could bruise more easily, or take longer to stop bleeding from a cut. However, this was found in patients taking more than twice the amount per day than you will be. As well, you will be asked to report if you have a change in bleeding times. If you bruise more easily or take longer to stop bleeding, we will notify your primary care physician for appropriate follow-up. If you are on warfarin, this may mean that your dose of warfarin may have to be adjusted. If a medical issue arises between study assessments, you are asked to contact the study investigators.
In the event that you take greater than the recommended dose of omega-3 supplements, you may experience an upset stomach and abnormal bleeding. Should this, or any other adverse event occur, please contact a member of the study staff immediately using contact information listed on the first page of this document. If the adverse event is severe, or to reach the study physician for urgent matters, please contact the locating number 416-480-4244 and ask for Dr. Herrmann to be paged. This is a 24 hr emergency contact number.

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. This may include new information about the risks and benefits of being a participant in this study. During the course of this study, any new information about the omega-3s and their safety will be communicated immediately to you and you will be asked if you would continue to participate in this study.

When your blood is drawn, there may be some discomfort and/or bruising; however, this is expected to be very mild.

What are the Benefits of Participating in this Study?

It is unknown whether you will benefit directly from participation in this study. There is a 50% chance that you will receive a placebo that is neutral to your cardiac and brain health.

Can Participation in this Study End Early?

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:
- The study doctor believes it is best for you to stop being in the study
- You do not follow directions about the study
- You require a change in your antidepressant medications (you will be asked about your medications at each visit)
- You start psychotherapy during the course of the study
- You become pregnant during the course of the study

If you are removed from this study, the investigator(s) will discuss the reasons with you and plans will be made for your continued care outside of the study. This will not affect your participation in your cardiac rehabilitation program.

Your participation in this study is voluntary. You may withdraw at any time. Thus, if you do not wish to take part in this study or wish to withdraw at any time after commencing the study, you and/or your family’s care will not be affected in any way. Your doctor will discuss alternate options for your care.

Should you choose to withdraw from the study you are encouraged to contact the study coordinator, Robert Mitchell, Department of Psychiatry at 416-480-6100 ext. 3185.
WHAT ARE THE COSTS OF PARTICIPATING IN THE STUDY?

You will incur no costs as a result of participation in this study. You will be reimbursed for any parking or travel expenses that are due to your participation in this study, upon providing a receipt. You will not receive any compensation or monetary benefits for participating in this trial.

Confidentiality

Your identity in this study will be treated as confidential. Certain research staff involved in this study may need to review your medical chart. If you agree to this study, we will look at your medical chart in order to record information on: blood pressure, heart rate, medications, mood symptoms and cardiopulmonary assessments that occur during the trial period. Data will be kept in password protected computer files and locked filing cabinets in a secure area. Data will only be accessed by the study investigators and by the research staff under their direct supervision. On all data collected for this study, your name will not be used, but instead you will be identified only by a unique assigned number. In the future, only the study investigators, members of the Toronto Rehab Research Ethics Board, Health Canada and other regulatory authorities will be granted direct access to your medical records. This access is to verify clinical trial procedures and/or data. This will be done without violating your confidentiality, to the extent permitted by the applicable laws and regulations.

If, during this study, you voice suicidal thoughts or an intention to harm yourself or others, we will notify your clinical care team at Toronto Rehab immediately. Your clinical team at Toronto Rehab will then be responsible for your care according to their usual protocol.

None of your personal information will be given to anyone without your permission unless required by law. When the results of this study are published, your identity will not be disclosed. The data for this study will be retained for 25 years.

Contacts

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 Toronto Rehab physician (study co-investigator) Dr. Paul Oh at 416-597-3422 x5263 or the person in charge of this study (Principal Investigator) Dr. Krista Lanctôt 416-480-6100 x2241.

If you have questions about your rights as a research participant, or about any ethical issues relating to this study, you can contact someone who is independent of the research team. Please call the Research Ethics Board Office at (416) 597-3422 x 3081.
Do The Investigators Have Any Conflicts Of Interest?

Dr. Paul Oh, the Toronto Rehab investigator of this study, is also the Chair of the Toronto Rehab Research Ethics Board. He has not been involved in the independent ethics review of this study. The other investigators declare that they have no conflicts of interest.
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.
2. By signing this consent form, you do not give up any of your legal rights.
3. If, as a result of your participation in this study, any new clinically important medical information about your health is obtained, you will be given the opportunity to decide whether you wish to be made aware of that information.
4. You have the right to access, review and request changes to your personal information (i.e. address, date of birth).
5. You have the right to be informed of the results of this study once the entire study is complete.
Consent to Participate in this Study:

I have read all 8 pages of the information and consent form and fully understand the nature and the purpose of the study in which I have been asked to take part. The explanation I have been given has mentioned both the possible risks and benefits of the study. I understand that I will be free to withdraw from the study at any time without affecting my subsequent treatment by my doctor in any way. I voluntarily consent to participate in this study. I understand that I have the right to receive a copy of this signed and dated informed consent package before participating in this study.

_________________________________
Name of Participant (typed or printed)

_________________________________
Signature of Participant Date

_________________________________
Name of Person obtaining Consent

_________________________________
Signature of Person obtaining Consent Date

_________________________________
Name of Investigator (typed or printed)

_________________________________
Signature of the Investigator Date
Appendix C – SCID-I Module for Depression
Structured Clinical Interview for the DSM-IV—Depression Module (SCID)

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

1. Has there been a period of time when you were feeling depressed or down most of the time nearly every day? What was that like?
   - If yes: How long did it last? As long as two weeks?
   - Rate if depression lasts most of the day, nearly every day

2. What about losing interest or pleasure in things you usually enjoyed?
   - If yes: Nearly every day? How long did it last? As long as two weeks?
   - Rate if markedly diminished pleasure in all or almost all activities most of every day, based on patient’s responses or on observations of others

The following seven questions focus on the worst two weeks in the past month. If the patient is depressed equally the whole month, focus on the last two weeks:

3. Did you lose or gain any weight? How was your appetite?
   - Rate if significant weight loss when dieting, weight gain, or change in appetite nearly every day
   - Check if: □ Weight Loss/Decreased Appetite
     □ Weight Gain/Increased Appetite

4. How were you sleeping?
   - Rate if insomnia or hypersomnia nearly every day
   - Check if: □ Insomnia
     □ Hypersomnia

5. Were you so fidgety or restless that you were unable to sit still? What about the opposite—talking or moving more slowly than normal for you?
   - Rate if evening agitation or retardation nearly every day, observable by others or according to behaviour during interview
   - Check if: □ Agitation
     □ Retardation

6. What was your energy like? Were you tired all the time? Nearly every day?
   - Rate if fatigue or loss of energy nearly every day

7. How did you feel about yourself? Worthless? Guilty about things you had or had not done?
   - Do not rate if merely self-reproach or guilt about being ill. Code as absent or equivocal if only low self-esteem
   - Check if: □ Worthlessness
     □ Guilt

8. Did you have trouble thinking or concentrating? Was it hard to make decisions about everyday things?
   - Check if: □ Diminished ability to think
     □ Indecisiveness

9. Were things so bad that you were thinking a lot about death or that you would be better off dead?
   - What about thinking about hurting yourself?
   - Do not rate if only fear of dying
   - Check if: □ Thoughts of own death
     □ Specific plan
     □ Suicidal ideation
     □ Suicide attempt

**CLASSIFY:**
□ Non-Depressed
□ Minor Depressed
□ Major Depressed (at least 5 of above are present, including #1 or #2)
Appendix D – 17-Item HAM-D
**Hamilton Depression Rating Scale (HAM-D)**

**Interviewer:** The questions in bold are to be read exactly as written. Additional questions are provided for further exploration or clarification as needed. You may add your own follow up questions if necessary.

**Overview:** I’d like to ask you some questions about the past week, since last (DAY OF THE WEEK). How have you been feeling since then?

<table>
<thead>
<tr>
<th>What’s your mood been this past week (compared to when you feel OK)?</th>
<th>Depressed Mood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you been feeling down or depressed? Sad? Hopeless? Helpless? Worthless?</td>
<td>0 - absent</td>
</tr>
<tr>
<td>If yes: Can you describe what this feeling has been like for you? How bad is this feeling? Does this feeling lift at all if something good happens? How are you feeling about the future?</td>
<td>1 - indicated on questioning</td>
</tr>
<tr>
<td>In the last week how often have you felt (own equivalent)? Every day? All day? Have you been crying at all?</td>
<td>2 - spontaneously reported verbally</td>
</tr>
<tr>
<td>Have you been especially critical of yourself this past week, feeling you’ve done things wrong, or let others down?</td>
<td>3 - communicated non verbally (facial expression, posture, voice, weeping)</td>
</tr>
<tr>
<td>If yes: What have your thoughts been? Have you been feeling guilty about anything you’ve done or not done? What about things that happened a long time ago? Have you thought that you’ve brought (this depression) on yourself in some way? Do you feel you’re punished by being sick? Do you feel your depression is a punishment for something bad that you’ve done? What?</td>
<td>4 - Virtually Only - spontaneous verbal and non-verbal communication</td>
</tr>
<tr>
<td>If scored 1-4: How long have you been feeling this way?</td>
<td><strong>Feelings of Guilt</strong></td>
</tr>
<tr>
<td>0 - absent</td>
<td>0 - absent</td>
</tr>
<tr>
<td>1 - self reproach, feels he has let people down</td>
<td>1 - self reproach, feels he has let people down</td>
</tr>
<tr>
<td>2 - ideas of guilt or rumination over past errors or sinful deeds</td>
<td>2 - ideas of guilt or rumination over past errors or sinful deeds</td>
</tr>
<tr>
<td>3 - present illness is punishment, delusions of guilt</td>
<td>3 - present illness is punishment, delusions of guilt</td>
</tr>
<tr>
<td>4 - hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations</td>
<td>4 - hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations</td>
</tr>
</tbody>
</table>
This past week have you had any thoughts that life is not worth living? What about thinking you’d be better off dead? Have you had thoughts of hurting or killing yourself? If yes:
What have you thought about?
Have you actually done anything to hurt yourself?

<table>
<thead>
<tr>
<th>Suicide</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - absent</td>
</tr>
<tr>
<td>1 - feel life is not worth living</td>
</tr>
<tr>
<td>2 - wishes he were dead or any thoughts of possible death to self</td>
</tr>
<tr>
<td>3 - suicidal ideas or gestures</td>
</tr>
<tr>
<td>4 - attempts at suicide</td>
</tr>
</tbody>
</table>

I’d like to ask you now about your sleeping during the past week.
What were your usual hours of going to sleep and waking up, before this began?
Have you had any trouble falling asleep at the beginning of the night? If yes:
After you go to bed, how long has it been taking to fall asleep?
Have you changed the time at which you try to get to sleep since you’ve been depressed?
How many nights this week have you had trouble falling asleep?

<table>
<thead>
<tr>
<th>Insomnia Early</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - no difficulty falling asleep</td>
</tr>
<tr>
<td>1 - complains of occasional difficulty falling asleep, i.e. more than half hour</td>
</tr>
<tr>
<td>2 - complains of nightly difficulty of falling asleep</td>
</tr>
</tbody>
</table>

During the past week have you been waking up in the middle of the night?
If yes:
Do you get out of bed? What do you do? (Only go to the bathroom?)
When you get back in bed, are you able to fall right back asleep?
How many nights this week have you had this kind of trouble?
Have you felt your sleeping has been restless or disturbed some nights?

<table>
<thead>
<tr>
<th>Insomnia Middle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - no difficulty</td>
</tr>
<tr>
<td>1 - complains of being restless and disturbed during the night</td>
</tr>
<tr>
<td>2 - waking during the night—any getting out of bed, except to void</td>
</tr>
</tbody>
</table>
### Hamilton Depression Rating Scale (HAM-D) Cont’d

<table>
<thead>
<tr>
<th><strong>What time have you been waking up in the morning for the last time, this past week?</strong></th>
<th><strong>Insomnia Late</strong></th>
</tr>
</thead>
</table>
| **If early:** | 0 - no difficulty  
1 - waking in early hours of morning but goes back to sleep  
2 - unable to fall asleep again if gets out of bed |
| Is that with an alarm clock or do you just wake up yourself? |  |
| What time do you usually wake up (when not depressed)? |  |
| How many mornings this week have you awakened early? |  |

<table>
<thead>
<tr>
<th><strong>If outpatient: Have you been working this week— in or out of the home?</strong></th>
<th><strong>Work and Activities</strong></th>
</tr>
</thead>
</table>
| **If not working:** | 0 - no difficulty  
1 - thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies  
2 - loss of interest in activity, hobbies or work— by direct report of the patient or indirect in listlessness, indecision and vacillation (needs to push to work)  
3 - decrease in actual time spent in activities or decrease in productivity  
4 - stopped working because of present illness |
| Why not? |  |
| **If working:** |  |
| Have you been able to get as much done as you usually do *(when you feel ok?)* |  |
| How have you been spending your time this past week when not at work? *(focus on primary activities such as family and frequent leisure activities)* |  |
| Have you felt interested in doing *(those things)*, or do you feel you have to push yourself to do them? |  |
| Have you stopped doing anything you used to? If yes: Why? |  |
| How much do you enjoy doing *(these things)* compared to usual? |  |
| Is there anything you look forward to? |  |

<table>
<thead>
<tr>
<th><strong>Rating Based on Observation During Interview</strong></th>
<th><strong>Retardation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>If telephone interview:</strong> <em>Do you feel that your speech or physical movements are sluggish?</em></td>
<td><em>slowness of thought and speech; impaired ability to concentrate; decreased motor activity</em></td>
</tr>
</tbody>
</table>
| Has anyone actually commented on this? | 0 - normal speech and thought  
1 - slight retardation at interview  
2 - obvious retardation at interview  
3 - interview difficult  
4 - complete stupor |
### Hamilton Depression Rating Scale (HAM-D) Cont'd

<table>
<thead>
<tr>
<th><strong>Rating Based on Observation During Interview</strong></th>
<th><strong>Agitation</strong></th>
</tr>
</thead>
</table>
| If telephone interview: As we talk, are you fidgeting at all, or having trouble sitting still? What are you doing? Do others notice that you are restless? | 0 - none  
1 - fidgetiness  
2 - playing with hands, hair, etc.  
3 - moving about, can’t sit still  
4 - hand-wringer, nail biting, hair pulling |

<table>
<thead>
<tr>
<th><strong>Have you been feeling especially tense or irritable this past week?</strong></th>
<th><strong>Anxiety Psychic</strong></th>
</tr>
</thead>
</table>
| If yes:  
Is this more than usual for you?  
Have you been worrying a lot about little things, things you don’t ordinarily worry about? | 0 - no difficulty  
1 - subjective tension and irritability  
2 - worrying about minor matters  
3 - apprehensive attitude apparent in face or speech  
4 - fears expressed without questioning |
| If yes:  
Like what, for example?  
How often have you felt this way the past week?  
Has this caused you any problems or difficulties? If yes: What? | |

<table>
<thead>
<tr>
<th><strong>In this past week, have you had any physical symptoms that sometimes go along with being nervous, like (read list, pause for reply)</strong></th>
<th><strong>Anxiety Somatic</strong></th>
</tr>
</thead>
</table>
| If yes:  
How much have these things been bothering you this past week?  
How bad have they gotten? How much of the time have you had them?  
Did these symptoms interfere with usual activities? If yes: Which activities? | Physiologic concomitants of anxiety, such as:  
*GI*—dry mouth, gas, indigestion, diarrhea, cramps, belching  
*CV*—heart palpitations, headaches  
*RESP*—hyperventilating, sighing, urinate frequently, sweating |
| NOTE: Don’t rate if clearly due to medication | 0 - absent  
1 - mild  
2 - moderate  
3 - severe  
4 - incapacitating |

104
**HAMilton Depression Rating Scale (HAM-D) Cont’d**

<table>
<thead>
<tr>
<th>How has your appetite been this past week?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(Compared to your usual appetite)</em></td>
</tr>
<tr>
<td>Have you had to force yourself to eat?</td>
</tr>
<tr>
<td>Have other people had to urge you to eat?</td>
</tr>
<tr>
<td>Have you had any stomach or intestinal problems? <em>If yes:</em> Have you needed to take anything for that?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Somatic Symptoms Gastrointestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - none</td>
</tr>
<tr>
<td>1 - loss of appetite but eating without encouragement</td>
</tr>
<tr>
<td>2 - difficulty eating without urging; request or requires laxatives or medication for G.I. symptoms</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How has your energy been this past week?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>If low energy:</em></td>
</tr>
<tr>
<td>Have you felt tired? <em>If yes:</em> How much of the time? How bad has it been?</td>
</tr>
<tr>
<td>This week, have you had any aches or pains?</td>
</tr>
<tr>
<td>What about backaches, headaches, or muscle aches?</td>
</tr>
<tr>
<td>Have you felt any heaviness in your limbs, back, or head?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Somatic Symptoms General</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - none</td>
</tr>
<tr>
<td>1 - heaviness in limbs, back or head. Backaches, headache, muscle aches. Loss of energy and fatigability</td>
</tr>
<tr>
<td>2 - any clear-cut symptom</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How has your interest in sex been this week?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(I’m not asking about actual sexual activity, but about your interest in sex—how much do you think about it?)</em></td>
</tr>
<tr>
<td>How much do you think about sex?</td>
</tr>
<tr>
<td>Has there been any change in your interest in sex <em>(from when you were not depressed)</em>?</td>
</tr>
<tr>
<td><em>If less:</em> How much less?</td>
</tr>
<tr>
<td>Is this something you’ve thought much about?</td>
</tr>
<tr>
<td><em>If no:</em> Is this unusual for you?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>General Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>loss of libido, menstrual disturbances</em></td>
</tr>
<tr>
<td>0 - absent</td>
</tr>
<tr>
<td>1 - mild</td>
</tr>
<tr>
<td>2 - severe</td>
</tr>
</tbody>
</table>
**Hamilton Depression Rating Scale (HAM-D) Cont’d**

<table>
<thead>
<tr>
<th>In the last week how much have your thoughts been focused on your physical health or how your body is working (compared to your normal thinking)?</th>
<th>Hypochondriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you been preoccupied with this?</td>
<td>0 - not present</td>
</tr>
<tr>
<td>Do you complain much about how you feel physically?</td>
<td>1 - self-absorption (bodily)</td>
</tr>
<tr>
<td>Have you seen a doctor about these problems? If yes:</td>
<td>2 - preoccupation with health</td>
</tr>
<tr>
<td>What did the doctor say?</td>
<td>3 - frequent complaints, requests for help</td>
</tr>
<tr>
<td>Have you found yourself asking for help with things you could really do for yourself?</td>
<td>4 - hypochondriacal delusions</td>
</tr>
<tr>
<td>If yes:</td>
<td></td>
</tr>
<tr>
<td>Like what, for example?</td>
<td></td>
</tr>
<tr>
<td>How often has that happened?</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Have you lost any weight in the past week?</th>
<th>Loss of Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>If yes:</td>
<td>A - Rating by history</td>
</tr>
<tr>
<td>Was it because of this depression? How much weight did you lose?</td>
<td>0 - no weight loss</td>
</tr>
<tr>
<td>If not sure:</td>
<td>1 - probable weight loss due to current depression</td>
</tr>
<tr>
<td>Do you think your clothes are any looser on you?</td>
<td>2 - definite (according to patient) weight loss due to depression</td>
</tr>
<tr>
<td>Did you lose any weight last week?</td>
<td>3 - not assessed</td>
</tr>
<tr>
<td>If yes:</td>
<td></td>
</tr>
<tr>
<td>Was it because you were depressed or down?</td>
<td></td>
</tr>
<tr>
<td>How much did you lose?</td>
<td></td>
</tr>
</tbody>
</table>

**Rating Based on Observation During Interview**

<table>
<thead>
<tr>
<th>Insight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - acknowledges being depressed and ill OR currently not depressed</td>
</tr>
<tr>
<td>1 - acknowledges illness but attributes cause to bad food, climate, overwork, need for rest, etc</td>
</tr>
<tr>
<td>2 - denies being ill</td>
</tr>
</tbody>
</table>

Total HAM-D Score

*Inform study physician if HAM-D score > 23*
Appendix E – BDI-II
**Beck Depression Inventory-II (BDI-II)**

**Instructions:** This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that describes the way you have been feeling during the **past two weeks, including today**. If several statements in the group seem to apply equally well, circle the highest number for that group.

<table>
<thead>
<tr>
<th>1. Sadness</th>
<th>7. Self-Dislike</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I do not feel sad.</td>
<td>0 I feel the same about myself as ever.</td>
</tr>
<tr>
<td>1 I feel sad much of the time.</td>
<td>1 I have lost confidence in myself.</td>
</tr>
<tr>
<td>2 I am sad all the time.</td>
<td>2 I am disappointed in myself.</td>
</tr>
<tr>
<td>3 I am so sad or unhappy that I can’t stand it.</td>
<td>3 I dislike myself.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Pessimism</th>
<th>8. Self-Criticalness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I am not discouraged about my future.</td>
<td>0 I don’t criticize or blame myself more than usual.</td>
</tr>
<tr>
<td>1 I feel more discouraged about my future than I used to.</td>
<td>1 I am more critical of myself than I used to be.</td>
</tr>
<tr>
<td>2 I do not expect things to work out for me.</td>
<td>2 I criticize myself for all my faults.</td>
</tr>
<tr>
<td>3 I feel my future is hopeless and will only get worse.</td>
<td>3 I blame myself for everything bad that happens.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Past Failure</th>
<th>9. Suicidal Thoughts or Wishes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I do not feel like a failure.</td>
<td>0 I don’t have any thoughts of killing myself.</td>
</tr>
<tr>
<td>1 I have failed more than I should have.</td>
<td>1 I have thoughts of killing myself, but I would not carry them out.</td>
</tr>
<tr>
<td>2 As I look back, I see a lot of failures.</td>
<td>2 I would like to kill myself.</td>
</tr>
<tr>
<td>3 I feel I am a total failure as person.</td>
<td>3 I would kill myself if I had the chance.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Loss of Pleasure</th>
<th>10. Crying</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I get as much pleasure as I ever did from the things I enjoy.</td>
<td>0 I don’t cry anymore than I used to.</td>
</tr>
<tr>
<td>1 I don’t enjoy things as much as I used to.</td>
<td>1 I cry more than I used to.</td>
</tr>
<tr>
<td>2 I get very little pleasure from the things I used to enjoy.</td>
<td>2 I cry over every little thing.</td>
</tr>
<tr>
<td>3 I can’t get any pleasure from the things I used to enjoy.</td>
<td>3 I feel like crying, but I can’t.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Guilty Feelings</th>
<th>11. Agitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I don’t feel particularly guilty.</td>
<td>0 I am no more restless or wound up than usual.</td>
</tr>
<tr>
<td>1 I feel guilty over many things I have done or should have done.</td>
<td>1 I feel more restless or wound up than usual.</td>
</tr>
<tr>
<td>2 I feel quite guilty most of the time.</td>
<td>2 I am so restless or agitated that it’s hard to stay still.</td>
</tr>
<tr>
<td>3 I feel guilty all the time.</td>
<td>3 I am so restless or agitated that I have to keep moving or doing something.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. Punishment Feelings</th>
<th>12. Loss of Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I don’t feel I am being punished.</td>
<td>0 I have not lost interest in other people or activities.</td>
</tr>
<tr>
<td>1 I feel I may be punished.</td>
<td>1 I am less interested in other people or things than before.</td>
</tr>
<tr>
<td>2 I expect to be punished.</td>
<td>2 I have lost most of my interest in other people or things.</td>
</tr>
<tr>
<td>3 I feel I am being punished.</td>
<td>3 It’s hard to get interested in anything.</td>
</tr>
<tr>
<td><strong>13. Indecisiveness</strong></td>
<td><strong>18. Changes in Appetite</strong></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>0 I make decisions about as well as ever.</td>
<td>0 I have not experienced any change in my appetite.</td>
</tr>
<tr>
<td>1 I find it more difficult to make decisions than usual.</td>
<td>1a My appetite is somewhat less than usual.</td>
</tr>
<tr>
<td>2 I have much greater difficulty in making decisions than I used to.</td>
<td>1b My appetite is somewhat more than usual.</td>
</tr>
<tr>
<td>3 I have trouble making any decisions.</td>
<td>2a My appetite is much less than before.</td>
</tr>
<tr>
<td></td>
<td>2b My appetite is much more than before.</td>
</tr>
<tr>
<td></td>
<td>3a I have no appetite at all.</td>
</tr>
<tr>
<td></td>
<td>3b I crave food all the time.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>14. Worthlessness</strong></th>
<th><strong>19. Concentration Difficulty</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I do not feel I am worthless.</td>
<td>0 I can concentrate as well as ever.</td>
</tr>
<tr>
<td>1 I don't consider myself as worthwhile and useful as I used to.</td>
<td>1 I can't concentrate as well as usual.</td>
</tr>
<tr>
<td>2 I feel more worthless as compared to other people.</td>
<td>2 It's hard to keep my mind on anything for very long.</td>
</tr>
<tr>
<td>3 I feel utterly worthless.</td>
<td>3 I find I can't concentrate on anything.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>15. Loss of Energy</strong></th>
<th><strong>20. Tiredness or Fatigue</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I have as much energy as ever.</td>
<td>0 I am no more tired or fatigued than usual.</td>
</tr>
<tr>
<td>1 I have less energy than I used to have.</td>
<td>1 I am more tired or fatigued more easily than usual.</td>
</tr>
<tr>
<td>2 I don't have enough energy to do very much.</td>
<td>2 I am too tired or fatigued to do a lot of the things I used to.</td>
</tr>
<tr>
<td>3 I don't have enough energy to do anything.</td>
<td>3 I am too tired or fatigued to do most of the things I used to do.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I have not experienced any change in my sleeping pattern.</td>
<td>0 I have not noticed any recent change in my interest in sex.</td>
</tr>
<tr>
<td>1a I sleep somewhat more than usual.</td>
<td>1 I am less interested in sex than I used to be.</td>
</tr>
<tr>
<td>1b I sleep somewhat less than usual.</td>
<td>2 I am much less interested in sex now.</td>
</tr>
<tr>
<td>2a I sleep a lot more than usual.</td>
<td>3 I have lost interest in sex completely.</td>
</tr>
<tr>
<td>2b I sleep a lot less than usual.</td>
<td></td>
</tr>
<tr>
<td>3a I sleep most of the day.</td>
<td></td>
</tr>
<tr>
<td>3b I wake up 1-2 hours early and can't get back to sleep.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>17. Irritability</strong></th>
<th><strong>TOTAL SCORE:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 I am no more irritable than usual.</td>
<td></td>
</tr>
<tr>
<td>1 I am more irritable than usual.</td>
<td></td>
</tr>
<tr>
<td>2 I am much more irritable than usual.</td>
<td></td>
</tr>
<tr>
<td>3 I am irritable all the time.</td>
<td></td>
</tr>
</tbody>
</table>
Appendix F – Bligh and Dyer Lipid Extraction Protocol
MODIFIED BLIGH AND DYER LIPID EXTRACTION

(Courtesy of Dr. Steffany AL Bennett/CIHR Training Program in Neurodegenerative Lipidomics)

Glassware

- Kimble 10 mL glass threaded tip conical tubes with black teflon-lined caps (Fisher, cat# 73785-10)
- Disposable 5 mL glass test tubes (e.g. VWR 47729-570)
- Amber 2 mL glass vials with teflon-lined caps (BioLynx Chromatographic Specialties C779100AW)
- Glass pipettes dedicated to lipid work (no contact with detergent) – note: if do not come in contact with lipids, let CHCl₃ and methanol evaporate before next use
- Any re-used glassware that been exposed to lipids (NB – if extensive residue, discard):
  o Wash in 10% HCl-ddH₂O
  o Rinse in ddH₂O (EXTENSIVELY), then rinse in MeOH or CHCl₃, let dry in fumehood

Per sample

- 1X (12X75mm) disposable glass test tube for sample homogenization
- 1X 10 mL Kimble tube for extraction & 1X 10 mL Kimble tube for collection
- 6X 2 mL amber vial tube for final lipid storage
- 1X 0.5 mL or 1.5 mL Eppendorf tube for protein sample

Solutions to prepare

1) *MADE FRESH* acidified methanol (AcMeOH): 2% acetic acid in methanol amt depending on (#) samples
2) *FILTERED* 0.1M Na acetate (MW 82.03) – 4.102 g in 500 mL ddH₂O
3) C13:0 lyso-PC 10 uM

Procedure (keep all tubes on ice):

Set-up and homogenization

- Set centrifuge to 4 °C
- Add 3.2 mL 0.1M Na acetate to all extraction tubes
- Put sample in the disposable glass tube
- Add 1 mL AcMeOH (rinse any extra sample down with the AcMeOH)
- Homogenize with tissue grinder for around 30 seconds (until well-homogenized)
  o Retain 25 μL of homogenate for protein quantification (Eppendorf)
- Add 41.3 uL of 10 uM C13:0 LPC (187.5 ng in 300 uL EtOH final volume) to the homogenate
-Transfer the sample with Pasteur pipette to collection tube
- Rinse with 1 mL AcMeOH and transfer to collection tube (repeat this 2 more times)
  o Total volume of AcMeOH is 4 mL

Extraction

- Add 3.8 mL CHCl₃ to extraction tube
- Swirl or invert 3X in the fume hood and 1X before placing in centrifuge
- Centrifuge 2 min @ 2000 rpm (800 rcf), 4°C
- Retain bottom phase with Pasteur pipette and transfer to collection tube
- **Re-extract with 2 ml of CHCl₃ (3 times total)**
- Swirl or invert 3X in the fume hood and 1X in front of the centrifuge
- Centrifuge 2 min @ 2000 rpm (800 rcf), 4°C
- Retain bottom phase again (collect all bottom phases in same collection tube)

### Solvent evaporation, resuspension and storage

- Evaporate chloroform under constant stream of N₂ gas
- Resuspend in 300 uL absolute EtOH and flush with N₂ gas and flick tube vigorously
- Incubate at 30 °C for 10 min and flick tube until lipid dissolves
- Centrifuge 1 min @ 2000 rpm to collect ethanol at bottom and transfer with Pasteur pipette to amber glass tube
- Aliquot into 6 X 50 uL aliquots and store at -80 °C in amber glass vials (under N₂)

**NB – important to keep lipids out of light and oxygen as much as possible!**

---

### Reagent and glassware preparation

| Na acetate | label Kimble tubes, Eppendorfs (also weigh), and amber vials |
| Acidified MeOH (fresh) |  |
| C13LPC |  |

### Sample prep

cultured cells (treatments?) - washes (extract from wash?) (days/minutes)
brain or other tissues - dissections (~60 min), measure wet weights

### Procedure

set-up and homogenization (~30-60 min)
extration (~30-60 min)
solvent evaporation, resuspension and storage (~3-4 hrs)
Other useful comments:

- Because of the general sensitivity of naturally occurring lipids to peroxidation and hydrolytic degradation \( \Rightarrow \) important that solvents be free from contaminants, \( \therefore \) use good reagent grade solvents, glass (preferably amber) containers
- Chloroform dissolves plastics
- Lipids adhere to plastics so limit their use
- If sample really doesn't dissolve well in ethanol, try 50:50 chloroform:ethanol mix
- Lipids should not be stored for longer that one to two years before analysis
- Use fresh or flash frozen \& -80C-stored tissues/cells
- Useful reference website - www.lipidmaps.org
Appendix G – Personal Communication (Dr. S.A.L Bennett)
Oct 25, 2012

Graham Mazareeuw
Neuropsychopharmacology Research Group
Sunnybrook Health Sciences Centre
2075 Bayview Ave, Suite EG-04
Toronto, Ont. M4N 3M5

Dear Graham,

This letter is to confirm that the following findings attributed to my research are correct:

- Our unpublished animal studies indicate that while PC (O-16:0/2:0) is neurotoxic when elevated in the CNS, this is not reflected by plasma concentrations.
- Plasma concentrations of PC(O-18:0/2:0) appear to be representative of neurodegeneration in animals.

Sincerely

Steffany A.L. Bennett, Ph.D.
Neural Regeneration Laboratory
Ottawa Institute of Systems Biology
Professor, University Research Chair in Neurolipidomics
Director, CIHR Training Program in Neurodegenerative Lipidomics
Email: sbennett@uottawa.ca
Tel: 613 562-5800 x8372, Fax: 613 562-5452