Omega-3 Fatty Acids and Depressive Symptoms in Coronary Artery Disease

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

Doctor of Philosophy

Graduate Department of Pharmacology and Toxicology

University of Toronto

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Abstract

Introduction: Depressive symptoms are highly incident among coronary artery disease (CAD) patients and are associated with reduced benefit from cardiac rehabilitation (CR) and increased mortality despite current treatments. Omega-3 fatty acid (ω-3 FA) deficits, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been independently linked with depression and with CAD. This research investigated the relevance of ω-3 FAs to the presence and treatment of depressive symptoms in CAD as well as predictors of ω-3 FA treatment efficacy.

Study 1: The relationship between the ratio of EPA+DHA to arachidonic acid (AA), a dietary fatty acid with opposing metabolism, in different erythrocyte phospholipid fractions and depressive symptoms in CAD was assessed. In a cross-sectional analysis of 76 CAD patients (age=61.9±8.5, 74% male, 43% depressed), lower EPA+DHA/AA ratios in erythrocyte phosphatidylinositol (B=-12.71, p<.01) and sphingomyelin (B=-2.52, p<.01) fractions were associated with greater depressive symptom severity. Study 2: The efficacy of ω-3 FA supplements for treating depressive symptoms in CAD was assessed. In a 12-week randomized placebo-controlled trial of 1.9 g/day, EPA-enriched ω-3 FA supplements in 86 CAD patients (age=61.8±8.9, 73% male, 43% depressed, n=41 ω-3 FA, n=45 placebo) participating in CR, ω-3 FA treatment increased plasma EPA and DHA concentrations, but did not reduce depressive
symptoms compared to placebo ($F_{3,35}=0.72$, $p=.40$). In all patients, greater plasma EPA+DHA concentrations at entry predicted greater reduction in depressive symptoms over 12 weeks of CR ($F_{1,35}=5.43$, $p=.02$). **Study 3:** Lipid peroxidation may influence ω-3 FA metabolism and may therefore affect antidepressant efficacy; however this interaction has yet to be investigated. In 62 randomized CAD patients (age=61.4±8.3, 74% male, 39% depressed, n=26 ω-3 FA, n=36 placebo), greater pre-treatment serum lipid hydroperoxide concentrations independently predicted worsening of depressive symptoms ($F_{1,25}=7.55$, $p=.01$), particularly depressive mood ($F_{1,25}=9.20$, $p<.01$), over 12 weeks in the ω-3 FA group, but not in the placebo group.

**Conclusions:** ω-3 FAs may be central to the pathophysiology and severity of depressive symptoms in CAD patients. However, the ability of ω-3 FA supplementation to confer antidepressant benefits in those with ω-3 FA deficits may be dependent on pre-treatment markers of lipid peroxidation.
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<th>Full Form</th>
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<tbody>
<tr>
<td>ω-3 FA</td>
<td>Omega-3 fatty acid</td>
</tr>
<tr>
<td>ω-6 FA</td>
<td>Omega-6 fatty acid</td>
</tr>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-linoleic acid</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>BDNF</td>
<td>Brain-derived neurotrophic factor</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>CAROTID</td>
<td>CAD Randomized Omega-3 Trial in Depression</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CR</td>
<td>Cardiac rehabilitation</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>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ENRICHED</td>
<td>ENhancing Recovery in Coronary Heart Disease</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HEPE</td>
<td>Hydroxyeicosapentaenoic acid</td>
</tr>
</tbody>
</table>
HETE  Hydroxyeicosatetraenoic acid
HHE  Hydroxyhexenal
HNE  Hydroxynonenal
HPA  Hypothalamic-pituitary-adrenal (axis)
HR  Heart rate
IDO  Indoleamine 2,3-dioxygenase
IHD  Ischemic heart disease
IL  Interleukin
ITT  Intention-to-treat
LOX  Lipoxygenase
LPH  Lipid hydroperoxides
Lyso  Lysophosphocholine
MI  Myocardial infarction
MIND-IT  Myocardial Infarction and Depression – Intervention Trial
MMSE  Mini-Mental State Examination
PC  Phosphatidylcholine
PE  Phosphatidylethanolamine
PI  Phosphatidylinositol
PS  Phosphatidylserine
PTCA  Percutaneous transluminal coronary angioplasty
RCT  Randomized controlled trial
ROS  Reactive oxygen species
SADHART  Sertraline AntiDepressant Heart Attack Randomized Trial
SBP  Systolic blood pressure
SM  Sphingomyelin
SSRI  Selective serotonin reuptake inhibitors
STAR*D  Sequenced Treatment Alternatives to Relieve Depression
Statin 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor
THP Trillium Health Partners
TNF Tumor necrosis factor
UHN Toronto Rehab Toronto Rehabilitation Institute at University Health Network
VO$_2$ peak Volume of oxygen peak
VO$_2$ peak fraction Fraction of age and gender expected VO$_2$ peak
Chapter I

Introduction

1.1 Statement of Problem

Coronary artery disease (CAD) is the leading cause of mortality in the developed world (The World Health Organization, 2011). Cardiovascular medications and lifestyle interventions are important secondary prevention strategies to reduce the risk of morbidity and mortality among CAD patients (Smith et al., 2011). One particularly important lifestyle intervention is cardiac rehabilitation (CR), a program of standardized exercise and dietary behaviours to improve fitness and health, which has been shown to reduce morbidity and the risk of mortality among attending CAD patients (Taylor, 2014).

Depressive symptoms are highly incident among CAD patients. Approximately 20% of CAD patients will experience a major depressive episode in the first year following an acute coronary syndrome (ACS), which is at least four-fold greater than the annual incidence in the general adult population (Patten et al., 2006). An additional 30-45% of CAD patients will experience minor depression (Celano and Huffman, 2011, Sowden and Huffman, 2009), a term representing the presence of sub-threshold depressive symptoms which can impact medical outcomes independently and are a risk factor for future major depressive episodes (Patten et al., 2012). Unresolved, depressive symptoms reduce adherence to medications (Blumenthal et al., 1982), can lead to accelerated cognitive decline (Freiheit et al., 2012), and increase the risk of dropout from CR (Swardfager et al., 2011). Ultimately, depression doubles the risk of mortality in CAD patients (Penninx et al., 2001).

Despite the importance of ameliorating depressive symptoms, current antidepressant interventions achieve only a modest benefit (26-64% remission under clinical trial conditions) in
those with CAD (Dowlati et al., 2010b) and many pharmacological options are limited by cardiovascular side effects. Psychotherapeutic trials demonstrate similarly modest efficacy (Mendes de Leon et al., 2006, Lesperance et al., 2007). As such, the development of more efficacious and effective CAD-relevant therapies for depressive symptoms remains an unmet medical need.

The possible role of omega-3 fatty acids (ω-3 FAs) in the pathophysiology and treatment of depressive symptoms in CAD patients is supported in current literature. Reductions in plasma and erythrocyte concentrations of ω-3 FAs have been observed in CAD patients (Harris et al., 2006) and in depressed patients (Lin et al., 2010), independently. Plasma and erythrocyte ω-3 FA concentrations appear to be further reduced in depressed CAD patients compared to non-depressed CAD patients (Amin et al., 2008, Chang et al., 2015, Frasure-Smith et al., 2004, Schins et al., 2007, Parker et al., 2006). Moreover, depressive symptoms in CAD patients have been linked with lower ratios of ω-3 to omega-6 (ω-6) fatty acids, a common dietary fatty acid with several functions opposing ω-3 FAs (Chang et al., 2015, Frasure-Smith et al., 2004, Vollmer-Conna et al., 2015). Accordingly, meta-analytic data indicate that ω-3 FA supplements may be efficacious for treating depressive symptoms in those without CAD, particularly when formulated with a high ratio of eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA) (Sublette et al., 2011, Martins et al., 2012, Grosso et al., 2014). Despite this evidence, there are several gaps in knowledge which hamper our understanding of the relevance of ω-3 FAs to depressive symptoms in CAD and the clinical antidepressant potential of ω-3 FA supplements.

1.1.1 Gaps in Knowledge

**Gap 1:** Despite several studies reporting reductions in plasma and erythrocyte ω-3 FA concentrations in depressed CAD patients compared to non-depressed CAD patients, none of those studies investigated the phospholipid fractions most relevant to ω-3 FA deficits. Different
phospholipid classes, such as the phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS), as well as sphingomyelin (of the sphingolipid class), and lysophospholipids, have diverse functional roles in membrane regulation and cellular signalling (van Meer et al., 2008). Among many processes, those diverse roles may have implications for the balance of pro- and anti-inflammatory signalling (Kita et al., 2006, Ma, 2007, Morrison et al., 2012), which is a proposed contributor to depressive symptoms in those with CAD (Dowlati et al., 2010a, Maes, 2008). As such, the ratio of ω-3 FA to ω-6 FA concentrations, particularly the ratio of EPA+DHA to arachidonic acid (AA), a potent ω-6 FA, in different phospholipid fractions may be relevant to different signalling pathways implicated in the mechanisms of depressive symptoms in CAD.

Additionally, it remains unclear which depressive symptoms are most closely associated with reduced EPA+DHA to AA ratios in erythrocyte phospholipids. Not only do symptoms of CAD, such as sleep disturbances, appetite changes, and inability to concentrate, overlap with depressive symptoms (Kohlmann et al., 2013, Sharma et al., 2014, Linke et al., 2009, Delisle et al., 2012), but different depressive symptoms have been shown to differentially predict cardiovascular outcomes (Stewart et al., 2007, Stewart et al., 2012) and antidepressant response (Trivedi et al., 2005). It is therefore important to identify which depressive symptoms are associated with reduced erythrocyte EPA+DHA to AA ratios in order to determine whether erythrocyte ω-3 FA fractions are a promising antidepressant target in CAD.

**Gap 2:** While ω-3 FA treatment has shown potential as an antidepressant intervention in the general adult population, treatment efficacy in CAD patients is unclear. Previous studies assessing ω-3 FA treatment efficacy in CAD patients were limited either by using a formulation that was low in EPA dose (thought to be the main antidepressant effector) or by using ω-3 FAs as an adjunctive treatment to other antidepressant medications (Grosso et al., 2014), which may...
limit observable treatment benefits. Furthermore, treatment efficacy in CAD patients with minor depressive symptoms remains uncertain, and the particular depressive symptoms which may be reduced have not been reported. Finally, ω-3 FA treatment efficacy in combination with an exercise intervention such as CR has not been investigated. CR may have antidepressant efficacy independently (Rutledge et al., 2013, Yohannes et al., 2010); however, not all CAD patients benefit and some may experience persistent depressive symptoms during CR. ω-3 FA treatment efficacy in combination with CR may support its use as an additional antidepressant intervention for CAD. Addressing these gaps in knowledge may clarify the potential antidepressant efficacy of EPA-enriched ω-3 FA treatment in CAD patients participating in CR.

**Gap 3:** Treatment efficacy of ω-3 FAs for depressive symptoms in non-CAD populations is variable (Grosso et al., 2014). Therefore, this study seeks to identify pre-treatment biomarkers predicting ω-3 FA antidepressant efficacy in CAD. Specifically, the pathways mediating oxidative stress to lipids will be investigated as these pathways are elevated in CAD (Maes et al., 2011) and may alter the metabolism of ω-3 FAs away from potentially beneficial anti-inflammatory and neurogenic pathways (Assies et al., 2014). Accordingly, a state of high lipid peroxidation, as measured by serum concentrations of lipid hydroperoxides (LPH) and hydroxynonenal (HNE) prior to ω-3 FA treatment may reduce antidepressant efficacy in CAD patients. The potential influence of pre-treatment lipid peroxidation on treatment efficacy for depressive symptoms in CAD patients has yet to be investigated. Addressing this gap in knowledge may identify a pre-treatment predictor of which CAD patients may benefit most, or least, from ω-3 FA treatment.
1.2 Study Objectives and Hypotheses

This work sought to investigate the relevance of the EPA+DHA to AA ratio in different erythrocyte phospholipid fractions to the presence of depressive symptoms in CAD, the efficacy of ω-3 FA (EPA+DHA) treatment for reducing depressive symptoms in CAD during CR, and the association between pre-treatment lipid peroxidation and subsequent ω-3 FA treatment efficacy.

**Study 1**: The objective of Study 1 was to investigate the cross-sectional relationship between depressive symptoms and EPA+DHA to AA ratios in different erythrocyte phospholipid fractions in CAD patients [cross-sectional].

*Primary Hypothesis*: PCs are the most abundant type of phospholipid in membranes and contain the greatest percentage of EPA, DHA, and AA (Raphael and Sordillo, 2013). It was therefore hypothesized that lower EPA+DHA to AA ratios in the erythrocyte PC fraction would be associated with greater depressive symptom severity as measured using the investigator-rated 17-item Hamilton Depression Rating Scale (HAM-D).

*Secondary Hypothesis*: Other membrane phospholipid classes, such as PE, PI, PS, SM, and lysophospholipids accept EPA, DHA, and AA and have diverse distributions and functions in peripheral and neural membranes. It was therefore hypothesized that lower EPA+DHA to AA ratios in those erythrocyte phospholipid fractions would be associated with greater depressive symptom severity as measured using the HAM-D.

*Exploratory Hypothesis*: EPA+DHA to AA ratios in erythrocyte phospholipid fractions would demonstrate associations with symptoms particular to depressive mood as measured using the HAM-D.
**Study 2:** The objective of Study 2 was to investigate the efficacy of ω-3 FA treatment for depressive symptoms in CAD patients over 12 weeks of CR [efficacy].

*Primary Hypothesis:* Depressive symptoms measured using the HAM-D would be lower over 12 weeks in ω-3 FA treated patients than in placebo treated patients.

*Secondary Hypothesis:* Depressive symptoms measured using the self-report Beck Depression Inventory (BDI)-II would be lower over 12 weeks in ω-3 FA treated patients than in placebo treated patients.

*Exploratory Hypothesis:* ω-3 FA treatment would demonstrate efficacy for reducing symptoms particular to depressive mood (measured using both the HAM-D and the BDI) over 12 weeks.

**Study 3:** The objective of Study 3 was to investigate whether pre-treatment markers of lipid peroxidation were associated with the antidepressant efficacy of ω-3 FA treatment over 12 weeks in CAD patients [predictor of efficacy].

*Primary Hypothesis:* Higher baseline serum LPH concentrations, a marker of early-stage lipid peroxidation, would be associated with reduced antidepressant efficacy of ω-3 FA treatment over 12 weeks.

*Secondary Hypothesis:* Higher baseline serum HNE concentrations, a marker of late-stage lipid peroxidation, would be associated with reduced antidepressant efficacy of ω-3 FA treatment over 12 weeks.

*Exploratory Hypothesis:* Baseline serum LPH and HNE concentrations would be associated with ω-3 FA treatment efficacy for symptoms particular to depressive mood over 12 weeks.
1.3 Review of the Literature

1.3.1 Clinical Characteristics and Management

1.3.1.1 Coronary Artery Disease

CAD is the leading cause of mortality in developed countries (The World Health Organization, 2011). In Canada, CAD is responsible for 30% of all deaths each year. Over 1.3 million Canadians suffer from CAD and as many as 9 in 10 have at least one risk factor (Public Health Agency of Canada, 2009), highlighting not only the current prevalence, but also the risk of CAD in our population. In addition to mortality, CAD contributes significantly to morbidity and disability among Canadians, and is associated with a high annual cost to the Canadian economy (~ $22.2 billion) (Public Health Agency of Canada, 2009).

CAD is characterized by a stenosis, or narrowing, of the arteries that supply blood to the heart, leading to reduced blood flow, and therefore oxygen transfer, to the cardiac muscle. Stenosis of the coronary arteries is caused by thickening of the artery walls due to the formation of atherosclerotic plaques. Cardiovascular risk factors such as hypertension, smoking, dyslipidemia, obesity, and type II (non-insulin dependent) diabetes mellitus are known contributors to atherosclerotic plaque development and are each highly prevalent among CAD patients (Criqui, 1986).

Stenosis can become great enough to necessitate a revascularization procedure such as percutaneous transluminal coronary angioplasty (PTCA), in which a stent is placed into the narrowed artery and opens the vessel to restore adequate blood flow, or coronary artery bypass graft (CABG), in which a healthy vessel is placed around the narrowed artery to provide an alternate route for blood flow to the myocardial tissue. Both PTCA and CABG procedures are intended to reduce the risk of myocardial infarction (MI). The clinical population of CAD
patients consists of those with a history of MI and/or previous revascularization procedure and/or angiographic evidence of at least 50% stenosis in one or more coronary arteries.

**Pharmacotherapy for CAD**

While revascularization procedures can alleviate the deficits in blood flow to the myocardium due to artery stenosis, pharmacotherapies are the first line strategy for management of cardiovascular risk factors that contribute to CAD severity and the risk of MI (Smith et al., 2011). Medications most commonly prescribed are low-dose (81mg) acetylsalicylic acid (ASA) for its anti-inflammatory and anti-platelet properties, the angiotensin converting enzyme (ACE) inhibitors, β-adrenergic receptor antagonists, calcium channel antagonists, and diuretics for controlling hypertension, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (“statins”) for controlling aberrant cholesterol and triglyceride concentrations associated with dyslipidemia, metformin, glyburide, peroxisome proliferator-activated receptor gamma agonists, and/or insulin for glycemic control in diabetes II, and nitroglycerin for angina pectoris. Platelet inhibitors such as clopidogrel or ticagrelor for are also typically prescribed for one year after PTCA to reduce blood clotting around the stent.

**ω-3 FA Supplements for CAD**

ω-3 FAs are essential polyunsaturated fatty acids, obtained only through diet. The classification of ω-3 is based on the presence of a double bond at the 3rd carbon from the end of the fatty acid carbon chain [Figure I]. Epidemiological evidence has shown that greater dietary ω-3 FA intake is associated with a reduced incidence of CAD (Harris et al., 2008). Accordingly, a meta-analysis of 11 case-control and 2 cohort studies showed that ω-3 FA concentrations were substantially reduced in both plasma and erythrocytes of CAD patients compared to medically healthy controls (Harris et al., 2006). Consistent with their role as part of a heart-healthy diet, ω-
ω-3 FA supplements have been approved by both Health Canada and the United States Food and Drug Administration for reducing plasma triglyceride concentrations. ω-3 FA supplements have also been recommended for reducing the risk of cardiovascular events and mortality in those with CAD (Marik and Varon, 2009, Di Minno et al., 2010), although meta-analytic evidence of efficacy for reducing those outcomes is mixed (Chowdhury et al., 2014, Rizos et al., 2012). In light of their ability to remediate ω-3 FA deficits and their potential cardiovascular benefits, ω-3 FA supplements are commonly used over-the-counter by CAD patients.

**Cardiac Rehabilitation**

CR is a recommended secondary prevention intervention for CAD (Smith et al., 2011). CR has consistently conferred a reduction in the incidence of premature mortality in patients with established CAD and those at risk for CAD due to vascular risk factors (Oldridge, 2012, Lawler et al., 2011).

CR is a medically supervised exercise and educational program designed to mitigate risk factors for CAD through the adoption of healthy lifestyle behaviours. Participating CAD patients are coached by trained health care professionals on strategies for establishing healthy exercise and dietary habits, attend weekly supervised exercise sessions, and complete additional unsupervised daily exercise (Hamm and Kavanagh, 2000).

In addition to the benefits of lifestyle coaching, the benefits of CR to mortality and morbidity are strongly correlated with improvements in cardiopulmonary fitness (Kavanagh et al., 2002, Kavanagh et al., 2003, Grazzi et al., 2014). Specifically, it has been shown that each 1% increase in the peak volume of oxygen [\( \text{VO}_2 \text{ peak} \)] utilized by a patient during a cardiac stress test (as measure of cardiopulmonary fitness) is associated with a 2% decrease in the risk of mortality among CAD patients (Vanhees et al., 1995).
1.3.1.2 Depressive Symptoms in CAD

Depressive symptoms, a term encompassing both major and minor depression, are a clinically important, common, and persistent problem associated with CAD. The life-time incidence of a major depressive episode in Canadian adults over 18 years of age is 7.9% to 8.6%, with between 4% and 5% of the population experiencing major depression over 12 months (Bland et al., 1988, Patten et al., 2006). In contrast, the incidence of depressive symptoms in CAD patients is at least four-fold greater. Approximately 20% of CAD patients experience a major depressive episode within one year following an ACS, while another 30-45% will not meet criteria for major depression, but will suffer from minor depression (Burg and Abrams, 2001, Carney et al., 1987, Frasure-Smith et al., 1993, Frasure-Smith et al., 1995a, Ladwig et al., 1991, Lesperance and Frasure-Smith, 2003, Lesperance et al., 1996, Schleifer et al., 1989, Strik et al., 2004, van Melle et al., 2004). Minor depression shares diagnostic criteria with major depression but satisfies fewer criterion symptoms (American Psychiatric Association, 2013), although it is a risk factor for major depressive episodes (Patten et al., 2012). While much of the previous literature has focused exclusively on major depression, the presence of minor depressive symptoms in CAD is clinically relevant and is studied in this Thesis.

Depressive symptoms, both major and minor, often persist for at least one year following hospitalization for an ACS (Frasure-Smith et al., 1999, Lauzon et al., 2003) and their persistence is associated with poorer psychosocial rehabilitation (Mayou et al., 1978, Stern et al., 1977), adherence to cardiac medications (Blumenthal et al., 1982), and overall quality of life (Ruo et al., 2003). As such, depressive symptoms can impede secondary prevention strategies for CAD patients. In the context of CR, depressive symptoms are associated with poorer cardiopulmonary fitness at program entry (Lavoie et al., 2004, Swardfager et al., 2008). Specifically, it has been shown that each point increase in depressive symptom severity is associated with more than a 2
ml/kg/min reduction in VO$_2$ peak (Swardfager et al., 2008). Greater depressive symptom severity at entry to CR also limits the cardiopulmonary benefit of CR in CAD patients. For example: in a recent study of 195 CAD patients in CR, major depression at program entry was not only associated with a greater likelihood of non-adherence (hazard ratio of 2.4) and dropout (hazard ratio 2.5) from CR, but also with a significantly reduced (50% lower than non-depressed patients) cardiopulmonary fitness benefit from CR in those who completed the program (Swardfager et al., 2011). Accordingly, depressive symptoms are a major independent contributor to premature mortality in CAD patients (meta-analyzed odds ratio of 2.6) (Barth et al., 2004). Depressive symptoms significantly and substantially increase the risk of MI independently of traditional cardiac risk factors in those with stable CAD (Frasure-Smith et al., 1993, Frasure-Smith et al., 1995a, Ladwig et al., 1991, Lesperance and Frasure-Smith, 2000, Murray and Lopez, 1997, Stern et al., 1977, Wassertheil-Smoller et al., 1996) and patients who are post-CABG (Blumenthal et al., 2003) or post-MI (Ahern et al., 1990, Frasure-Smith et al., 1993, Frasure-Smith et al., 1995a, Frasure-Smith et al., 1995b, Ladwig et al., 1991) (odds ratios between 1.6 and 2.6). Depressive symptoms also increase the risk of stroke (odds ratio of 2.1) (Whooley et al., 2008) in CAD patients, independently of traditional cardiac risk factors. The negative impact of depressive symptoms on cardiovascular outcomes increases with symptom severity in a dose-dependent manner (Penninx et al., 2001, Lesperance et al., 2000).

Importantly, depressive symptoms significantly increase the risk of morbidity and mortality regardless of whether depressive symptom onset is pre- or post-ACS (Leung et al., 2012).

The relationships between depressive symptoms and poor medical and quality of life outcomes appear to be differentially related to the types of depressive symptoms involved (or symptom clusters). For example, depressive mood symptoms have been associated with incident coronary artery calcification (Stewart et al., 2012), whereas somatic depressive symptoms have been
associated with an increased risk of acute ischaemic events (Davidson et al., 2005, Deverts et al., 2010, Stewart et al., 2007, Stewart et al., 2009). Furthermore, depressive symptom clusters may demonstrate different changes in response to antidepressant pharmacotherapy and may predict non-response (Trivedi et al., 2005). As such, depressive symptom clusters may be important to identify and monitor in future studies of depression.

**Current Treatments for Depressive Symptoms in CAD**

Selective serotonin reuptake inhibitors (SSRIs) and noradrenergic and specific serotonergic antidepressants are recommended by consensus guidelines (Canadian Network for Mood and Anxiety Treatments) for management of depression in patients with cardiovascular disease (Ramasubbu et al., 2012). Tricyclic antidepressants are not recommended for use in CAD due to their potential cardiovascular side effects (Mavrides and Nemeroff, 2013). There is little clinical trial evidence describing the safety or efficacy of other antidepressant agents, such as the serotonin-norepinephrine reuptake inhibitors, norepinephrine-dopamine reuptake inhibitors, or monoamine oxidase inhibitors in CAD (Mavrides and Nemeroff, 2013), although antidepressant medications from those classes may be prescribed clinically to CAD patients at the discretion of a physician.

Despite those guidelines, a large proportion of CAD patients do not respond to the recommended antidepressant interventions. Two large-scale randomized controlled trials (RCTs), the CREATE (Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy) (Lesperance et al., 2007) and the SADHART (Sertraline AntiDepressant Heart Attack Randomized Trial) (Glassman et al., 2002) established the general safety of SSRIs in CAD patients with major depression but found limited efficacy. A third, smaller study reported similar findings with fluoxetine (Strik et al., 2000). Those studies reported remission in 26-64% of patients with only modest differences between groups of
patients receiving an SSRI vs. placebo at 12, 24, and 25 weeks, respectively. For example: in CREATE, 64.1% of patients remained symptomatic after 12 weeks of citalopram treatment and only 52.8% of patients met criteria for treatment response. Those trials included only those who met diagnostic criteria for major depression even though minor depression is especially prevalent among older patients suffering chronic medical conditions (Koenig et al., 1997, Koenig et al., 1991) such as CAD and it may exist on a continuum of depressive symptom severity with major depression (Penninx et al., 2001). Only one antidepressant study in CAD, the MIND-IT (Myocardial Infarction and Depression - Intervention Trial) (Honig et al., 2007), included patients with minor depression, finding that mirtazapine had a modest effect in patients with CAD, comparable to that of the SSRIs, though no subgroup analysis on the efficacy in those with minor depression was reported. However, meta-analytic findings from antidepressant interventions such as duloxetine have not demonstrated efficacy for reducing symptoms of minor depression in those without CAD (Schacht et al., 2014), further supporting the limitations of available antidepressant pharmacotherapies for those clinically meaningful depressive symptoms in CAD patients. Though it should be noted that symptom remission rates may improve clinically with the use of additional or alternative antidepressant interventions, the clinical effectiveness of currently available antidepressant interventions appears to be a limiting factor in providing optimal medical care for depressed patients. For example: the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) (Rush et al., 2006) trial showed that up to 50% of patients may experience symptom remission after only 2 levels of antidepressant intervention, though up to 33% of patients may not remit despite up to 4 levels of treatment, leaving a considerable number of patients with residual depressive symptoms.

Although the safety of SSRIs in CAD has been generally established, some SSRIs demonstrate adverse cardiometabolic effects. For example, some SSRIs, such as citalopram, are associated
with a dose-dependent prolongation of the QT interval (Cooke and Waring, 2012, Beach et al., 2014). Weight gain and increased circulating concentrations of cholesterols and triglycerides (Beyazyuz et al., 2013) are also associated with certain SSRIs and may exacerbate adverse cardiometabolic profiles already of substantial burden in CAD patients.

Recognized psychotherapeutic approaches are minimally effective in reducing depressive symptoms in CAD patients. In the ENRICHD (ENhancing Recovery in Coronary Heart Disease) study (Mendes de Leon et al., 2006), patients with major or minor depression randomized to cognitive behavioural therapy had a slightly greater reduction in depressive symptoms compared to those receiving usual care. The CREATE trial found no evidence to support the benefit of interpersonal psychotherapy. Similarly, no effect of cognitive behavioural stress management was observed in the Women’s Heart Study (Claesson et al., 2005). Thus, studies investigating non-drug interventions for depression in CAD have yet to demonstrate a clinical benefit.

Exercise interventions may be a potential strategy for reducing depressive symptoms. A 2013 review from the Cochrane Collaboration summarized 35 studies (n=1356 patients) and found a moderate benefit of exercise interventions compared to non-exercise control interventions, with similar effectiveness to pharmacotherapies (Cooney et al., 2013). While each of the included studies demonstrated a benefit of exercise on depressive symptoms, there was significant variability in the degree of benefit, which was unrelated to the intervention duration or method of assessing depression (clinical criteria or scale cut-point). Exercise interventions ranged from aerobic only, to resistance training only, and to combinations of aerobic and resistance training, with different levels of intensity. The variability in intervention type may have been a source of the variability in antidepressant effects; however, there is presently no consensus on the ideal exercise intervention for treating depressive symptoms. A recent meta-analysis of 13 studies
including CAD patients found that exercise in the form of CR can reduce depressive symptoms, irrespective of the duration of CR (Rutledge et al., 2013), and findings from another study indicate that these benefits may be long-lasting (Yohannes et al., 2010). However, as mentioned, depression at entry to CR predicts subsequent dropout from CR (Swardfager et al., 2011) and therefore a lack of potential antidepressant benefit from CR. Furthermore, not all CAD patients experience an antidepressant benefit from CR, despite program compliance (Milani and Lavie, 2007).

Collectively, antidepressant pharmacotherapies, psychotherapeutic approaches, and exercise interventions in the form of CR or otherwise appear to demonstrate high variability in treatment efficacy for reducing depressive symptoms in CAD patients. As approximately 40% of patients who achieve remission of depressive symptoms will experience a relapse within 1 year (Blumenthal, 2011, Hamer et al., 2011), identifying effective treatments remains a clinically important and presently unmet medical need for CAD patients.

1.3.1.3 ω-3 FA Supplements for Depressive Symptoms

The potential antidepressant efficacy of ω-3 FA supplements is currently an active area of research and efficacy in RCTs has been summarized by several meta-analytic reviews (Freeman et al., 2006, Lin and Su, 2007, Ross et al., 2007, Grosso et al., 2014, Martins et al., 2012, Sublette et al., 2011). Most recently, a meta-analysis of 11 RCTs including patients with clinically defined major depression and 8 RCTs including patients with minor depression found that ω-3 FA supplements were efficacious in reducing depressive symptoms in both major and minor depressed groups compared to placebo (Grosso et al., 2014). Meta-analytic data suggest that ω-3 FA efficacy is most pronounced in studies using an ω-3 FA formulation that is enriched with EPA compared to DHA (Sublette et al., 2011, Martins et al., 2012, Grosso et al., 2014). Specifically, it was found that formulations containing at least 60% EPA of the total EPA+DHA
concentrations, with an EPA dose range of 200mg/d to 2000 mg/d in excess of DHA were efficacious for reducing depressive symptoms, whereas formulations not meeting those criteria were generally not efficacious (Sublette et al., 2011). Despite that evidence, few studies have investigated the antidepressant efficacy of ω-3 FA supplements in CAD patients (Carney et al., 2009, Zimmer et al., 2013, Giltay et al., 2011, Haberka et al., 2013), and none of those studies used an EPA-enriched formulation. The antidepressant efficacy of ω-3 FA supplements adjunctive to CR also remains unstudied, despite the clinical relevance of depressive symptoms during CR (Swardfager et al., 2008, Swardfager et al., 2011). As ω-3 FA supplements may have cardiovascular benefits (DiNicolantonio et al., 2014), they may be an ideal investigational intervention for depressive symptoms in CAD and additional studies are needed to characterize efficacy in that population.

The investigation of ω-3 FAs for treating depressive symptoms is supported by clinical observation of ω-3 FA deficits in depressed patients. Reduced concentrations of phospholipids and cholesterol esters containing ω-3 FAs, and EPA and DHA in particular, are observed in plasma and serum, as well as in erythrocyte membranes of adults with major depression compared to healthy controls (Lin et al., 2010). Lower erythrocyte ω-3 FA fractions have been associated with SSRI treatment resistance in depressed patients (McNamara et al., 2014), indicating that ω-3 FA may be a possible predictor of response to currently recommended antidepressant interventions. The association between ω-3 FAs and depressive symptoms may be relevant in CAD given the consistently observed reductions in ω-3 FAs among CAD patients (Harris et al., 2006). As such it has been hypothesized that reduced ω-3 FA tissue concentrations increase the susceptibility of CAD patients to depressive symptoms (Frasure-Smith et al., 2004, Peet and Stokes, 2005, Severus et al., 2001). Consistent with this, depressive episodes are associated with a further reduction of plasma and erythrocyte EPA+DHA concentrations in
CAD patients (Amin et al., 2008, Chang et al., 2015, Frasure-Smith et al., 2004, Schins et al., 2007, Parker et al., 2006), with one study demonstrating that plasma phospholipid EPA+DHA concentrations were reduced by a magnitude of -11.6% in depressed CAD patients compared to those without depression (Frasure-Smith et al., 2004).

Another marker that is relevant to ω-3 FAs is the ratio of EPA+DHA to AA in plasma or membrane phospholipids. This ratio indicates the availability of EPA and DHA as substrates for anti-inflammatory mediators produced by eicosanoid pathways relative to the availability of AA as a substrate for pro-inflammatory mediators produced by those same eicosanoid pathways (Schmitz and Ecker, 2008). Beyond plasma and erythrocyte EPA and DHA concentrations which suggest ω-3 FA deficits, the EPA+DHA to AA ratio may provide a more informative marker of the relevance of those deficits for balancing pro- and anti-inflammatory processes. Accordingly, lower EPA+DHA to AA ratios have been associated with greater depressive symptom severity in those with CAD (Chang et al., 2015, Schins et al., 2007, Vollmer-Conna et al., 2015) and those without CAD (Adams et al., 1996, Maes et al., 1996, Kiecolt-Glaser et al., 2007, Liu et al., 2013, Conklin et al., 2007), and have been implicated as predictors of reduced response to antidepressant pharmacotherapies (Dinan et al., 2009).

The particular phospholipid fractions that are most relevant to the relationships between EPA, DHA, and AA metabolism and depressive symptoms have not been investigated. PCs are the most abundant membrane phospholipid class and integrate the largest amount of EPA, DHA, and AA (Raphael and Sordillo, 2013). PCs and their fatty acid derivatives have been implicated in depression and in various inflammatory processes related to depression (Demirkan et al., 2013, Mazereeuw et al., 2014, Schiepers et al., 2009) as well as other systemic and neurodegenerative diseases (Bennett et al., 2013, Farooqui et al., 2007, McIntyre, 2012). Other lipid fractions, such PE, PS, PI, and SM have also been associated with depression (Demirkan et
al., 2013, Kornhuber et al., 2005, Schiepers et al., 2009) and also take up EPA, DHA, and AA (Raphael and Sordillo, 2013). Indeed, the PI fraction is highly enriched with AA and is therefore an important fraction for AA metabolism, despite it being a minor fraction in membranes (D’Souza and Epand, 2014). Different phospholipid classes perform different functions within the cellular membrane (van Meer et al., 2008), with some fractions such as SM and PI implicated in lipid raft formation, second messenger signalling, and membrane binding of phospholipase enzymes that are important for pro- and anti-inflammatory phospholipid remodelling (van Meer et al., 2008, Ma, 2007, Morrison et al., 2012). Accordingly, the fatty acid composition of different membrane phospholipids may point to different roles for those fatty acids. The particular fractions in which the EPA+DHA to AA ratios are most strongly associated with depressive symptoms may have mechanistic implications and may therefore be worth exploring.

Furthermore, the particular depressive symptoms that are associated with lower erythrocyte EPA+DHA to AA ratios are unclear. As symptoms of CAD may overlap with depressive symptoms (Kohlmann et al., 2013, Sharma et al., 2014, Linke et al., 2009, Delisle et al., 2012), evidence regarding the particular depressive symptoms that are associated with lower erythrocyte EPA+DHA to AA ratios in CAD may be needed in order to identify whether the ratios are relevant to depressed mood, or whether they reflect increased somatic and/or sleep disturbances in CAD.

1.3.1.4 ω-3 FA Intake, Biotransformation, and Distribution

ω-3 are derived from the essential dietary precursor alpha-linolenic acid (ALA; C18:3ω-3) (Nettleton, 1991). Dietary ALA is then transformed in vivo by a series of desaturase and elongase enzymes to produce the long-chain ω-3 FAs EPA (C20:5-ω3) and DHA (C22:6ω-3) (Schaeffer et al., 2006) [Figure 1]. In humans, the conversion from ALA to EPA and DHA is
insufficient to provide adequate amounts of EPA and DHA (Emken et al., 1994, Pawlosky et al., 2001). Therefore, the primary means of EPA and DHA intake in humans is through dietary fish consumption (Nettleton, 1991), albeit in much lower doses than those shown to have cardiac (840-1000 mg/day) (Cunnane, 2004, Yokoyama et al., 2007) or neuropsychiatric (1000-9000 mg/day) benefits (Freeman et al., 2006, Nemets et al., 2002, Su et al., 2003). As such, ω-3 FA supplements are a promising means of substantially increasing EPA and DHA intake.

The ω-3 FAs are distributed to all tissues and are regularly taken up into the brain from peripheral fatty acid pools in order to maintain their brain concentrations (Chen et al., 2008, Freund Levi et al., 2014, Watkins et al., 2001). While different fatty acid pools have been suggested, recent evidence from rats suggests that brain fatty acids are primarily derived from peripheral pools of unesterified fatty acids (Chen et al., 2008). Both EPA and DHA are taken up by the brain; however, EPA uptake is considerably lower than DHA uptake (Igarashi et al., 2013). Once in the brain, DHA is predominantly incorporated into cell membranes, whereas EPA is predominantly (>99%) oxidized (Rapoport, 2013). Accordingly, DHA makes up the majority of fatty acids in the brain while EPA is negligible (Jicha and Markesbery, 2010). Once incorporated, DHA is an important constituent of neuronal cell membranes and membrane structures, including lipid rafts (Duraisamy et al., 2007, Li et al., 2006, Ma, 2007, Stillwell et al., 2005), where it influences multiple signal transduction pathways (Ma et al., 2004).

Brain uptake of ω-3 FAs appears to be sensitive to dietary deficits. For example: in models of chronic ω-3 FA dietary deficiency, ω-3 FA brain uptake was reduced 40-fold (Contreras et al., 2000). Accordingly, chronic administration of dietary ω-3 FAs has been shown to increase their abundance in cortical tissue (Minami et al., 1997, Okada et al., 1996). While steady state concentrations in plasma may be reached within 2 weeks of ω-3 FA supplementation (Rusca et
al., 2009), it may be many weeks before normal concentrations of fatty acids are recovered in the brain (Rapoport, 2001).

In clinical samples, blood concentrations of ω-3 FAs reflect both dietary intake and biological processes using ω-3 FAs (Davidson, 2013). The total concentration of ω-3 FAs (EPA+DHA) in blood correlates with the concentrations of DHA in cerebrospinal fluid (Guest et al., 2013). There does not appear to be a correlation between EPA alone in the blood and any ω-3 FA in the cerebrospinal fluid.

1.3.1.5. ω-3 FA Supplementation: Metabolism and Safety

According to analyses of ω-3 FA clinical pharmacology studies, oral ω-3 FA supplementation specifically providing the end products of ω-3 FA metabolism, EPA and DHA, is effective at increasing plasma deficits in EPA and DHA (Rupp et al., 2004). When provided by supplements, EPA and DHA are absorbed in the small intestine and have good oral bioavailability. Daily administration of EPA and DHA in the form of ethyl esters has been shown to increase serum phospholipid, serum cholesterol ester, and erythrocyte membrane concentrations (Krokan et al., 1993) providing both sustained daily uptake and adequate incorporation into blood lipids to replenish tissue stores for subsequent release (Rupp et al., 2006).

ω-3 FA supplements administered as an ethyl ester appear to be absorbed more slowly than ω-3 FAs from fish oil triacylglycerols, and it is thought that these pharmacokinetics may be beneficial in maintaining more consistent plasma concentrations (Rupp et al., 2006, Yokoyama et al., 2007). EPA from the ethyl ester exhibits a relatively long plasma half-life in plasma phospholipids, triacylglycerols and cholesteryl esters, permitting infrequent dosing (Zuijdegeist-van Leeuwen et al., 1999). Upon emulsification and hydrolysis in the small intestine, ω-3 FAs
from ethyl esters are absorbed as unesterified fatty acids. Clinical studies show that blood concentrations of EPA and DHA are highly correlated with incorporation of EPA and DHA into peripheral tissues (Harris et al., 2004), plasma EPA+DHA concentrations to be used as a measure of compliance with study medication in addition to capsule counts.

EPA and DHA are well tolerated at doses of 100-3000 mg per day, alone or in combination with other cardiac medications (Davidson et al., 2007, Yokoyama et al., 2007). Side effects are typically reported in fewer than 7% of subjects. There have been no significant differences between ω-3 FAs and placebo in the incidence of serious adverse events or adverse events affecting any organ class. While it has been suggested that fish oils may increase the risk of bleeding, human clinical data show little effect of ω-3 FAs on platelet aggregation (Balk et al., 2004) or on concentrations of platelet-derived growth factor (Wallace et al., 2000) and large clinical trials have shown high-dose ω-3 FA fish oil to be safe, even when administered concurrently with other agents that increase bleeding, such as ASA and warfarin (Eritsland et al., 1996, Leaf et al., 1994, Bender et al., 1998), which are commonly used by CAD patients.

1.3.2 Pathophysiology and Proposed Mechanisms

1.3.2.1 CAD

The etiopathophysiology of CAD is understood to involve aberrant activity of pro-inflammatory (Ridker, 2007) and oxidative stress processes (Nemeroff and Goldschmidt-Clermont, 2012). Dysregulation of inflammatory and oxidative processes in CAD may stem from and/or contribute to metabolic syndrome, consequences of genetic risk factors, psychological stress, or a collection of those and/or other processes (Negi and Anand, 2010). Inflammatory and oxidative stress mediators are integral to the development of atherosclerotic plaques along the coronary artery wall, which can perpetuate their own production (Weber and Noels, 2011, Young et al., 2002), leading to progressive atherosclerosis. Accordingly, elevated circulating
concentrations of inflammatory markers such as C-reactive protein (CRP) (Kaptoge et al., 2010), tumor necrosis factor (TNF) (Bruunsgaard et al., 2000), and interleukin (IL)-6 (Lindmark et al., 2001), as well as oxidative stress mediators such as LPH (Meigs et al., 2007, Fujita et al., 2006, Kovacs et al., 1997, Walter et al., 2008) have been observed in CAD patients.

1.3.2.2 Depressive Symptoms in CAD

The underlying mechanisms of depressive symptoms in CAD are currently unclear and are likely multifactorial (Stapelberg et al., 2011). In some CAD patients, depressive symptoms may reflect an emotional adjustment to experiencing an ACS (Alonzo and Reynolds, 1998). However, the persistence of depressive symptoms for as long as one year post-ACS (Frasure-Smith et al., 1999, Lauzon et al., 2003), and the bi-directional relationship between depressive symptoms and CAD (Nemeroff and Goldschmidt-Clermont, 2012) indicates potentially overlapping mechanisms. As extensively reviewed elsewhere (Carney et al., 2005, Celano and Huffman, 2011, Davidson et al., 2005, de Jonge et al., 2010, Khan et al., 2010, Mazereeuw et al., 2013, Nemeroff and Goldschmidt-Clermont, 2012, Poole et al., 2011, Stapelberg et al., 2011), depressive symptoms in CAD may be related to the dysfunction of several mechanisms such as dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response; dysregulation of autonomic tone, as evidenced by reduced heart rate variability; aberrant platelet reactivity and coagulation processes, which may influence inflammatory signalling; and/or vascular endothelial dysfunction, which can reduce cerebral perfusion, a consistent feature of depression (Ota et al., 2014, Orosz et al., 2012, Colloby et al., 2012). Dysfunction of those key mechanisms may be particularly relevant in patients with vulnerabilities to depressive symptoms (genetic or otherwise), such as carriers of the short serotonin transporter allele or the met allele of the brain-derived neurotrophic factor (bdnf) gene (Levinson, 2006, Homberg et al., 2014), both of which appear to reduce neurogenesis (Haase
and Brown, 2015), which is an important process for neural adaptation and resilience to stress. Two processes central to those key mechanisms and to the overlap between depressive symptoms and CAD are the inflammatory response and oxidative stress (Maes et al., 2011). Together with those other mechanisms, inflammation and oxidative stress characterize the systemic metabolic perturbations observed in depressed patients with CAD, and are associated with neuroinflammatory and neurodegenerative processes in the brain, as well as alterations to mood-regulating circuitry (Maes et al., 2009, Mazereeuw et al., 2013, Miller et al., 2013). As such, inflammation and oxidative stress are relevant to depressive symptoms in CAD, and may be particularly harmful under conditions of reduced neurogenesis.

Depression is associated with pro-inflammatory activity. Elevated concentrations of TNF, IL-6, IL-1, and the soluble IL-2 receptor have been consistently detected in the blood of depressed patients when compared to non-depressed controls (Dowlati et al., 2010a, Howren et al., 2009, Liu et al., 2011). Elevated pro-inflammatory activity in the central nervous system of depressed patients has also been reported (Lindqvist et al., 2009, Pandey et al., 2012, Shelton et al., 2011, Tonelli et al., 2008). Whether inflammation precedes, or is a consequence of depression is controversial (Poole et al., 2011) and is likely not consistent across cases. For example: evidence suggests that the relationships between IL-6, CRP, and depressive symptoms in CAD might be mediated by poor health behaviours, such as physical inactivity, smoking, and higher body mass index (Duivis et al., 2011), rather than mechanisms of depression per se. Conversely, depressive symptoms are a consistent side-effect of interferon-α treatment for hepatitis C (Udina et al., 2012) and a recent meta-analysis found that elevated IL-6 and CRP concentrations could predict incident depression in over 14,000 adults (Valkanova et al., 2013). The variability in those relationships is in keeping with the bi-directionality of the depression-CAD relationship, and may indicate that depressive symptom onset is mediated by different mechanisms post-ACS.
than it might have been pre-ACS. Consistent with this, depression in CAD patients has been associated with elevated blood concentrations of IL-6, CRP, IL-8, and TNF compared to non-depressed CAD patients (Duivis et al., 2011, Tajfard et al., 2014). Furthermore, elevated blood concentrations of CRP appear to be specifically associated with somatic depressive symptoms (Deverts et al., 2010), which include malaise, fatigue, reduced appetite, and gastrointestinal disturbances, suggesting that inflammatory processes might be more relevant to the presence of certain depressive symptoms compared to others.

In animals, induction of an inflammatory cytokine response by lipopolysaccharide administration, generating the release of mediators such as IL-1, IL-6, and TNF, leads to depression- and anxiety-like behaviours (Dunn et al., 2005, Sperner-Unterweger et al., 2014). Those behaviours are referred to as “sickness behaviours” and include weakness, malaise, reduced interest in surroundings, reduced appetite, disrupted sleep, listlessness, and inability to concentrate, among others (Hart, 1988). In animals, sickness behaviours are the result of peripherally induced cytokines acting on cytokine receptors in the brain (Dantzer, 2004). There is considerable overlap between sickness behaviours and many depressive symptoms, such as weakness, malaise, anhedonia, changes in appetite, changes in sleep, and inability to concentrate (Dantzer et al., 2008), supporting the involvement of inflammatory activity in depressive symptom etiology and its possible relevance to certain depressive symptom clusters.

Inflammatory processes interact with metabolism of key monoamines thought to be involved in mechanisms of depressive symptoms (Schildkraut, 1965, Hirschfeld, 2000, Miller et al., 2013). Pro-inflammatory cytokines appear to have a reciprocal relationship with dopamine release (Felger and Miller, 2012, Ghosh et al., 2003), which may pertain to sickness behaviours such as anhedonia and malaise. Pro-inflammatory cytokines can also reduce available synaptic concentrations of serotonin by increasing serotonin uptake into synaptosomes (Zhu et al., 2006),
by increasing serotonin catabolism (Dunn et al., 2005), and by activating the tryptophan metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) (Dantzer et al., 2008). IDO converts tryptophan, a precursor amino acid for serotonin production, into kynurenine, a pro-inflammatory mediator with various pro- and anti-inflammatory metabolites (reviewed (Dantzer et al., 2011)). Accordingly, the ratio of kynurenine to its precursor tryptophan in plasma indicates IDO activity, and elevated plasma kynurenine/tryptophan ratios have been associated with greater depressive symptom severity in CAD (Swardfager et al., 2009).

Oxidative stress has also been consistently associated with depression. Meta-analytic data support elevated concentrations of circulating oxidative stress markers in depressed patients (Black et al., 2015, Palta et al., 2014). Of particular relevance to dysregulated lipid metabolism in CAD and depression, meta-analytic data also support specific elevations of circulating lipid peroxidation markers in depressed patients (Black et al., 2015, Palta et al., 2014). Oxidative stress activity and/or a compromised antioxidant system has also been observed in post-mortem brain tissue of depressed patients (Gawryluk et al., 2011, Shelton et al., 2011, Michel et al., 2010), and has been linked with the general dysfunction of inflammatory processes and neurotransmission associated with depression. For example: reduced antioxidant activity may be associated with increased pro-inflammatory responsivity and associated depressive symptoms in animals and humans (Szewczyk et al., 2011, Leonard and Maes, 2012). Collectively, these findings support oxidative stress as a CAD-relevant mechanism of depressive symptoms.

Inflammatory and oxidative stress processes may interact with neurotrophic factors important for neurogenesis and cell fate (Goldman and Chen, 2011). Accordingly, a large meta-analysis demonstrated that serum concentrations of BDNF are significantly lower in depressed patients relative to non-depressed healthy controls (Molendijk et al., 2014). Similarly, reduced concentrations of other neurotrophic factors such as glial cell line-derived neurotrophic factor
(Lin and Tseng, 2015), insulin-like growth factor 1 (van Varsseveld et al., 2015), and vascular endothelial growth factor (Clark-Raymond and Halaris, 2013) have been associated with depressive symptoms. Prolonged exposure to inflammatory processes and oxidative stress, especially in combination with reduced concentrations of neurotrophic factors may be associated with neurodegeneration (Moylan et al., 2012), particularly to vulnerable brain regions such as the hippocampus (Anacker et al., 2011).

The hippocampus is not only a regulatory region for the HPA axis (Jacobson and Sapolsky, 1991), but it is also one of the few sites for neurogenesis in the adult brain (Fuchs and Flugge, 2014). While a causal role for the hippocampus in depression is unclear, hippocampal atrophy is a consistent feature in patients with depression (Du et al., 2012), even in those experiencing their first depressive episode (Cole et al., 2011), and may be indicative of reduced neurogenesis. As hippocampal atrophy has been associated with CAD (Koschack and Irle, 2005), it may be another CAD-relevant mechanism of depressive symptoms.

In summary, inflammation and oxidative stress are central mechanisms in CAD pathophysiology, and are integrated with key mechanisms thought to mediate depressive symptoms in CAD. Reduced neurogenesis is another feature of depression which, in combination with inflammation and oxidative stress is linked with neurodegeneration in the hippocampus and other brain regions, which is a prevalent finding among CAD patients. Thus, inflammation, oxidative stress, and neurogenesis are processes that may be relevant to depressive symptoms in CAD.

1.3.3 Pathophysiological Relevance of ω-3 FAs

ω-3 FAs are integral components of key mechanisms associated with depressive symptoms in CAD. ω-3 FA deficiency is associated with the dysregulation of dopamine (Zimmer et al.,
neurotransmission in animals. Accordingly, ω-3 FA supplementation is associated with the restoration of dopamine (Chalon, 2006, Shin and Dixon, 2011, Davis et al., 2010, Song et al., 2007) and serotonin (McNamara et al., 2010, Chalon, 2006, Vancassell et al., 2008, Song et al., 2008) neurotransmission in animals, with associated reductions in depression- and anxiety-like behaviours (Vancassell et al., 2008, Pudell et al., 2014). ω-3 FAs are also implicated in the HPA axis regulation (Mocking et al., 2013), improvements in autonomic tone (La Rovere et al., 2013, Sauder et al., 2013, Billman and Harris, 2011, Carney et al., 2010), reduced platelet reactivity (Gajos et al., 2010, Svaneborg et al., 2002, Guillot et al., 2009) and improvements in vascular endothelial function (Ahmadi et al., 2014, Miyoshi et al., 2014, Kondo et al., 2014, Tousoulis et al., 2014, Toyama et al., 2014) and cerebral perfusion (Jackson et al., 2012, Hamazaki-Fujita et al., 2011). ω-3 FAs are also highly relevant inflammatory and oxidative stress activity, as well as neurogenesis.

1.3.3.1 Inflammation

ω-3 FAs regulate immune functions both in the brain and in the periphery (Levant, 2013). ω-3 FA deficiency is associated with elevated plasma concentrations of IL-6, TNF, and CRP in adult rats (McNamara et al., 2010), as well as elevated concentrations of plasma and brain arachidonic acid (McNamara et al., 2010, McNamara et al., 2008, Mathieu et al., 2008), which is the precursor lipid for the pro-inflammatory series of prostaglandins, isoprostanes, and leukotrienes (Janssen and Kiliaan, 2014). ω-3 FA supplementation has been consistently shown to reduce the release of pro-inflammatory cytokines (Lu et al., 2010, Paterniti et al., 2014, Chang et al., 2013, Hall et al., 2012, Antonietta Ajmone-Cat et al., 2012, McNamara et al., 2010, Sekhon-Loodu et al., 2014, Mark et al., 2014) and pro-inflammatory lipids, such as AA-derived prostaglandins, leukotrienes, and thromboxanes (Hung et al., 2000, James et al., 1993, Ferretti and Flanagan,
1990), as well as increase concentrations of anti-inflammatory lipids such as the EPA- and DHA-derived resolvin and protectin eicosanoids and docosanoids (Yates et al., 2014). As mentioned, the ratio of EPA and DHA to AA in plasma and membranes may indicate the balance of those pro- and anti-inflammatory eicosanoids (Schmitz and Ecker, 2008). This is particularly relevant to the production of inflammatory cytokines as AA concentrations have been consistently associated with IL-6 and CRP concentrations in plasma (Dinan et al., 2009, Lotrich et al., 2013). The anti-inflammatory effects of ω-3 FAs have been consistently reported in the brain in animal models (reviewed (Orr et al., 2013b, Farooqui, 2012)), where DHA and DHA-related resolvins may be particularly neuroprotective (Orr et al., 2013a). Protective effects have also been demonstrated in the context of depressive symptoms. For example: in hepatitis C patients, lower plasma ω-3 FA concentrations, and EPA+DHA to AA ratios, prior to interferon α treatment was predictive of depressive symptom onset following treatment (Lotrich et al., 2013). ω-3 FA supplementation prior to interferon α treatment can prevent the onset of depressive symptoms (Su et al., 2014). Collectively, ω-3 FAs appear to be key participants in the regulation of inflammatory activity and protection from inflammatory-induced metabolic changes.

1.3.3.2 Oxidative Stress

ω-3 FAs are also involved in antioxidant processes. Enzymatic oxidation of ω-3 FAs yields generally anti-inflammatory eicosanoid and docosanoid mediators, such as the E- and D-series resolvins and protectins (Schmitz and Ecker, 2008). This is in contrast to the enzymatic oxidation of arachidonic acid, which yields generally pro-inflammatory eicosanoids, such as the prostaglandins, leukotrienes, and thromboxanes (Schmitz and Ecker, 2008). Accordingly, ω-3 FA supplementation has been shown to protect against or reduce the onset of oxidative stress in cells and animals (Mori and Beilin, 2004). Clinically, dietary ω-3 FA intake and erythrocyte ω-3
FA fractions are inversely associated with oxidative stress (Baek and Park, 2013, Lee et al., 2013a). Of relevance to depressive symptoms, ω-3 FA-rich diets have been shown to protect against increased lipid peroxidation and depressive symptom-like behaviours related to chronic mild stress in rats (de Mello et al., 2014). Collectively, attenuation of oxidative stress through enzymatic ω-3 FA metabolism may implicate ω-3 FAs in the mechanisms of depressive symptoms in CAD.

1.3.3.3. Neurogenesis and Neuroprotection

As integral components of neuronal membranes, ω-3 FAs can facilitate membrane fluidity, which enhances cellular plasticity, and can be rapidly metabolised to protect the cell from inflammatory and oxidative insults (Crupi et al., 2013). ω-3 FAs and their eicosanoid and docosanoid derivatives can also enhance neuronal stem cell proliferation and differentiation, thereby increasing neurogenesis (Kang et al., 2014). Accordingly, animal studies indicate that ω-3 FA deficiency can impair neurogenesis (Brand et al., 2010), while ω-3 FA supplementation can enhance neurogenesis and cellular plasticity (Sakayori et al., 2011, Schipper et al., 2011, Dagai et al., 2009, Kawakita et al., 2006), even in aged animals (Cutuli et al., 2014, Dyall et al., 2010), and in animal models of brain injury (Hu et al., 2013, Lei et al., 2013). Consistent with their neurogenic and neuroprotective roles, ω-3 FAs may protect the brain from acute inflammatory and oxidative stress insults. For example: preclinical evidence suggests that chronic ω-3 FA administration may be neuroprotective in models of cerebral ischemia (Belayev et al., 2005, Cao et al., 2005, Cao et al., 2007, Okada et al., 1996), possibly attributable to its antithrombotic and anti-inflammatory effects (Kalman et al., 1992, Umemura et al., 1995). In the context of neurodegeneration associated with those processes, both greater erythrocyte ω-3 FA fractions and ω-3 FA supplements have been associated with greater volume in the hippocampal region (Pottala et al., 2014, Daiello et al., 2015) and medial temporal lobe (Samieri
et al., 2012) in humans, which are two areas of relevance to depressive symptoms.

1.3.4 Potential Biological Predictors of Treatment Efficacy: Role of Oxidative Stress

Although ω-3 FAs appear to be mechanistically related to depressive symptoms, the clinical antidepressant efficacy of ω-3 FA supplements is variable. Biological predictors of ω-3 FA treatment efficacy may help clinicians and scientists identify subgroups of patients who are most likely to experience an antidepressant benefit from ω-3 FA supplements.

Pre-treatment oxidative stress may be relevant to the treatment efficacy of ω-3 FAs. As mentioned, oxidative stress is implicated in the pathophysiology of CAD and of depressive symptoms in CAD. Reported antioxidant effects of ω-3 FA supplementation in clinical samples are mixed. Some studies have found that ω-3 FA supplementation can reduce oxidative stress (Maffei et al., 2014, Daak et al., 2013, Azizi-Soleiman et al., 2013, Bozcali et al., 2013, McDonald et al., 2013, Takaki et al., 2011, Gajos et al., 2011, Tayyebi-Khosroshahi et al., 2010, Bouzidi et al., 2010, Sarbolouki et al., 2010, Taccone-Gallucci et al., 2006, Barbosa et al., 2003, Jain et al., 2002), while others have found no antioxidant effects, particularly in medically ill populations (Darghosian et al., 2015, Taheri et al., 2014, Freund-Levi et al., 2014, Firuzi et al., 2013, Hassan Eftekhar et al., 2013, Stanger et al., 2014, Ramirez-Ramirez et al., 2013, Mocking et al., 2012, Kooshki et al., 2011, Agouridis et al., 2011, Dawczynski et al., 2009, Nakamura et al., 2005). Furthermore, some studies indicate that ω-3 FA supplementation can increase oxidative stress markers, particularly lipid peroxidation markers such as malondialdehyde or 8-isoprostane compared to placebo (Carrepeiro et al., 2011, Ramezani et al., 2011).

Although ω-3 FAs demonstrate anti-inflammatory and antioxidant properties when enzymatically metabolized (Farooqui, 2012), ω-3 FAs and ω-6 FAs are also prime targets for non-enzymatic oxidation by reactive oxygen species (ROS) due to the presence of several
double-bonds on their carbon chains (Hulbert et al., 2007). When those fatty acids are attacked by ROS, they produce harmful LPH free radical species, indicating the early stages of lipid peroxidation. Antioxidants such as glutathione peroxidase scavenge the body for LPH and neutralize those radicals to protect against further oxidative damage (Forman et al., 2014). During periods of increased oxidative stress and/or reduced antioxidant activity, the production of LPH can overwhelm the antioxidant defenses and contribute to further oxidative damage to lipids and proteins (Forman et al., 2014, Moylan et al., 2014). Indicators of this later-stage lipid peroxidation include the AA-derived HNE, which are a later-stage peroxidation product of ω-6 FAs, or such as the ω-3 FA-derived hydroxyhexenals (HHE) (Assies et al., 2014).

Conditions of high lipid peroxidation, such as CAD, may present an environment of high non-enzymatic oxidation that may shift the metabolism of ω-3 FA supplements away from the primarily enzymatic production of anti-inflammatory, antioxidant, and neuroprotective mediators, and toward the non-enzymatic production of harmful LPH and HNE/HHE species [Figure I]. That shift in metabolism may be reflected by the variability in clinical antioxidant effects of ω-3 FA supplements in medically ill populations, and may accordingly underlie the variability in the treatment efficacy for depressive symptoms. As such, pre-treatment lipid peroxidation may be highly relevant to ω-3 FA treatment efficacy for depressive symptoms in CAD patients; however, this has not presently been investigated.
Figure 1. Metabolism of EPA, DHA, and AA in Plasma and Membranes.

EPA, DHA, and AA are present in plasma and in membrane phospholipids in the brain and periphery, where they can enhance membrane fluidity and cellular plasticity. EPA may be converted to DHA through a series of elongase and desaturase enzymes. EPA and DHA can be metabolized by COX, LOX, and CYP enzymes to produce anti-inflammatory mediators such as resolvins, protectins, maresins, series 3 prostaglandins, series 5 leukotrienes, HETEs, and epoxides. AA can be metabolized by COX, LOX, and CYP enzymes to produce pro-inflammatory mediators such as series 2 prostaglandins, series 4 leukotrienes, thromboxanes, HETEs, and epoxides. As such, the balance between EPA and DHA to AA in plasma and membranes indicates the likelihood of pro- or anti-inflammatory mediators being produced by COX, LOX, and CYP enzymes. EPA, DHA, and AA are each vulnerable to ROS due to their many double bonds; while this protects cells from ROS, it can lead to the formation of oxidative lipid peroxidation by-products such as LPH and, should antioxidant defenses be unable to neutralize LPH, later-stage by-products such as HHE (DHA) or HNE (AA) may be formed. Lipid peroxidation may therefore redirect EPA and DHA metabolism away from the production of anti-inflammatory mediators, and in the case of EPA and DHA supplements, away from potentially antidepressant mediators. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; COX, cyclooxygenase; LOX, lipoxygenase; CYP, cytochrome P450; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acids; ROS, reactive oxygen species; LPH, lipid hydroperoxides; HHE, hydroxyhexenal; HNE, hydroxynonenal.
1.4 Summary of Knowledge and Thesis Objectives

Depressive symptoms are common among CAD patients and can independently contribute to poor rehabilitation outcomes, reduced quality of life, and mortality. While presently available antidepressant interventions are generally safe for CAD patients, they demonstrate only modest efficacy and symptom relapse is common. As such, more effective interventions for depressive symptoms in CAD patients are a presently unmet medical need.

ω-3 FAs are anti-inflammatory, antioxidant, and pro-neurogenic lipids which may counteract the pro-inflammatory, oxidative stress, and neurodegenerative mechanisms associated with depressive symptoms in CAD. However, the clinical relationship between depressive symptoms in CAD patients and ω-3 FA deficits, particularly the ratio of ω-3 to ω-6 FAs in erythrocyte phospholipids, remains unclear. ω-3 FA supplements are effective at increasing ω-3 FA concentrations in humans, are safe for use in CAD, and are well tolerated. ω-3 FA supplements may be efficacious for reducing depressive symptoms in patients without CAD, but their efficacy in patients with CAD also remains unclear.

Oxidative stress processes are common to CAD and depressive symptoms. Polyunsaturated fatty acids, such as ω-3 FAs, are highly vulnerable to ROS. Lipid peroxidation is associated with the production of lipid free-radicals which can exacerbate oxidative stress and create lipid-protein adducts affecting protein function, with possible relevance to neurotrophic proteins such as BDNF. Accordingly, a high pre-treatment state of lipid peroxidation may alter the metabolism of ω-3 FA supplements and reduce their efficacy for reducing depressive symptoms in CAD patients. However, the predictive association between pre-treatment lipid peroxidation and ω-3 FA treatment efficacy has not been investigated.
The following studies investigated the cross-sectional relationship between depressive symptoms and the EPA+DHA to AA ratio in erythrocyte phospholipid fractions in those with CAD [Study 1], the longitudinal efficacy of ω-3 FA treatment on depressive symptoms in those with CAD [Study 2], and the association between pre-treatment lipid peroxidation and ω-3 FA treatment efficacy for depressive symptoms in those with CAD [Study 3]. Collectively, these studies aimed to address gaps in knowledge regarding the potential of ω-3 FAs as an intervention for depressive symptoms in CAD patients.
Chapter II

Materials and Methods

2.1 Overview of Setting: Cardiac Rehabilitation

This Thesis included CAD patients participating in CR programs at the Toronto Rehabilitation Institute at University Health Network (UHN Toronto Rehab) and Trillium Health Partners (THP) in the Greater Toronto Area. Both CR programs are funded by the Ontario Ministry of Health and Long-Term Care, and patients assume no costs associated with participation in CR provided that they are valid members of the Ontario Health Insurance Plan. Referral to the program must be prescribed by a licenced physician. All CAD patients are accepted into the CR programs unless they have a specific contraindication to exercise, such as aortic stenosis, uncontrolled hypertension, or uncontrolled heart failure as outlined by the Canadian Association of Cardiac Rehabilitation guidelines (Stone et al., 2001).

The CR protocols at UHN Toronto Rehab and THP consisted of both aerobic and resistance training in a group setting, with supervision by exercise and medical specialists. Following a group intake session in which they were oriented to the program and given a series of short lectures on the risk factors for CAD and the value of exercise, patients attended supervised exercise visits consisting of an aerobic walk/jog or resistance training once per week for 18 weeks (THP) or 24 weeks (UHN Toronto Rehab). Additionally, patients were given personalized at-home exercise plans, which they carried out 4 days per week to supplement their supervised in-class training.

The cardiopulmonary fitness benefits of exercise from both the supervised and unsupervised program components were operationalized using the VO$_2$ peak during exercise stress tests.
(Milani et al., 2006) conducted at entry to CR and after 12 weeks of CR. Symptom-limited graded exercise stress tests were performed using a cycle ergometer (such as the Ergoline 800 EL at UHN Toronto Rehab). The work load of the cycle was increased each minute by 16.7 Watts until maximal exertion was determined. Each patient’s ventilatory capacity at maximal exertion was determined through breath samples collected and calibrated during the exercise stress test, resulting in a VO₂ peak (Hamm and Kavanagh, 2000). The VO₂ peak is a reliable indicator of cardiopulmonary fitness (Milani et al., 2006) that is normalized to body mass (ml of O₂/kg of body mass/minute of exercise).

2.2 Overview of Studies

Each aim and hypothesis was investigated by conducting the CAD Randomized Omega-3 Trial in Depression (CAROTID). CAROTID was a double-blind placebo-controlled RCT investigating the efficacy of 1.9 g/day ω-3 FA supplements for reducing depressive symptoms over 12 weeks in CAD patients who were participating in CR. Collection and measurement of patient characteristics and depressive symptoms at 5 time points during the 12 week trial (screening, baseline, week 4, week 8, and week 12), as well as fasting blood samples collected at the trial baseline and week 12 visits provided appropriate data for each study in this Thesis. In describing the CAROTID trial methods, the foundation for the methods of each study will be introduced.

2.3 CAROTID Trial

2.3.1 Patient Selection

Patients were recruited from UHN Toronto Rehab and THP. CAROTID was approved by the Research Ethics Boards of both centres as well as Sunnybrook Research Institute, the principal trial site [Appendix A]. Recruitment from CR enabled the trial to investigate ω-3 FA treatment
benefits in CAD patients already receiving the current standard of clinical management for CAD.

Consecutive patients with evidence of CAD (history of MI, prior CABG or PTCA, ischemic heart disease, or at least a 50% stenosis in one or more major coronary artery as documented in the referral note and chart and assessed by the medical director) were invited to participate in research at entry to CR. Patients providing consent to be contacted by a research associate were then invited to discuss the CAROTID trial details with a study associate at a screening visit (week -2) where full informed consent was provided by each patient prior to study initiation.

At the screening visit, patients were assessed for trial eligibility based on the following criteria:

**Trial inclusion criteria:**
- Language (speaks and understands English)
- Between 45-80 years old
- Stable CAD (based on no hospitalization for cardiac events for at least 7 weeks prior)
- Angiographic documentation of presence and extent of CAD (number of vessels involved, extent of stenosis, etc.)
- Written, informed consent [Appendix B]

**Trial exclusion criteria:**
- Significant acute medical illness (sepsis, autoimmune condition, drug overdose, uncontrolled diabetes, severely disturbed liver, kidney or lung function, anemia, uncontrolled hypothyroidism)
- Clinically significant cognitive impairment (Mini-Mental State Examination [MMSE] < 24 (Cacciatore et al., 2005, 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)
• Ventricular tachycardia and/or implantable cardioverter defibrillator (Jenkins et al., 2008)
• 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
• Current use of a concentrated ω-3 FA supplement, or contraindication to soybean/corn oil
• Women of childbearing potential
• Allergies or hypersensitivity to fish
• Pre-existing bleeding disorder
• History of electroconvulsive therapy
• Suicidal ideation or a history of suicidal ideation/Attempts
• Severe depression, defined by HAM-D score >23
• Current or history of psychotic episode or personality disorder

Concomitant antidepressant interventions were allowed if used for at least 3 months at a stable dose as the antidepressant efficacy of ω-3 FAs has been found in patients using maintenance medications for recurrent depression (Nemets et al., 2002). Those characteristics were noted as possible confounders. Any changes to antidepressant medications or initiation of over-the-counter ω-3 FA supplement use were tracked at each study visit.
Once trial eligibility was confirmed, detailed demographic and anthropomorphic data (exercise parameters such as resting and maximal blood pressure and heart rate, cardiopulmonary fitness (VO$_2$ peak), and body mass index [BMI]), medical history, and concomitant medications were recorded from each patient. General cognitive performance was also assessed using a global cognitive screening measure, the MMSE (Folstein et al., 1975), to ensure that each patient was cognitively intact.

Patients were then entered into a 2 week single-bind placebo lead-in (screening) phase, during which they were asked to use the study supplements daily under the impression that they may have already been allocated to the ω-3 FA supplementation or the placebo arms. The 2-week screening phase enabled the confirmation of trial eligibility at a second visit (the baseline visit) and ensured that “depression” at baseline was determined after 2 weeks of placebo use.

2.3.2 Schedule of Assessments

Baseline

Upon completion of the screening phase, patients were invited to the trial baseline visit (week 0) at which study eligibility was re-assessed. Patients who continued to be eligible were enrolled into the “randomization” phase of the trial and assessed further [Figure II].

Patients were assessed for the presence of a depressive episode and for depressive symptom severity (whether depressed or not). The presence of a depressive episode was assessed using standardized criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV (First et al., 1996). The determination of a depressive episode was primarily based on the presence of a depressed mood and/or anhedonia for the majority of the most recent month; however, those symptoms must have been combined with the presence of secondary symptoms such as weight and/or appetite changes, sleep schedule changes, agitation or retardation, fatigue,
feelings of worthlessness or guilt about past errors, reduced decision making ability or focus, and/or suicidal ideation in the 2 week period in which the patient was most depressed. Patients who were experiencing a depressed mood and/or anhedonia in addition to at least 3-4 of the secondary symptoms (totalling at least 5 symptoms) were classified as experiencing a major depressive episode at the baseline visit. Patients who were experiencing a depressed mood and/or anhedonia in addition to 1-2 of the secondary symptoms (totalling at least 3, but less than 5) were classified as experiencing minor depression at the baseline visit. The presence of a major or minor depressive episode was determined so that “depression” could be included as an a priori treatment interaction term in the analysis of ω-3 FA treatment effects on depressive symptoms [Study 2]. It was also used to identify a depressed subgroup for post-hoc analyses.

Depressive symptoms were measured by the study investigator using the 17-Item HAM-D. The HAM-D is highly reliable and sensitive (Strik et al., 2001), and was chosen as the primary continuous measure of depressive symptoms. As recommended by expert consensus, the HAM-D is the gold standard for assessing antidepressant efficacy in trials with CAD patients (Davidson et al., 2006). The HAM-D was 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 ω-3 FAs (Nemets et al., 2002, Stoll et al., 1999, Su et al., 2003). The HAM-D was conducted in a structured manner (Williams, 1988), under the training and supervision of an experienced geriatric psychiatrist. Depressive symptoms were also reported by each patient using the self-report 21-Item BDI-II (Beck et al., 1961, Davidson et al., 2006), which has shown appropriate sensitivity in previous antidepressant trials in CAD patients (Lesperance et al., 2007, Honig et al., 2007, Glassman et al., 2002) and provided a complementary measure of depressive symptoms.
At the completion of the baseline trial visit, randomized patients were assigned (1:1 ratio) to receive either the study ω-3 FA supplement (1.9 g/day) or the placebo for the remainder of the trial (see description of randomization below), to which both the study investigators and the patients were blind (double-blind).

**Weeks 4 and 8**

At each follow-up visit (weeks 4 and 8), patients were assessed for treatment compliance (capsule count) and for changes to medications or their medical condition. Each patient was monitored for the safety and tolerability of the ω-3 FA treatment using a standard adverse event checklist according to consensus guidelines (Bays, 2007, Harris, 2007).

Patients were also reassessed for the severity of depressive symptoms using the HAM-D and BDI-II. A new supply of study medication was provided to each patient at the end of each follow-up visit.

**Week 12**

Week 12 was the final trial visit. Patients were reassessed for treatment compliance (capsule count), changes to medications or their medical condition, and the experience of adverse events. As week 12 coincided with a follow-up cardiac stress test from each patient’s CR program, those follow-up data were collected from medical records. Patients were also reassessed for the severity of depressive symptoms using the HAM-D and BDI-II.
Figure II. Schedule of Assessments and Randomization for the CAROTID trial.
Patients were screened at entry to CR (week -2) and then enrolled in the trial during the first week of exercise (week 0). Patients were then randomized to receive ω-3 FA treatment (1.9 g/day) or placebo in a 1:1 ratio for 12 weeks concurrently with CR. Study 1 used data from the CAROTID baseline visit (cross-sectional analysis), Study 2 used data from the baseline, week 4, week 8, and week 12 CAROTID visits (longitudinal analysis), and Study 3 used data from the baseline and week 12 CAROTID visits (longitudinal analysis). Abbreviations: DSM-IV, Diagnostic and Statistical Manual of Mental Disorders 4th Edition; HAM-D, 17-Item Hamilton Depression Rating Scale; BDI-II, 21-Item Beck Depression Inventory II; ω-3 FA, omega-3 fatty acid
2.3.3 Study Intervention: ω-3 FA Supplementation

The experimental intervention was 3 capsules (3x1g) of fish oil-derived concentrated ethyl esters, providing 1.9 g ω-3 FAs daily (1.2 g EPA and 0.6 g DHA, with 0.1 g other ω-3 FAs) (Davidson et al., 2006). A recent meta-analysis found non-significant support for higher doses (Lin and Su, 2007), and an available dose-ranging study with depression showed no increased benefit above 1.0 g EPA (Peet and Horrobin, 2002). The formulation of EPA (1.2 g; 66.7%) + DHA (0.6 g; 33.3%) was chosen based on dose-optimization studies indicating antidepressant benefits of EPA formulations (Nemets et al., 2002, Peet and Horrobin, 2002) and reports of plasma DHA deficits in depressed patients (Lin et al., 2010).

The matching placebo was 3 capsules (3x1g) of 50/50 soybean/corn oil blend containing less than 0.12 g ω-3 FAs with negligible EPA and DHA. This placebo was suitable for patients with CAD (O'Keefe et al., 2006) as it contained predominantly (56%) polyunsaturated FAs while contributing less than 2% of the recommended daily intake of saturated fat. To make experimental and placebo capsules indistinguishable with regard to taste and appearance, carob (dark brown) and lemon-lime flavoring was added to the gelatin encapsulation as Ocean Nutrition Canada (Dartmouth, Nova Scotia) has done before (Nemets et al., 2006, O'Keefe et al., 2006, Stark et al., 2000). ω-3 FA and placebo were equicaloric, each contributing only 9 calories/capsule. ω-3 FA and placebo capsules were manufactured by Ocean Nutrition in a Canadian site licensed in accordance with Natural Health Products Regulations using a preparation that has been filed with the Natural Health Products Directorate (NPN 80000901). Patients were advised to take the study medication with their first meal of the day to minimize any possible variations in response based on time of day.
Randomization

A block randomization code was independently computer-generated by the Pharmacy Department at Sunnybrook Research Institute, the principal trial site. Kits with study medication were consecutively pre-packaged as per the randomization sequence prior to commencement of the trial. Study medication kits were administered in order as consecutive patients were enrolled. All study personnel remained blind to treatment allocation until the final patient had completed follow-up, plasma analyses had been performed, and the database was “locked”.

Compliance

Compliance with the study medication was assessed through capsule counts at week 4, week 8, and week 12 trial visits. Total compliance percentage was determined by the number of capsules used compared to the number assigned over 12 week trial.

2.3.4 Biological Assays

At the baseline and week 12 visits, 34 mL of fasting (12 hours overnight) blood was drawn from the antecubital vein by a trained study associate. Erythrocytes and plasma/serum from fasting blood samples were isolated by centrifugation (Model 614B, The Drucker Company) at 3,150 (SD 100) RPM for 10 minutes and each fraction was frozen separately at -80°C until analysis. Those samples enabled the examination of biological markers of ω-3 FA fractions in erythrocytes and concentrations in plasma [Studies 1, 2, and 3] as well as serum concentrations of lipid peroxidation markers [Study 3].

Erythrocyte and Plasma Fatty Acid Analyses

Erythrocyte fractions of EPA, DHA, and AA are reliable indicators of long-term EPA, DHA, and AA intake (Harris and Von Schacky, 2004) and were used as baseline covariates in the study analyses. The percentage of EPA, DHA, and AA in each phospholipid fraction (PC, PE,
PI, PS, SM, lysophospholipid) was determined using gas chromatography using a validated technique (Ma et al., 2007). Percentage composition of EPA and DHA in each fraction was then summed and divided by the percentage composition of AA in that fraction, generating the EPA+DHA to AA ratio. Total plasma EPA and DHA concentrations were assessed at baseline and week 12 and are a reliable indicator of total erythrocyte percentages (Harris and Von Schacky, 2004). All analyses were performed blinded to patient characteristics.

Changes in plasma EPA and DHA concentrations enabled a complementary measure of compliance with the study intervention, as well as accounting for any dietary sources of EPA and DHA in both the ω-3 FA and placebo groups. EPA+DHA to AA ratios in erythrocyte phospholipid fractions and total plasma EPA+DHA concentrations were each assessed as baseline predictors of ω-3 FA treatment efficacy over 12 weeks, while total plasma EPA+DHA measurements from week 12 blood samples enabled changes in plasma EPA+DHA associated with ω-3 FA treatment to be measured.

**Lipid Peroxidation Markers**

Lipid peroxidation was measured using spectrophotometric assays from serum samples provided by study patients at baseline. Early-stage lipid peroxidation was evaluated by measuring the concentrations of LPH (Cayman; Item No. 705003). LPH was extracted from samples by the addition of 1 unit of LPH (also known as LPO) Assay Extract R and 2 units of cold chloroform per unit of sample. Assay tubes were then centrifuged at 1500 x g to isolate the bottom chloroform extract layer, which was used in the assay. The isolated sample was then combined with 0.9 unit of 2:1 chloroform-methanol solvent mixture and 0.1 unit of chromogen mixture per unit of chloroform extract. Following an incubation period at room temperature, samples were loaded into 96-well plates and absorbance of each sample was read at 500 nm. Absorbances
were compared with a standard hydroperoxide curve to determine the amount of LPH in samples. To assess the levels of late-stage lipid peroxidation we measured HNE (Cell Biolabs, Inc.; STA-338), which is a lipid peroxidation by-product that can conjugate to protein amino acid residues, forming stable adducts which can alter the structure and function of the protein. HNE was quantified by standard sandwich ELISA designed to detect HNE protein adducts.

2.3.5 Statistical Transformations

Adjusted Cardiopulmonary Fitness

For each patient, the VO$_2$ peaks measured from the exercise stress tests coinciding with the baseline and week 12 assessments were recalculated into a fraction of the expected VO$_2$ peak [VO$_2$ peak fraction] based on available age and gender norms (Jones and Campbell, 1982). VO$_2$ peak fraction is a clinically meaningful measurement of cardiopulmonary fitness enabling comparison across age and gender (Jones and Campbell, 1982). VO$_2$ peak fraction was calculated using the following formulas:

For males:

\[
\text{VO}_2 \text{ peak fraction} = \frac{\text{VO}_2 \text{ peak from stress test}}{60 - (0.55 \times \text{age})}
\]

For females:

\[
\text{VO}_2 \text{ peak fraction} = \frac{\text{VO}_2 \text{ peak from stress test}}{48 - (0.37 \times \text{age})}
\]

VO$_2$ peak fraction was used as the measure of cardiopulmonary fitness in each study.

Depressive Symptom Clusters

In addition to measuring depressive symptom severity using HAM-D and BDI-II total scores, scores from clusters of similar depressive symptoms measured by each scale were explored in the study analyses.
Depressive symptoms measured by the HAM-D were organized into three symptom clusters: mood symptoms, sleep and psychic anxiety symptoms, and somatic symptoms, as has been determined previously (O'Brien and Glaudin, 1988, Gullion and Rush, 1998). HAM-D mood symptoms were items 1, 2, 3, 7, 8, and 13; HAM-D sleep and psychic anxiety symptoms were items 4, 5, 6, 9, and 10; HAM-D somatic symptoms were items 11, 15, and 17. The HAM-D appetite item was not a member of any cluster in this Thesis.

Depressive symptoms measured using the BDI-II were organized into two symptom clusters: cognitive symptoms and somatic symptoms, as has been determined previously (Bekke-Hansen et al., 2012). BDI-II cognitive symptoms were items 1-15 and BDI-II somatic items were 16-21. Scores for each item of a cluster were totalled, yielding a symptom cluster total score that was used for study analyses.

**Multiple Imputation**

Missing data from week 4, 8, and/or 12 visit assessments were imputed using a multiple imputation procedure (SPSS, version 20; SPSS Inc., Chicago, IL). For each visit, each missing variable was imputed using that variable’s score at the prior visit as a predictor in addition to the sample mean and trends for that variable at the imputed visit. Each imputation was generated 5 times. Outcome parameters from study analyses were pooled using SPSS or Microsoft Excel.

2.4  **Summary of Study Designs and Statistical Approaches**

2.4.1 Study 1: EPA+DHA to AA Ratios in Erythrocyte Phospholipids and Depressive Symptoms in CAD

Study 1 was a cross-sectional investigation of the association between EPA+DHA to AA ratios in erythrocyte phospholipid fractions and depressive symptom severity in CAD patients using the following data from the CAROTID baseline visit:
- Patient sociodemographic, medical, medication, and cardiopulmonary fitness parameters (VO₂ peak fraction and other physiological parameters)
- Depressive symptom severity (HAM-D total score and symptom cluster scores)
- EPA, DHA, and AA percentage of total phospholipids in each erythrocyte fraction (PC, PE, PI, PS, SM, and lyso) from fasting blood

Individual linear regressions were conducted to investigate the association between HAM-D total scores and the EPA+DHA to AA ratios in the PC fraction (primary) and the other phospholipid fractions (secondary). Significant associations identified in the secondary analysis were corrected for multiple comparisons (Benjamini and Hochberg, 1995).

Erythrocyte phospholipid fractions remaining significant predictors of HAM-D total score were then investigated in the context of other known predictors of depressive symptoms in CAD patients using a backward elimination linear regression model. The following predictors were considered for inclusion:

**Initial Predictors**

- Age
- Sex
- Living alone
- CAD intervention
- VO₂ peak fraction
- Antidepressant use
- History of a depressive episode
- Obesity
- History of cigarette smoking
- Hypertension
- Diabetes mellitus
- Dyslipidemia
- Cognition (MMSE)

Collinear predictors were excluded from the final model. Predictors below 5% significance were omitted from the model until all were significant predictors of depressive symptoms.

Relationships between HAM-D symptom cluster scores and significant erythrocyte EPA+DHA to AA ratios were then explored using a backward elimination linear regression as above.
2.4.2 Study 2: ω-3 FA Treatment and Depressive Symptoms in CAD

Study 2 was a longitudinal investigation of the efficacy of 1.9 g/day ω-3 FA supplements for reducing depressive symptoms in CAD patients over 12 weeks compared to placebo. This study used the following patient data from each visit of the CAROTID trial over 12 weeks:

- Patient sociodemographic, medical, medication data
- Baseline and week 12 cardiopulmonary fitness parameters
- The presence of a DSM-IV confirmed depressive episode (baseline)
- Depressive symptom severity (baseline and weeks 4, 8, and 12)
  - HAM-D total score and symptom cluster scores
  - BDI-II total score and symptom cluster scores
- Treatment assignment (ω-3 FA or placebo)
- Baseline EPA+DHA/AA in erythrocyte PI and SM fractions
- Plasma EPA+DHA concentrations from baseline and week 12

An intention-to-treat (ITT) analysis of treatment efficacy was conducted using a repeated measures general linear regression with HAM-D total score (primary outcome) or BDI-II total score (secondary outcome) as dependent variables with 4 observations (baseline, week 4, week 8, and week 12). Covariates were selected based on significant and/or potentially clinically meaningful differences in patient characteristics between the ω-3 FA and placebo groups at baseline.

Exploratory analyses of treatment efficacy on depressive symptoms measured using HAM-D total score, BDI-II total scores, or HAM-D or BDI-II symptom cluster scores were conducted using a univariate general linear model with depressive symptom change scores over 12 weeks as dependent variables. Covariates were selected as per the primary and secondary outcomes.
Exploratory analyses of baseline ω-3 FAs (EPA+DHA to AA ratio in erythrocyte PI and SM fractions as well as total plasma EPA+DHA concentrations) as predictors of ω-3 FA treatment efficacy were conducted in each treatment group using repeated measures general linear regression with HAM-D or BDI-II scores as dependent variables with 4 observations (baseline, week 4, week 8, and week 12) and with either baseline EPA+DHA to AA ratio in erythrocyte PI and SM fractions or baseline total plasma EPA+DHA concentrations as a predictor in addition to the planned covariates age, sex, and antidepressant use which have been previously associated with depressive symptoms in CAD (Swardfager et al., 2008). Predictive associations between baseline ω-3 FAs and changes in HAM-D or BDI-II scores over 12 weeks were also explored using linear regression to provide context for the direction of the association.

**Sample Size Calculation**

A sample size of 80 (40 per group) provides 80% power to detect a 4-5 point difference in HAM-D total score between the ω-3 FA and placebo groups at week 12 at a significance level (α) of 0.05 in a population with a standard deviation in HAM-D score of 6 points. This sample size corresponds with a large effect size (0.8-1.0) between the treatment groups, which is in line with previous RCTs using EPA-enriched ω-3 FA supplements to treat depressive symptoms (Peet and Horrobin, 2002, Nemets et al., 2002). To account for an expected patient dropout rate of 6-7% (Swardfager et al., 2011, Carney et al., 2009), the target same size was inflated to 86 patients.

**2.4.3 Study 3: Lipid Peroxidation and ω-3 FA Antidepressant Efficacy**

Study 3 was a longitudinal investigation of baseline lipid peroxidation as a predictor of 1.9 g/day ω-3 FA treatment efficacy for reducing depressive symptoms in CAD patients over 12 weeks of CR. This study used the following patient data from the baseline and week 12 visits of the CAROTID trial:
• Patient sociodemographic, medical, medication data
• Baseline and week 12 cardiopulmonary fitness parameters
• The presence of a DSM-IV criteria confirmed depressive episode (baseline)
• Depressive symptom severity (baseline and week 12)
  • HAM-D total scores and symptom cluster scores
• Treatment assignment (ω-3 FA or placebo)
• Baseline plasma EPA+DHA concentrations
• Baseline serum concentrations of lipid peroxidation markers (LPH and HNE)

Antidepressant users were excluded due to potential confounding effects on depressive symptoms and baseline concentrations of lipid peroxidation markers (Lee et al., 2013b).

Baseline LPH was assessed as a predictor of depressive symptom change using a repeated measures general linear model with HAM-D total score (primary outcome) and HAM-D symptom cluster scores (exploratory outcomes) as dependent variables with 2 observations (baseline and week 12). Predictive associations between baseline LPH and change in depressive symptom scores over 12 weeks were tested in the total sample and each treatment group separately using the same model. The covariate was planned to be baseline plasma EPA+DHA concentrations due to its previously identified relationship with depressive symptom change in CAD [Study 2]. Predictive associations between baseline LPH and change in depressive symptom scores over 12 weeks were also investigated using linear regression to provide context for the direction and increment of the association. Baseline HNE concentrations were explored as predictors of depressive symptom change over 12 weeks using the same methods.

All study analyses were two-tailed with a significance value of p<.05. Statistical models were computed using SPSS statistical software, version 20.0, Chicago, IL, USA.
Chapter III

Results

3.1 Study 1: EPA+DHA to AA Ratios in Erythrocyte Phospholipids and Depressive Symptoms in CAD

3.1.1 Study 1 Patient Characteristics

Between August 2010 and February 2014, 645 patients were assessed for eligibility and 121 met study criteria at the screening visit. Of those, 76 CAD patients met criteria at the baseline visit and provided erythrocyte ω-3 FA samples [Figure III].

Figure III. Patient Flow through Study 1.
Patients were mostly male (74%), were aged 61.9 (SD 8.5) years, and had an average of 3.1 (SD 1.5) vascular risk factors. Patients on average demonstrated resting heart rate and blood pressure parameters in the normal range. The mean VO$_2$ peak during exercise stress testing was 18.6 (SD 5.5) ml/kg/minute, which corresponds to 73% of age- and sex-adjusted VO$_2$ peak norms. The mean BMI was 28.5 (SD 5.0) kg/m$^2$, with 40% of patients considered obese (BMI ≥ 30 kg/m$^2$). The majority of patients were using medications affecting the cardiovascular system, such as antihypertensive medications (78%) and β-adrenergic receptor blockers (74%). Sixty-two (82%) of the patients were recruited from the UHN Toronto Rehab. Patient sociodemographic and medical characteristics are summarized in Table I.

<table>
<thead>
<tr>
<th>Table I. Study 1 Patient Characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
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<td>-------------------------------------------</td>
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<td><strong>Demographics</strong></td>
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<td>Ethnicity</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td>African American</td>
</tr>
<tr>
<td>Asian</td>
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<td>South Asian</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Marital status/living situation</td>
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<tr>
<td>Married/living with others</td>
</tr>
<tr>
<td>Employment status</td>
</tr>
<tr>
<td>Employed full-time</td>
</tr>
<tr>
<td>Employed part-time</td>
</tr>
<tr>
<td>Not employed/retired</td>
</tr>
<tr>
<td>Smoking history</td>
</tr>
<tr>
<td>Previous smoker</td>
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<tr>
<td>Current smoker</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
</tr>
<tr>
<td>Fish intake, servings/week, mean (SD)</td>
</tr>
<tr>
<td>Annual family income ($) (median)</td>
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</table>
**Cardiovascular Characteristics**

<table>
<thead>
<tr>
<th>CAD event</th>
<th>MI/IHD</th>
<th>PTCA</th>
<th>CABG</th>
<th>Other</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Dyslipidemia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>27</td>
<td>28</td>
<td>18</td>
<td>3</td>
<td>51</td>
<td>21</td>
<td>56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI (kg/m(^2)) (SD)</th>
<th>28.5 (5.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vascular risk factors (SD)</td>
<td>3.1 (1.5)</td>
</tr>
<tr>
<td>Resting systolic BP (mm Hg) (SD)</td>
<td>124.0 (20.5)</td>
</tr>
<tr>
<td>Resting diastolic BP (mm Hg) (SD)</td>
<td>74.0 (11.1)</td>
</tr>
<tr>
<td>Resting HR (beats/minute) (SD)</td>
<td>70.5 (14.1)</td>
</tr>
<tr>
<td>VO(_2) peak (ml/kg/minute) (SD)</td>
<td>18.6 (5.5)</td>
</tr>
<tr>
<td>Fractional VO(_2) peak (%) (SD)</td>
<td>73 (23)</td>
</tr>
</tbody>
</table>

**Concomitant Medications**

<table>
<thead>
<tr>
<th>Medication</th>
<th>Count</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiarrhythmia</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>59</td>
<td>78</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>ASA</td>
<td>66</td>
<td>87</td>
</tr>
<tr>
<td>β-adrenergic receptor blockers</td>
<td>56</td>
<td>74</td>
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<tr>
<td>Calcium channel blockers</td>
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<td>22</td>
</tr>
<tr>
<td>Diuretics</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Platelet inhibitor</td>
<td>53</td>
<td>70</td>
</tr>
<tr>
<td>Statin</td>
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<td>97</td>
</tr>
<tr>
<td>Thyroid replacement</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; MI, myocardial infarction; IHD, ischemic heart disease; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft; BMI, body mass index; BP, blood pressure; HR, heart rate; VO\(_2\), volume of oxygen; fVO\(_2\), VO\(_2\) peak fraction
3.1.2 Study 1 Depressive Symptom Characteristics

Patients were all cognitively healthy (MMSE score >24) and 43% demonstrated depressive symptoms consistent with a major or minor depressive episode (DSM-IV), with a range of HAM-D total scores between 0 and 20. Depressive mood symptoms and sleep and psychic anxiety symptoms contributed the majority of depressive symptom scores. Twenty-eight (37%) patients had a history of a previous depressive episode [Table II]. Only 12% were using an antidepressant and 7% were using anxiolytic medications.

Table II. Study 1 Depressive Symptom and Cognitive Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM-IV depressive episode</td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>26% (n=20)</td>
</tr>
<tr>
<td>Minor</td>
<td>17% (n=13)</td>
</tr>
<tr>
<td>Not depressed</td>
<td>57% (n=43)</td>
</tr>
<tr>
<td>History of depression</td>
<td>37% (n=28)</td>
</tr>
<tr>
<td>HAM-D symptom scores</td>
<td></td>
</tr>
<tr>
<td>HAM-D total score</td>
<td>7.2 (5.9)</td>
</tr>
<tr>
<td>HAM-D mood cluster score</td>
<td>3.4 (3.3)</td>
</tr>
<tr>
<td>HAM-D sleep/anxiety cluster score</td>
<td>2.4 (2.1)</td>
</tr>
<tr>
<td>HAM-D somatic cluster score</td>
<td>1.3 (1.3)</td>
</tr>
<tr>
<td>MMSE total score</td>
<td>28.8 (1.2)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; HAM-D, 17-Item Hamilton Depression Rating Scale; MMSE, Mini-Mental State Examination

3.1.3 Study 1 Outcomes

Primary Outcome

In an unadjusted linear regression analysis, EPA+DHA to AA ratios in the erythrocyte PC fraction were not associated with HAM-D total scores in CAD patients [Table III].

Secondary Outcomes

In unadjusted linear regression analyses, lower EPA+DHA to AA ratios in the erythrocyte PI and SM fractions were significantly associated with greater HAM-D total scores, greater
EPA+DHA to AA ratios in the erythrocyte PS fraction were significantly associated with greater HAM-D total scores, and EPA+DHA to AA ratios in the erythrocyte PE and lyso fractions were not associated with HAM-D total scores in CAD patients [Table III]. After correcting for multiple comparisons, the associations between lower EPA+DHA to AA ratios in the erythrocyte PI and SM fractions and greater HAM-D total scores remained significant.

**Table III.** Correlations between HAM-D Total Score and Erythrocyte EPA+DHA to AA Ratios in Individual Phospholipid Fractions in Study 1.

<table>
<thead>
<tr>
<th>PL Fraction</th>
<th>EPA+DHA/AA Ratio (SD)</th>
<th>Pearson r</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC <em>(primary)</em></td>
<td>0.32 (0.16)</td>
<td>0.03</td>
<td>.81</td>
</tr>
<tr>
<td>PE</td>
<td>0.36 (0.12)</td>
<td>-0.07</td>
<td>.57</td>
</tr>
<tr>
<td>PI</td>
<td>0.23 (0.15)</td>
<td>-0.36</td>
<td>&lt;.01*</td>
</tr>
<tr>
<td>PS</td>
<td>0.34 (0.14)</td>
<td>0.24</td>
<td>.04</td>
</tr>
<tr>
<td>SM</td>
<td>0.93 (0.89)</td>
<td>-0.41</td>
<td>&lt;.01*</td>
</tr>
<tr>
<td>Lyso</td>
<td>0.28 (0.50)</td>
<td>-0.17</td>
<td>.16</td>
</tr>
</tbody>
</table>

Abbreviations: HAM-D, 17-Item Hamilton Depression Rating Scale; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; lyso, lysophospholipid; SD, standard deviation

* Associations survived correction for multiple comparisons

In the final covariate-adjusted model, each unit lower EPA+DHA to AA ratio in the erythrocyte PI fraction was associated with 12.71 points higher on the HAM-D. Each year of younger age was associated with 0.19 points higher on the HAM-D. A history of a previous depressive episode was associated with 5.53 points higher on the HAM-D. Finally, each point lower in MMSE total score was associated with 1.07 points higher on the HAM-D [Table IV]. Collectively, these predictors accounted for 39% of the variance in HAM-D total scores (F=12.92, p<.01).
Table IV. Covariates in Final Linear Regression Model of PI Fraction EPA+DHA to AA Ratios Predicting HAM-D Total Scores in Study 1.

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Coefficients (B)</th>
<th>Standard Errors</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI EPA+DHA to AA ratio</td>
<td>-12.71</td>
<td>3.52</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Age</td>
<td>-0.19</td>
<td>0.07</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>History of depression</td>
<td>5.53</td>
<td>1.13</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>MMSE total score</td>
<td>-1.07</td>
<td>0.45</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: HAM-D, 17-Item Hamilton Depression Rating Scale; PI, phosphatidylinositol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; MMSE, Mini-Mental State Examination; Final model: R=0.65, R²=0.42, adjusted R²=0.39, Standard error of the estimate=4.68, p<.01

In the final covariate-adjusted model, each unit lower EPA+DHA to AA ratio in the erythrocyte SM fraction was associated with 2.52 points higher on the HAM-D. Each year of younger age was associated with 0.22 points higher on the HAM-D. Female sex was associated with 2.04 points higher on the HAM-D. A history of a previous depressive episode was associated with 5.10 points higher on the HAM-D. Finally, each point lower in MMSE total score was associated with 1.10 points higher on the HAM-D [Table V]. Collectively, these predictors accounted for 45% of the variance in HAM-D total scores (F=12.74, p<.01).

Table V. Covariates in Final Linear Regression Model of SM Fraction EPA+DHA to AA Ratios Predicting HAM-D Total Scores in Study 1.

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Coefficients (B)</th>
<th>Standard Errors</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM EPA+DHA to AA ratio</td>
<td>-2.52</td>
<td>0.59</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Age</td>
<td>-0.22</td>
<td>0.07</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Sex</td>
<td>2.04</td>
<td>1.22</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>History of depression</td>
<td>5.10</td>
<td>1.15</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>MMSE total score</td>
<td>-1.10</td>
<td>0.43</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: HAM-D, 17-Item Hamilton Depression Rating Scale; SM, sphingomyelin; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; MMSE, Mini-Mental State Examination; Final model: R=0.70, R²=0.49, adjusted R²=0.45, Standard error of the estimate=4.45, p<.01
**Exploratory Outcomes**

The associations between EPA+DHA to AA ratios in the erythrocyte PI and SM fractions and specific depressive symptom clusters were explored. Each unit decrease in PI EPA+DHA to AA ratio corresponded with 6.43 points higher in HAM-D mood cluster score, 3.46 points higher in HAM-D sleep and psychic anxiety cluster score, and 2.36 points higher in HAM-D somatic cluster score. Each unit decrease in SM EPA+DHA to AA ratio corresponded with 1.24 points higher in HAM-D mood cluster score, 0.70 points higher in HAM-D sleep and psychic anxiety cluster score, and 0.58 points higher in HAM-D somatic cluster score [Table VI].

**Table VI.** PI and SM EPA+DHA to AA Ratios Predict HAM-D Symptom Cluster Scores in Study 1.

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Coefficients (B)</th>
<th>Standard Errors</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI Fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood</td>
<td>-6.43</td>
<td>1.91</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Sleep and psychic anxiety</td>
<td>-3.46</td>
<td>1.40</td>
<td>.02</td>
</tr>
<tr>
<td>Somatic</td>
<td>-2.36</td>
<td>0.88</td>
<td>.01</td>
</tr>
<tr>
<td>SM Fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood</td>
<td>-1.24</td>
<td>0.32</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Sleep and psychic anxiety</td>
<td>-0.70</td>
<td>0.25</td>
<td>.01</td>
</tr>
<tr>
<td>Somatic</td>
<td>-0.58</td>
<td>0.15</td>
<td>&lt;.01</td>
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</tbody>
</table>

Abbreviations: HAM-D, 17-Item Hamilton Depression Rating Scale; PI, phosphatidylinositol; SM, sphingomyelin; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid

**3.1.4 Summary of Study 1**

Greater depressive symptom severity was associated with smaller ratios of EPA+DHA to AA in erythrocyte PI and SM phospholipid fractions, independently of other known predictors such as history of previous depression, younger age, female sex, and poorer global cognitive performance. EPA+DHA to AA ratios in those fractions were associated with each depressive symptom cluster. This study supports the EPA+DHA to AA ratio as a marker of depressive symptoms across a range of severity and symptom types in CAD patients.
3.2 Study 2: ω-3 FA Treatment and Depressive Symptoms in CAD

3.2.1 Study 2 Patient Characteristics

Between August 2010 and February 2014, 645 patients were assessed for study eligibility and 121 were entered into the screening phase. Of those, 86 CAD patients met study criteria at the baseline visit and provided baseline plasma ω-3 FA samples [Figure IV].

![Patient Flow and Treatment Group Randomization in Study 2](image)

**Figure IV.** Patient Flow and Treatment Group Randomization in Study 2.
Seventy-three percent were male and eighty-four percent were living with a partner/spouse. The mean age of patients was 61.8 (SD 8.9) years old. Between the treatment groups at baseline, patients randomized to receive ω-3 FA treatment were older, fewer were using ASA, and a greater number were using antihypertensive medications (trend level) compared to those randomized to receive placebo. A greater number of diabetic patients were randomized to ω-3 FA treatment than placebo and accordingly, a greater number (trend level) of patients in the ω-3 FA group were using antidiabetic agents. Patients randomized to ω-3 FA treatment had lower mean MMSE scores at baseline than those randomized to placebo. Although baseline erythrocyte ω-3 FA fractions were not different between the treatment groups, baseline plasma EPA+DHA concentrations were significantly lower among patients randomized to receive ω-3 FAs. No other between-group patient differences were observed [Table VII].

**Table VII.** Study 2 Baseline Patient Demographic and Clinical Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=86)</th>
<th>ω-3 FA (n=41)</th>
<th>Placebo (n=45)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
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<td></td>
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<tr>
<td>Study site</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>UHN Toronto Rehab</td>
<td>69</td>
<td>33</td>
<td>36</td>
<td>0.03 1 .96</td>
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<td>Trillium Health Partners</td>
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<td>9</td>
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<tr>
<td>Asian</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>15</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Marital status/living situation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/living with others</td>
<td>72</td>
<td>32</td>
<td>40</td>
<td>1.85 1 .17</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed full-time</td>
<td>42</td>
<td>18</td>
<td>24</td>
<td>2.81 3 .42</td>
</tr>
<tr>
<td>Employed part-time</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Not employed/retired</td>
<td>38</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
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</table>
### Smoking History

<table>
<thead>
<tr>
<th></th>
<th>Previous smoker</th>
<th>Current smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>3</td>
</tr>
</tbody>
</table>

### Mean Age (SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous smoker</td>
<td>61.8 (8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>63.9 (9.3)</td>
<td>4.95</td>
<td>1</td>
<td>.03</td>
</tr>
</tbody>
</table>

### Fish Servings/Week (SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous smoker</td>
<td>1.6 (1.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.7 (1.3)</td>
<td>1.17</td>
<td>1</td>
<td>.28</td>
</tr>
</tbody>
</table>

### Median Income ($000)

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (IQR)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous smoker</td>
<td>80 (10–500)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>65 (12–200)</td>
<td>1.22</td>
<td>1</td>
<td>.27</td>
</tr>
</tbody>
</table>

### Cardiovascular Characteristics

<table>
<thead>
<tr>
<th>Event</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI/IHD</td>
<td>30</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>PTCA</td>
<td>32</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>CABG</td>
<td>21</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension</td>
<td>56</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Diabetes</td>
<td>23</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Obese (BMI&gt;30)</td>
<td>30</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>66</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

### Vascular Risk Factors (#) (SD)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean (SD)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting systolic BP (mm Hg)</td>
<td>123.3 (19.8)</td>
<td>1.53</td>
<td>1</td>
<td>.22</td>
</tr>
<tr>
<td>Resting diastolic BP (mm Hg)</td>
<td>73.9 (10.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO\textsubscript{2} peak</td>
<td>18.6 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO\textsubscript{2} peak fraction (%)</td>
<td>73 (23)</td>
<td>0.00</td>
<td>1</td>
<td>.96</td>
</tr>
</tbody>
</table>

### Psychometric Characteristics

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (SD)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>28.8 (1.2)</td>
<td>3.99</td>
<td>1</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>HAM-D\textsubscript{17} total score</td>
<td>7.3 (6.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI-II total score</td>
<td>13.0 (11.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### History of Depression

<table>
<thead>
<tr>
<th>Category</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major depressive episode</td>
<td>21</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Minor depression</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

### Biochemical Characteristics

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (SD)</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma EPA+DHA (μg/ml)</td>
<td>74.0 (31.9)</td>
<td>5.23</td>
<td>1</td>
<td>.03</td>
</tr>
<tr>
<td>Plasma EPA+DHA/AA</td>
<td>0.33 (0.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC PI EPA+DHA/AA</td>
<td>0.22 (0.15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC SM EPA+DHA/AA</td>
<td>0.95 (0.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Concomitant Medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>χ²</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiarrhythmia</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.26</td>
<td>1</td>
<td>.61</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>0.64</td>
<td>1</td>
<td>.43</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>18</td>
<td>12</td>
<td>6</td>
<td>3.29</td>
<td>1</td>
<td>.07</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>72</td>
<td>35</td>
<td>31</td>
<td>3.26</td>
<td>1</td>
<td>.07</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.26</td>
<td>1</td>
<td>.61</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>1.63</td>
<td>1</td>
<td>.20</td>
</tr>
<tr>
<td>ASA</td>
<td>79</td>
<td>31</td>
<td>42</td>
<td>5.25</td>
<td>1</td>
<td>.02</td>
</tr>
<tr>
<td>B-adrenergic receptor blockers</td>
<td>65</td>
<td>29</td>
<td>30</td>
<td>0.17</td>
<td>1</td>
<td>.69</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>0.06</td>
<td>1</td>
<td>.81</td>
</tr>
<tr>
<td>Diuretics</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>0.23</td>
<td>1</td>
<td>.63</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0.32</td>
<td>1</td>
<td>.57</td>
</tr>
<tr>
<td>Platelet inhibitor</td>
<td>65</td>
<td>29</td>
<td>31</td>
<td>0.04</td>
<td>1</td>
<td>.85</td>
</tr>
<tr>
<td>Statin</td>
<td>90</td>
<td>39</td>
<td>45</td>
<td>2.25</td>
<td>1</td>
<td>.13</td>
</tr>
<tr>
<td>Thyroid replacement</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>1.12</td>
<td>1</td>
<td>.29</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>0.27</td>
<td>1</td>
<td>.60</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>11</td>
<td>3</td>
<td>8</td>
<td>2.11</td>
<td>1</td>
<td>.15</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; df, degrees of freedom; ACS, acute coronary syndrome; MI, myocardial infarction; IHD, ischemic heart disease; PTCA, percutaneous transluminal coronary angioplasty; CAGB, coronary artery bypass graft; BMI, body mass index; BP, blood pressure; VO₂, volume of oxygen; MMSE, Mini-Mental Sate Examination; HAMD, 17-Item Hamilton Depression Rating Scale; BDI-II, Beck Depression Inventory II; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cell (erythrocyte); PI, phosphatidylinositol; SM, sphingomyelin; ASA, acetylsalicylic acid.

*a Group differences determined using an independent samples median test

### 3.2.2 Study 2 Depressive Symptom Characteristics

Of the eighty-six patients, twenty-one (24%) met DSM-IV criteria for a major depressive episode and an additional sixteen (19%) reported symptoms consistent with minor depression. Of all patients, 39% reported experiencing a previous depressive episode. Only 14% of patients were using an antidepressant medication at baseline. The proportion of depressed patients, severity of depressive symptoms (measured using the HAM-D and the BDI-II), and history of prior depression were not different between groups at baseline [Table VII].

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3.2.3 Study 2 Compliance and Trial Completion

Seventy-seven of the eighty-six (90%) randomized patients completed the trial. The dropout rate in the ω-3 FA treatment arm (n=5) did not differ from that in the placebo arm (n=4) ($\chi^2=0.25$, p=.62). Adverse event reporting was not different between treatment groups at any of the 3 follow-up visits [Appendix C]. The most commonly reported adverse symptoms were general pain, nasopharyngitis, and fatigue; however, none of the study dropouts cited those symptoms as the reason for withdrawal. Of the nine study dropouts, two began using an antidepressant during the study (one from each treatment group), three dropped out of CR, one was excluded due to a PTCA procedure shortly after randomization, one was excluded due to recent use of a recreational drug, one no longer wished to participate in the study due to time constraints, and one placebo user discontinued the study medication due to frequent eructation.

Of the study completers, sixty-three (73%) demonstrated adequate compliance (at least 80% of allocated study capsules) as determined by capsule counts at each visit. Compliance was not different between treatment groups ($\chi^2=0.29$, p=.59).

3.2.4 Study 2 Outcomes

Over 12 weeks in the total sample, a trending decrease of -1.1 ± 4.6 points in HAM-D total scores (primary outcome) was observed ($t_{85}=1.89$, p=.06) and HAM-D total scores changed significantly within subjects ($F_{3,85}=4.81$, p=.03), ranging from a decrease of -11 points to an increase of 10 points. BDI-II total scores (secondary outcome) significantly decreased by -2.5 ± 8.0 points ($t_{85}=3.09$, p<.01) and changed significantly within subjects over 12 weeks ($F_{3,85}=11.83$, p<.01), ranging from a decrease of -23 points to an increase of 19 points.

ω-3 FA treatment was associated with a significant increase in plasma concentrations of EPA ($F_{1.29}=33.29$, p<.01) and DHA ($F_{1.29}=15.29$, p<.01). ω-3 FA treatment was not related to
changes in VO₂ peak fraction over 12 weeks (treatment X time interaction: F₁,₈₅=1.14, p=.29). Changes in VO₂ peak fraction over 12 weeks of CR were not correlated with changes in HAM-D total score (n=86, R=-0.03, p=.82) or BDI-II total score (n=86, R=-0.08, p=.53).

**Primary Outcome**

There was no statistically significant difference in HAM-D total scores between the ω-3 FA treatment group and placebo group over 12 weeks of CR (treatment X time interaction: F₃,₈₅=0.72, p=.40)² [Table VIII] [Figure V].

![Course of HAM-D Scores Over 12 Weeks by Treatment Group](image)

**Figure V.** The Course of Depressive Symptoms Measured by HAM-D Total Scores over 12 Weeks of CR in ω-3 FA Treated and Placebo Treated Patients in Study 2. The green line represents the ω-3 FA treated group and the blue line represents the placebo treated group. HAM-D scores in this figure (y axis) are estimates adjusted for the included covariates².

---

¹,² Determined using a repeated measures general linear regression model. Covariates included: age, diabetes mellitus, baseline MMSE total score, ACE inhibitor use, ASA use, and baseline plasma EPA+DHA concentrations as those characteristics were different between treatment groups at baseline.
Secondary Outcome

There was no statistically significant difference in BDI-II total scores between the ω-3 FA treatment group and placebo group over 12 weeks of CR (treatment X time interaction: F_{3,85}=0.52, p=.47) \[^3\] [Table VIII] [Figure VI].

![Course of BDI-II Scores Over 12 Weeks by Treatment Group](image)

**Figure VI.** The Course of Depressive Symptoms Measured by BDI-II Total Scores over 12 Weeks of CR in ω-3 FA Treated and Placebo Treated Patients in Study 2. The green line represents the ω-3 FA treated group and the blue line represents the placebo treated group. BDI-II scores in this figure (y axis) are estimates adjusted for the included covariates\(^3\).

\(^3\) Determined using a repeated measures general linear regression model. Covariates included: age, diabetes mellitus, baseline MMSE total score, ACE inhibitor use, ASA use, and baseline plasma EPA+DHA concentrations as those characteristics were different between treatment groups at baseline.
**Exploratory Outcomes**

*Treatment Efficacy for Depressive Symptom Clusters*

There were no statistically significant differences in HAM-D total, BDI-II total, or depressive symptom cluster scores between the ω-3 FA treatment group and placebo group over 12 weeks of CR when pre- and post-trial change scores were compared [Table VIII]. The trending reduction of symptoms in the HAM-D sleep and psychic anxiety cluster observed in the placebo group compared to the ω-3 FA treatment group was most likely due to greater symptom severity among placebo users at baseline. Accordingly, mean symptom scores in that cluster were not different between groups at week 12 ($F_{1,85}=0.30$, $p=.59$).

**Table VIII.** Primary, Secondary, and Exploratory Outcomes in Study 2 ITT Analysis.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model Parameter of Interest</th>
<th>Adjusted Mean Group Difference (SE)</th>
<th>F Statistic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM-D total(^a)</td>
<td>Treatment X time</td>
<td>0.37 (1.31)(^c)</td>
<td>0.72</td>
<td>.40</td>
</tr>
<tr>
<td>Secondary Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI-II total(^a)</td>
<td>Treatment X time</td>
<td>0.27 (2.40)(^c)</td>
<td>0.52</td>
<td>.47</td>
</tr>
<tr>
<td>Exploratory Outcomes(^b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM-D total pre-post</td>
<td>Treatment group</td>
<td>1.07 (1.11)(^d)</td>
<td>0.98</td>
<td>.33</td>
</tr>
<tr>
<td>HAM-D mood</td>
<td>Treatment group</td>
<td>0.82 (0.68)(^d)</td>
<td>1.64</td>
<td>.20</td>
</tr>
<tr>
<td>HAM-D sleep/anxiety</td>
<td>Treatment group</td>
<td>0.75 (0.46)(^d)</td>
<td>3.11</td>
<td>.08</td>
</tr>
<tr>
<td>HAM-D somatic</td>
<td>Treatment group</td>
<td>-0.25 (0.30)(^d)</td>
<td>0.89</td>
<td>.35</td>
</tr>
<tr>
<td>BDI-II total pre-post</td>
<td>Treatment group</td>
<td>0.39 (1.68)(^d)</td>
<td>0.17</td>
<td>.68</td>
</tr>
<tr>
<td>BDI-II cognitive</td>
<td>Treatment group</td>
<td>0.19 (0.93)(^d)</td>
<td>0.17</td>
<td>.68</td>
</tr>
<tr>
<td>BDI-II somatic</td>
<td>Treatment group</td>
<td>-0.57 (0.88)(^d)</td>
<td>0.45</td>
<td>.50</td>
</tr>
</tbody>
</table>

*a: Within-subjects repeated measures linear regression using baseline, week 4, week 8, and week 12 as time points
b: Within-subjects repeated measures linear regression using baseline and week 12 as time points
c: Covariate-adjusted mean difference between treatment groups at week 12 (ω-3 FA – Placebo)
d: Covariate-adjusted mean difference in change over 12 weeks between treatment groups
**ω-3 FAs as a Predictor of Depressive Symptom Changes**

Higher baseline plasma EPA+DHA concentrations significantly predicted reductions in HAM-D total scores over 12 weeks of CR in the total sample ($F_{1,85}=5.43$, $B=-0.04$, SE=0.02, $p=.02$) [Figure VII] when adjusting for age, sex, and antidepressant use at baseline.⁴

**Figure VII.** Association between Baseline Plasma EPA+DHA Concentrations and Changes in Depressive Symptoms over 12 weeks of CR in all Study 2 Patients. Depressive symptoms were measured using HAM-D total scores. Summary statistics: $n=86$, $R^2=0.07$, $R=-0.27$, $p=.02$.

Younger age also significantly predicted reductions in HAM-D total scores in that model ($F_{1,85}=6.00$, $B=0.13$, SE=0.02, $p=.01$). Higher baseline plasma EPA+DHA concentrations appeared to be most strongly associated with reductions in depressive symptoms from the HAM-D somatic cluster ($F_{1,85}=7.69$, $B=-0.01$, SE<0.01, $p<.01$) compared to symptoms from the

---

⁴ Age, sex, and antidepressant use were chosen as covariates due to previously documented relationships with depressive symptoms and depressive symptom change over time (Materials and Methods, Section 2.4.2).
HAM-D mood ($F_{1,85}=1.91, p=.17$) or HAM-D sleep and psychic anxiety ($F_{1,85}=2.52, p=.12$) clusters. The associations between baseline plasma EPA+DHA concentrations and changes in HAM-D scores were not particular to either treatment group. Baseline plasma EPA+DHA to AA ratios did not predict changes in HAM-D total scores over 12 weeks in the total group ($F_{1,85}=1.91, p=.23$) or in either treatment group.

Baseline erythrocyte EPA+DHA to AA ratios in the PI ($F_{1,75}=0.81, p=.37$) and SM ($F_{1,75}=1.95, p=.17$) fractions did not predict changes in HAM-D total scores over 12 weeks in the total sample or in either treatment group. Similar results for both plasma and erythrocyte markers were observed for depressive symptoms measured by the BDI-II.

**Subgroup Analysis**

Patients were subgrouped into those who met DSM-IV criteria for the presence of a major or minor depressive episode at baseline and those who did not. Whether or not a patient met DSM-IV criteria for a major or minor depressive episode at baseline did not influence treatment efficacy over 12 weeks of CR (treatment X depressive episode interaction: $F_{3,85}=1.08, p=.30$). Efficacy was not detected for any of the depressive symptom clusters in either subgroup [Table IX].
### Table IX. Study 2 Subgroup ITT Analysis: Treatment Efficacy by Baseline Depression Status.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Model Parameter of Interest</th>
<th>Adjusted Mean (SE)</th>
<th>Group Differencea</th>
<th>F Statistic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met DSM-IV Depressive Episode Criteria (n=37)⁵</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM-D total</td>
<td>Treatment group</td>
<td>1.86 (1.85)</td>
<td>1.03</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>HAM-D mood</td>
<td>Treatment group</td>
<td>-0.28 (0.79)</td>
<td>2.60</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>HAM-D sleep/anxiety</td>
<td>Treatment group</td>
<td>0.74 (0.77)</td>
<td>1.01</td>
<td>.32</td>
<td></td>
</tr>
<tr>
<td>HAM-D somatic</td>
<td>Treatment group</td>
<td>-0.39 (0.52)</td>
<td>0.71</td>
<td>.41</td>
<td></td>
</tr>
<tr>
<td>BDI-II total</td>
<td>Treatment group</td>
<td>1.79 (2.71)</td>
<td>0.54</td>
<td>.47</td>
<td></td>
</tr>
<tr>
<td>BDI-II cognitive</td>
<td>Treatment group</td>
<td>1.39 (1.66)</td>
<td>0.79</td>
<td>.38</td>
<td></td>
</tr>
<tr>
<td>BDI-II somatic</td>
<td>Treatment group</td>
<td>0.29 (1.42)</td>
<td>0.05</td>
<td>.82</td>
<td></td>
</tr>
<tr>
<td>Did Not Meet DSM-IV Depressive Episode Criteria (n=49)⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM-D total</td>
<td>Treatment group</td>
<td>0.36 (1.16)</td>
<td>0.15</td>
<td>.70</td>
<td></td>
</tr>
<tr>
<td>HAM-D mood</td>
<td>Treatment group</td>
<td>0.42 (0.76)</td>
<td>0.37</td>
<td>.55</td>
<td></td>
</tr>
<tr>
<td>HAM-D sleep/anxiety</td>
<td>Treatment group</td>
<td>0.45 (0.48)</td>
<td>1.06</td>
<td>.31</td>
<td></td>
</tr>
<tr>
<td>HAM-D somatic</td>
<td>Treatment group</td>
<td>-0.09 (0.27)</td>
<td>0.17</td>
<td>.68</td>
<td></td>
</tr>
<tr>
<td>BDI-II total</td>
<td>Treatment group</td>
<td>-0.13 (1.69)</td>
<td>0.02</td>
<td>.89</td>
<td></td>
</tr>
<tr>
<td>BDI-II cognitive</td>
<td>Treatment group</td>
<td>0.40 (0.94)</td>
<td>0.24</td>
<td>.63</td>
<td></td>
</tr>
<tr>
<td>BDI-II somatic</td>
<td>Treatment group</td>
<td>-0.17 (1.00)</td>
<td>0.04</td>
<td>.84</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HAM-D, 17-Item Hamilton Depression Rating Scale; BDI-II, Beck Depression Inventory II. a positive mean differences reflect higher scores in ω-3 FA treatment group.

Excluding antidepressant users from the analyses did not reveal any efficacy of ω-3 FA treatment on depressive symptoms. In a subgroup of patients for whom treatment compliance data were available (n=81), including percentage compliance as a covariate did not reveal ω-3 FA treatment efficacy (treatment X time interaction: \( F_{3,85} = 0.62, p = .43 \)) for any depressive symptom measure and percentage compliance was not a significant predictor of changes in depressive symptoms (\( F_{3,85} = 1.36, p = .26 \)). Changes in the plasma EPA+DHA to AA ratio with ω-3 FA treatment did not correlate with changes in HAM-D total score (\( r = -0.14, p = .47 \)).

---

⁵ Determined using a repeated measures general linear regression model. Covariates included: baseline plasma EPA+DHA concentrations.

⁶ Determined using a repeated measures general linear regression model. Covariates included: age, diabetes mellitus, baseline MMSE total score, ACE inhibitor use, and baseline plasma EPA+DHA concentrations.
3.2.5 *Post-Hoc* Power Calculation for Study 2 Primary Outcome

The unadjusted between-group difference in HAM-D total score at week 12 was 0.84 (SD 1.35). Using a significance level ($\alpha$) of 0.05 and a sample of 86 patients, this study could detect the between group difference in HAM-D total score at week 12 with 11.9% power. A sample size of 924 patients (462 in each treatment group) would be required to detect that between-group difference with 80% power and address the primary hypothesis appropriately.

The National Institute for Clinical Excellence guidelines for the management of depression specify a clinically significant effect size to be 0.5 or greater (National Institute for Clinical Excellence, 2004). This corresponds with a 2-3 point mean difference on the HAM-D. This is comparable to the stringency defined in previous antidepressant trials in CAD patients (Frasure-Smith et al., 2006, Strik et al., 2000, van den Brink et al., 2002) and meta-analytic data on $\omega$-3 FA treatment of patients with major depression show a moderate effect size of 0.61 (95% CI 0.21-1.01) (Lin and Su, 2007) supporting the feasibility of this endpoint. In the present study, the observed between-group difference in HAM-D total score at week 12 corresponds with a small effect size (0.17) (Cohen, 1988). As such, even an appropriately powered study would have likely found a small effect size for treatment efficacy. Therefore, despite the low power, this study provides evidence that the potential treatment efficacy of 1.9 g/day EPA-enriched $\omega$-3 FA supplements for a range of depressive symptoms in CAD may not be of clinical significance.

3.2.6 Summary of Study 2

$\omega$-3 FA treatment increased plasma EPA and DHA concentrations over 12 weeks; however, it did not result in lower depressive symptom severity over 12 weeks compared to placebo when measured using either the HAM-D (primary outcome) or BDI-II (secondary outcome). No treatment efficacy was observed for the other HAM-D or BDI-II depressive symptom clusters (exploratory outcomes). No treatment efficacy was observed in the subgroup of patients who
met DSM-IV criteria for a major or minor depressive episode at baseline or in the subgroup of patients who did not meet DSM-IV depressive episode criteria at baseline (subgroup analysis).

In all patients, higher baseline plasma EPA+DHA concentrations significantly predicted reduction in depressive symptom severity over 12 weeks of CR when measured using either the HAM-D or BDI-II total scores.
3.3 Study 3: Lipid Peroxidation and ω-3 FA Antidepressant Efficacy

3.3.1 Study 3 Patient Characteristics

Between August 2010 and February 2014, 645 patients were assessed for study eligibility, 121 patients were entered into the screening phase, and 86 patients met study criteria at the baseline visit and were randomized. Of those, 62 patients provided baseline serum lipid peroxidation samples and were not using an antidepressant\(^7\) [Figure VIII].

![Figure VIII. Patient Flow through Study 3.](image)

\(^7\) Antidepressant users were excluded due to potentially confounding effects on lipid peroxidation status and/or changes in depressive symptoms over 12 weeks.
All patient characteristics and between-group comparisons are listed in Table X. Forty-six (74%) of the sixty-two patients were male and fifty-four (87%) were living with a partner/spouse. The mean age of patients was 61.4 (SD 8.3) years old. Between the treatment groups at baseline, patients randomized to receive ω-3 FA treatment were older and fewer were using ASA. A greater number of diabetic patients (trend level) were randomized to ω-3 FA treatment than placebo; however, no difference in the use of anti-diabetic agents was observed between groups. Baseline MMSE scores were lower (trend level) among patients randomized to receive ω-3 FAs compared to placebo, although all patients were cognitive healthy (MMSE > 24). Baseline plasma EPA+DHA concentrations were significantly lower among patients randomized to receive ω-3 FAs. No other between-group differences in patient characteristics were observed.

3.3.2 Study 3 Depressive Symptom Characteristics

Of the sixty-two patients, twelve (19%) met DSM-IV criteria for a major depressive episode, an additional twelve (19%) reported symptoms consistent with minor depression, and the remaining thirty-eight (61%) did not report significant depressive symptoms. Of all patients, twenty-one (34%) reported experiencing a previous depressive episode. The proportion of depressed patients, severity of depressive symptoms (HAM-D), and history of prior depression were not different between groups at baseline [Table X].
Table X. Study 3 Baseline Patient Demographic and Clinical Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=62)</th>
<th>ω-3 FA (n=26)</th>
<th>Placebo (n=36)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>21</td>
<td>25</td>
<td>1.01 1 .32</td>
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<td>Ethnicity</td>
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<td>2.62 4 .62</td>
</tr>
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<td>African American</td>
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<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>11</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Marital status/living situation</td>
<td></td>
<td></td>
<td></td>
<td>1.60 1 .21</td>
</tr>
<tr>
<td>Married/living with others</td>
<td>54</td>
<td>21</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed full-time</td>
<td>30</td>
<td>9</td>
<td>21</td>
<td>3.54 3 .32</td>
</tr>
<tr>
<td>Employed part-time</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Not employed/retired</td>
<td>26</td>
<td>14</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td>1.95 2 .38</td>
</tr>
<tr>
<td>Previous smoker</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>61.4 (8.3)</td>
<td>64.4 (8.5)</td>
<td>59.2 (7.7)</td>
<td>6.44 1 .01</td>
</tr>
<tr>
<td>Fish servings/week (SD)</td>
<td>1.4 (1.0)</td>
<td>1.6 (1.1)</td>
<td>1.4 (1.0)</td>
<td>0.53 1 .47</td>
</tr>
<tr>
<td>Median family income(^a) ($ 000)</td>
<td>80 (10–500)</td>
<td>65 (12–200)</td>
<td>80 (10–500)</td>
<td>1.22 1 .27</td>
</tr>
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<td><strong>Cardiovascular Characteristics</strong></td>
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<td></td>
<td></td>
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<tr>
<td>CAD event</td>
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<td></td>
<td></td>
<td>2.80 3 .42</td>
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<tr>
<td>MI/IHD</td>
<td>22</td>
<td>7</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>PTCA</td>
<td>21</td>
<td>9</td>
<td>12</td>
<td></td>
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<td>CABG</td>
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<td>9</td>
<td>9</td>
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<td>Other</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>39</td>
<td>16</td>
<td>23</td>
<td>0.04 1 .85</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td>3.75 1 .05</td>
</tr>
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<td>Obese (BMI&gt;30)</td>
<td>19</td>
<td>7</td>
<td>12</td>
<td>0.29 1 .59</td>
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<td>Dyslipidemia</td>
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<td>22</td>
<td>27</td>
<td>0.84 1 .36</td>
</tr>
<tr>
<td>Vascular risk factors (#) (SD)</td>
<td>2.9 (1.3)</td>
<td>3.1 (1.3)</td>
<td>2.8 (1.4)</td>
<td>0.89 1 .35</td>
</tr>
<tr>
<td>Fractional VO(_2) peak (%)</td>
<td>75 (22)</td>
<td>76 (16)</td>
<td>74 (25)</td>
<td>0.14 1 .71</td>
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</tbody>
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### Psychometric Characteristics

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<td>MMSE</td>
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<td>.05</td>
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<td>HAM-D&lt;sub&gt;17&lt;/sub&gt; total score</td>
<td>0.00</td>
<td>1</td>
<td>.98</td>
</tr>
<tr>
<td>HAM-D&lt;sub&gt;17&lt;/sub&gt; mood score</td>
<td>0.02</td>
<td>1</td>
<td>.90</td>
</tr>
<tr>
<td>HAM-D&lt;sub&gt;17&lt;/sub&gt; sleep/anxiety score</td>
<td>0.01</td>
<td>1</td>
<td>.93</td>
</tr>
<tr>
<td>HAM-D&lt;sub&gt;17&lt;/sub&gt; somatic score</td>
<td>0.47</td>
<td>1</td>
<td>.50</td>
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<th>P</th>
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<td>History of depression</td>
<td>0.01</td>
<td>1</td>
<td>.92</td>
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<tr>
<td>Met DSM-IV/V criteria</td>
<td>0.02</td>
<td>2</td>
<td>.99</td>
</tr>
<tr>
<td>Major depressive episode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor depression</td>
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### Biochemical Characteristics

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<tr>
<td>Plasma EPA+DHA (μg/ml)</td>
<td>5.15</td>
<td>1</td>
<td>.03</td>
</tr>
<tr>
<td>Serum LPH (μmol)</td>
<td>0.56</td>
<td>1</td>
<td>.46</td>
</tr>
<tr>
<td>Serum HNE (finol/μg)</td>
<td>0.01</td>
<td>1</td>
<td>.95</td>
</tr>
</tbody>
</table>

### Concomitant Medications

<table>
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</thead>
<tbody>
<tr>
<td>Antiarrhythmia</td>
<td>1.49</td>
<td>1</td>
<td>.22</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>2.60</td>
<td>1</td>
<td>.11</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>2.60</td>
<td>1</td>
<td>.11</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>1.51</td>
<td>1</td>
<td>.22</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>0.10</td>
<td>1</td>
<td>.76</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>1.08</td>
<td>1</td>
<td>.30</td>
</tr>
<tr>
<td>ASA</td>
<td>7.83</td>
<td>1</td>
<td>.01</td>
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<tr>
<td>B-adrenergic receptor blockers</td>
<td>1.01</td>
<td>1</td>
<td>.32</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>0.00</td>
<td>1</td>
<td>.98</td>
</tr>
<tr>
<td>Diuretics</td>
<td>0.01</td>
<td>1</td>
<td>.93</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>0.79</td>
<td>1</td>
<td>.37</td>
</tr>
<tr>
<td>Platelet inhibitor</td>
<td>0.42</td>
<td>1</td>
<td>.52</td>
</tr>
<tr>
<td>Statin</td>
<td>1.41</td>
<td>1</td>
<td>.24</td>
</tr>
<tr>
<td>Thyroid inhibitor</td>
<td>0.20</td>
<td>1</td>
<td>.65</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.11</td>
<td>1</td>
<td>.74</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1.08</td>
<td>1</td>
<td>.30</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; df, degrees of freedom; MI, myocardial infarction; IHD, ischemic heart disease; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft; BMI, body mass index; BP, blood pressure; $\text{VO}_2$, volume of oxygen; MMSE, Mini-Mental State Examination; HAMD, 17-Item Hamilton Depression Rating Scale; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cell (erythrocyte); PI, phosphatidylinositol; SM, sphingomyelin; LPH, lipid hydroperoxides; HNE, 4-hydroxynonenal; ASA, acetylsalicylic acid

<sup>a</sup> Group differences determined using an independent samples median test
3.3.3 Study 3 Outcomes

Over 12 weeks in the total sample, HAM-D total scores significantly decreased by -1.73 (SD 4.76) points (t_{61}=2.93, p<.01) and changed significantly within subjects (F_{1,61}=8.56, p=.01), ranging from a decrease of -11 points to an increase of 10 points. Changes in HAM-D total scores over 12 weeks were not correlated with changes in VO₂ peak fraction (n=62, R=0.03, p=.85). At least 80% treatment compliance was observed in 88% of the ω-3 FA treated patients.

LPH and HNE as Predictors in the Total Sample

Baseline serum LPH (F_{1,61}=0.56, p=.46) and HNE (F_{1,61}=0.01, p=.95) concentrations were not different between treatment groups [Table X]. Higher baseline serum LPH concentrations significantly predicted worsening HAM-D total scores over 12 weeks in the total sample (F_{1,61}=7.15, p=.01) independently of the significant predictive effect of lower baseline plasma EPA+DHA concentrations (F_{1,61}=5.04, p=.03)\(^8\). Baseline serum HNE concentrations did not predict HAM-D total score trajectory over 12 weeks (F_{1,61}=1.99, p=.16).

Primary Outcome: Baseline LPH and ω-3 FA Antidepressant Treatment Efficacy

There was no significant treatment X LPH interaction on changes in HAM-D total scores over 12 weeks (F_{1,61}=0.25, p=.62). This relationship was then investigated in each treatment group.

ω-3 FA Group (outcome of interest): Higher baseline serum LPH concentrations significantly predicted worsening of HAM-D total scores over 12 weeks (F_{1,25}=7.55, p=.01) [Figure IX] after adjusting for the trending association between lower baseline plasma EPA+DHA concentrations and worsening HAM-D total scores over 12 weeks (F_{1,25}=3.43, p=.08).

---

\(^8\) Determined using a repeated measures general linear regression model. Covariates were baseline serum LPH concentrations and baseline plasma EPA+DHA concentrations.
Placebo Group: Baseline LPH concentrations did not predict changes in HAM-D total scores over 12 weeks ($F_{1,35}=1.85$, $p=.18$) after adjusting for the potential influence of baseline plasma EPA+DHA concentrations ($F_{1,35}=1.50$, $p=.23$).

**Figure IX.** Baseline LPH Concentrations and Changes in Depressive Symptoms in $\omega$-3 FA Treated Patients in Study 3. In the $\omega$-3 FA group (n=26), higher baseline serum LPH concentrations were associated with worsening HAM-D total scores over 12 weeks (unadjusted: $R^2=0.38$, $R=0.61$, $p<.01$; adjusted: $R^2=0.39$, $B=0.12$, $p=.02$)

*Secondary Outcome: Baseline HNE and $\omega$-3 FA Antidepressant Treatment Efficacy*

There was no significant treatment X LPH interaction on changes in HAM-D total scores over 12 weeks ($F_{1,61}=1.41$, $p=.24$). This relationship was then investigated in each treatment group.

$\omega$-3 FA Group (outcome of interest): Baseline serum HNE concentrations did not predict HAM-D total score trajectory over 12 weeks in the $\omega$-3 FA treated group ($F_{1,25}=0.91$, $p=.35$).

Placebo Group: Baseline serum HNE concentrations did not predict HAM-D total score trajectory over 12 weeks in the placebo group ($F_{1,35}=1.72$, $p=.20$).
**Exploratory Outcomes**

The association between baseline serum LPH concentrations and HAM-D score trajectory was explored by depressive symptom cluster. Higher baseline LPH concentrations specifically predicted worsening of HAM-D mood symptoms in the ω-3 FA treatment group and did not predict changes in the other symptom clusters. Baseline LPH concentrations did not predict changes in any of the HAM-D symptom clusters in the placebo group [Table XI].

**Table XI.** Higher Baseline LPH Concentrations Predict Worsening HAM-D Scores over 12 weeks in ω-3 FA Treated Patients in Study 3.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>ω-3 FA</th>
<th></th>
<th>Placebo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,25}$</td>
<td>$P$ Value</td>
<td>$F_{1,25}$</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>HAM-D total</td>
<td>7.55</td>
<td>.01</td>
<td>1.85</td>
<td>.18</td>
</tr>
<tr>
<td>HAM-D mood</td>
<td>9.20</td>
<td>&lt;.01</td>
<td>1.94</td>
<td>.17</td>
</tr>
<tr>
<td>HAM-D sleep/anxiety</td>
<td>0.00</td>
<td>.96</td>
<td>0.28</td>
<td>.60</td>
</tr>
<tr>
<td>HAM-D somatic</td>
<td>2.27</td>
<td>.15</td>
<td>2.79</td>
<td>.10</td>
</tr>
</tbody>
</table>

Note: Repeated measures general linear regression models were used. Each model adjusted for the influence of baseline plasma EPA+DHA concentrations on HAM-D scores.

Baseline HNE concentrations did not predict changes in any of the HAM-D symptom clusters in either treatment group [Table XII].

**Table XII.** Higher Baseline HNE Concentrations Do Not Predict Worsening HAM-D Scores over 12 weeks in ω-3 FA Treated Patients in Study 3.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>ω-3 FA</th>
<th></th>
<th>Placebo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,25}$</td>
<td>$P$ Value</td>
<td>$F_{1,25}$</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>HAM-D total</td>
<td>0.91</td>
<td>.35</td>
<td>1.72</td>
<td>.20</td>
</tr>
<tr>
<td>HAM-D mood</td>
<td>0.08</td>
<td>.78</td>
<td>0.49</td>
<td>.49</td>
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<tr>
<td>HAM-D sleep/anxiety</td>
<td>0.57</td>
<td>.46</td>
<td>2.28</td>
<td>.14</td>
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<tr>
<td>HAM-D somatic</td>
<td>1.29</td>
<td>.27</td>
<td>1.13</td>
<td>.30</td>
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</tbody>
</table>

Note: Repeated measures general linear regression models were used. Each model adjusted for the influence of baseline plasma EPA+DHA concentrations on HAM-D scores.

The relationships between higher baseline LPH concentrations and worsening HAM-D total scores and HAM-D mood symptom cluster scores appeared to be strongest in a subgroup of ω-3 FA treated patients who met DSM-IV depression criteria at baseline (n=11; HAM-D total: 78
$R^2=0.49$, $R=0.70$, $p=.02$; HAM-D mood: $R^2=0.43$, $R=0.66$, $p=.03$) compared subgroup of patients who did not meet depression criteria at baseline (n=15; HAM-D total: $R^2=0.16$, $R=0.40$, $p=.14$; HAM-D mood: $R^2=0.24$, $R=0.49$, $p=.07$). Subgrouping by the presence of a depressive episode at baseline did not reveal any other relationships between baseline LPH concentrations and HAM-D symptom clusters.

### 3.3.4 Summary of Study 3

Higher baseline serum LPH concentrations significantly predicted worsening HAM-D total scores over 12 weeks in all patients. This association appeared to be driven by a significant predictive effect in the $\omega$-3 FA treated group specifically: although baseline serum LPH concentrations were not different between the treatment groups, higher baseline serum LPH concentrations significantly predicted worsening of HAM-D scores over 12 weeks in the $\omega$-3 FA treatment group and not in the placebo group. The predictive effect of higher baseline serum LPH concentrations on worsening HAM-D scores in $\omega$-3 FA treated patients remained significant after adjusting for the predictive effect of lower baseline plasma EPA+DHA concentrations on worsening HAM-D scores in that group. Baseline serum LPH concentrations appeared to be most strongly related to changes in depressive mood symptoms. The relationship also appeared to be stronger among $\omega$-3 FA treated patients who met DSM-IV criteria for a major or minor depressive episode at baseline than in those who did not. No relationships between baseline HNE concentrations and HAM-D score trajectory were observed in either treatment group.
Chapter IV
Discussion of Findings and Recommendations for Future Studies

4.1 Study Findings and Interpretation

The patients in each study were representative of the CAD population participating in CR with respect to mean age, percentage male, the presence and severity of depressive symptoms, and the types of medications used (Alter et al., 2014, Swardfager et al., 2011).

In a cross-sectional analysis of CAD patients at entry to CR, lower EPA+DHA to AA ratios in erythrocyte PI and SM fractions were associated with greater depressive symptom severity [Study 1]. This is consistent with meta-analytic data showing lower total plasma concentrations and erythrocyte ω-3 FA fractions in depressed patients without CAD (Lin et al., 2010). It is also consistent with previously reported associations between depressive symptoms and lower ω-3 FA to ω-6 FA ratios, whether measured by ratios of total ω-3 FAs, EPA, or EPA+DHA relative to AA or total ω-6 FAs (Adams et al., 1996, Maes et al., 1996, Kiecolt-Glaser et al., 2007, Liu et al., 2013, Conklin et al., 2007). Furthermore, lower ω-3 FA to ω-6 FA ratios have previously been observed in CAD patients (Chang et al., 2015, Vollmer-Conna et al., 2015, Schins et al., 2007), in whom lower ω-3 FA to ω-6 FA ratios have been implicated as part of a biological profile of depressive symptoms including elevated CRP, poor sleep quality, and reduced vitamin D concentrations (Vollmer-Conna et al., 2015).

The balance between EPA+DHA and AA suggests a balance between EPA- and DHA-metabolized anti-inflammatory eicosanoids and AA-metabolized pro-inflammatory eicosanoids, which may indicate their respective availabilities to cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes which are implicated in inflammatory cascades. Accordingly, lower EPA+DHA to AA ratios prior to interferon α treatment for hepatitis C have
been shown to predict the onset of depressive symptoms (Lotrich et al., 2013). Lower EPA+DHA to AA ratios were associated with greater IL-6 concentrations in that study, pointing to EPA and DHA as anti-inflammatory mediators that may protect against inflammation-related depressive symptoms, which may be relevant in CAD.

The roles of EPA and DHA in mitigating depressive symptoms may be different. Evidence suggests that metabolism of EPA and its downstream effectors is primarily peripheral, and that very little EPA is transported to the brain (Igarashi et al., 2013), supporting the hypothesis that EPA is relevant to a systemic anti-inflammatory response to the onset of depressive symptoms. However, EPA is also converted to DHA in the plasma (Schmitz and Ecker, 2008), the latter being transported to the brain through free fatty acid pools (Chen et al., 2008) during periods of inflammatory activity or increased brain DHA utilization. As such, the anti-inflammatory properties of EPA-derived mediators and the conversion of EPA to DHA for brain uptake may collectively be responsible for purported antidepressant efficacy in depressed patients.

One advantage of the present study is that EPA+DHA to AA ratios were measured in erythrocytes. Erythrocytes provide a more stable marker of blood ω-3 FAs than plasma, which can demonstrate up to 4-fold greater within-subjects variability in concentrations than erythrocytes (Harris and Thomas, 2010). Lower erythrocyte EPA+DHA to AA ratios may indicate a consistent deficit in ω-3 FA intake relative to AA intake, which is common in a western diet, and may predispose patients to depressive symptoms (Lin et al., 2010). It may also indicate increased utilization of ω-3 FAs from tissue stores in a biological attempt to resolve inflammation (Kidd, 2007) or increase ω-3 FA supply to the brain (Chen et al., 2008, Purdon et al., 1997).
Of potential mechanistic relevance, reduced EPA+DHA to AA ratios from erythrocyte PC or PE fractions, the most abundant fractions in erythrocytes and most other tissues, were not correlated with depressive symptoms in CAD patients. Instead, it was ratios in the PI and SM fractions that were most strongly correlated with depressive symptoms. These different relationships with depressive symptoms may simply reflect the hierarchy in which fatty acids integrate into phospholipid types, with ω-3 FA deficits possibly being revealed in phospholipid fractions that integrate EPA, DHA, and AA less readily, such as PI and SM (Raphael and Sordillo, 2013). As the PI fraction is typically rich in AA (Hicks et al., 2006), the ratio of EPA+DHA to AA in that fraction may be particularly indicative of the balance between pro- and anti-inflammatory fatty acid mediators in erythrocyte membranes. In light of this, it has been suggested that enrichment of EPA and DHA in the PI fraction may have meaningful implications on inflammatory signalling (D'Souza and Epand, 2014), with particular relevance to the brain, where the membrane PI fraction is greater than it is in other tissues (Christie, 2014).

The EPA+DHA to AA ratio in the erythrocyte SM fraction is also of potential mechanistic relevance to depressive symptoms. A recent study implicated sphingomyelin species, such as SM 23:1 and SM 16:0, as correlates of depressive symptoms (Demirkan et al., 2013); however, that study was unable to characterize the fatty acid chains involved, which is a limitation that may have been overcome by the present study. More broadly, SM lipids have been associated with several neurodegenerative diseases, such as depression and Alzheimer’s disease, suggesting that SM metabolism may be dysregulated by aberrant inflammatory and oxidative stress process which characterize those diseases (Kornhuber et al., 2005, Mielke and Lyketsos, 2010). Furthermore, SM is a prominent fraction in circulating lipoproteins implicated in atherosclerosis and vascular inflammatory processes (Chen et al., 2011b). Accordingly, the ratio of EPA+DHA to AA in the erythrocyte SM fraction may indicate the balance of inflammatory
eicosanoid mediators in circulating lipoproteins or in other SM rich tissues, with relevance to those neurodegenerative or atherosclerotic mechanisms.

Though the mechanistic importance of the relationships between depressive symptoms and different phospholipid fractions remains largely unclear, the present findings support a growing need for lipidomic approaches to enhance biomarker identification (Xu et al., 2013, Fonteh et al., 2006). Such approaches may also link the fatty acid content of different phospholipid fractions to other inflammatory or genetic factors thought to underlie depressive symptoms, and may help to construct a clearer mechanistic understanding.

Supporting the relevance of EPA+DHA to AA ratios in erythrocyte PI and SM fraction as CAD-relevant markers of depressive symptoms were the consistent associations across each depressive symptom cluster, including depressive mood symptoms. This suggests that the associations were likely driven not by symptom clusters such as sleep and psychic anxiety or somatic symptoms, which although related to depression, are not hallmark features of depressive episodes, but by depressive mood symptoms which are more congruent with hallmark diagnostic symptoms of low mood and/or anhedonia in those experiencing major or minor depressive episodes (American Psychiatric Association, 2013). Furthermore, the HAM-D depressive mood cluster contains symptoms of reduced interest in activities or surroundings, fatigue, and malaise, which, when combined with sleep disturbances, are key symptoms consistent with the sickness behaviour model of depressive symptoms (Dantzer, 2004). Although the mechanisms by which depressive mood may develop in those with CAD are unclear, the present findings suggest that EPA and DHA deficits may be associated with depressive symptoms related to an inflammatory event. In line with this, EPA and DHA supplementation may ameliorate mood and sleep symptoms through the restoration of systemic
and central inflammatory and oxidative stress processes linked to mood regulation through monoaminergic and inflammatory circuits in the brain [Section 1.3.3].

As consistently described in the literature, younger age (Chang et al., 2015, Amin et al., 2008, Gottlieb et al., 2004, Lavie and Milani, 2006, Swardfager et al., 2008), a history of a previous depressive episode (Katon et al., 1994, Barkow et al., 2003), and poorer cognitive performance (McDermott and Ebmeier, 2009, Ravnkilde et al., 2002) were each associated with greater depressive symptom severity in this study. The retention of those characteristics as predictors of depressive symptoms in this study supports the validity of the study sample for investigating depressive symptoms in CAD.

In summary, Study 1 found that lower EPA+DHA to AA ratios in erythrocyte PI and SM fractions were independently associated with greater depressive symptom severity overall, and in each depressive symptom cluster, in CAD patients. These findings support lower membrane phospholipid EPA+DHA to AA ratios as deficits that are relevant to the presence of depressive symptoms in CAD patients, which may be amenable to EPA and DHA supplementation.

In a treatment X time analysis [Study 2], ω-3 FA treatment (high ratio EPA to DHA formulation) did not result in lower depressive symptom severity over 12 weeks of CR compared to placebo, despite ω-3 FA treatment significantly increasing plasma EPA and DHA concentrations. Furthermore, changes in depressive symptoms over 12 weeks were not related to changes in the EPA+DHA to AA ratio. This finding is in keeping with several RCTs demonstrating that ω-3 FA treatment was not efficacious for reducing depressive symptoms (Grosso et al., 2014). However, it is in contrast to the meta-analytic findings that high ratio EPA to DHA formulations demonstrate efficacy for reducing depressive symptoms in the general adult population (Sublette et al., 2011, Martins et al., 2012).
The lack of antidepressant efficacy demonstrated in this study may be related to several factors. Firstly, the relatively mild depressive symptom severity of the included patients may have limited the detection of efficacy. Available meta-analytic data suggest that ω-3 FA treatment demonstrates the most consistent efficacy in major depressed patients without co-morbid medical and/or psychiatric conditions, and that greater efficacy is generally found in samples of more severely depressed patients than in samples of mildly depressed patients (Grosso et al., 2014). As such, including CAD patients with a range of depressive symptom severity may have inherently limited its potential for identifying treatment efficacy in CAD. While this study did not observe any treatment efficacy in CAD patients meeting DSM-IV depressive episode criteria at baseline, it is still possible that ω-3 FA treatment may be most relevant to that subgroup, although the small sample size in that subgroup may have precluded the detection of efficacy. Consistent with previous literature (Grosso et al., 2014), ω-3 FA treatment did not demonstrate efficacy in maintaining or lowering depressive symptom scores over 12 weeks among patients who did not meet DSM-IV depression criteria at baseline.

Secondly, the results of this study may suggest that our formulation of ω-3 FA treatment (1.2 g EPA + 0.6 g DHA per day) may not be suitable for reducing depressive symptoms across this symptom range in CAD patients, despite the formulation being supported by meta-analytic findings. Although, the efficacy of EPA-enriched ω-3 FA monotherapy for depressive symptoms in a well-powered group of CAD patients with depressive symptoms remains largely unexplored, limiting the available evidence from which to identify an appropriate formulation. Furthermore, as some studies have reported ω-3 FA supplement efficacy when used in combination with another antidepressant intervention (Grosso et al., 2014, Zimmer et al., 2013), it remains unclear whether ω-3 FA supplements are most efficacious as a monotherapy or as an
adjunctive therapy. Additional research may be needed in order to determine the most suitable antidepressant role for ω-3 FA supplements.

Thirdly, it is possible that 12 weeks of treatment was not sufficient to observe measurable efficacy, although several other RCTs using ω-3 FAs to treat depressive symptoms have observed efficacy in 12 weeks or shorter (meta-analyzed by (Grosso et al., 2014)). As mentioned, despite ω-3 FA plasma concentrations reaching steady state after approximately two weeks of supplementation, it may be several weeks before brain concentrations are sufficiently restored. Additional evidence regarding the mechanisms by which ω-3 FAs may achieve antidepressant benefits is likely needed to accurately predict the appropriate RCT duration.

Fourthly, the potential effects of CAD as a medical condition, or the presence of obesity, diabetes mellitus, and/or associated use of cardiovascular or lipid modifying medications (such as statins and/or ASA) on the magnitude and timescale of treatment efficacy may be worthwhile to explore in future studies; however, none of those factors appeared to influence treatment efficacy in this study.

Finally, the observation of ω-3 FA treatment efficacy may have been limited by the potential antidepressant efficacy of CR (Rutledge et al., 2013, Yohannes et al., 2010). However, as mentioned, not all CAD patients experience antidepressant benefits from CR and changes in cardiopulmonary fitness did not correlate with changes in depressive symptom severity over 12 weeks in the present studies. Furthermore, many patients experienced an increase in depressive symptom severity over 12 weeks, suggesting that antidepressant benefits of CR were not experienced by all included patients. Given those findings, it is unlikely that the presence of CR clouded ω-3 FA treatment efficacy in Study 2.
In addition to the lack of ω-3 FA treatment efficacy when measuring depressive symptoms collectively using HAM-D and BDI-II total scores, no statistically significant efficacy was observed for any of the depressive symptom cluster scores, further suggesting that treatment was generally inefficacious in this CAD sample.

A potential source of variability in treatment efficacy across ω-3 FA trials might be the variability in baseline ω-3 FA deficits among their included patients. Although lower baseline EPA+DHA to AA ratios in erythrocyte PI and SM fractions were associated with greater depressive symptom severity in Study 1, neither marker predicted ω-3 FA treatment efficacy or change in depressive symptoms in the ω-3 FA treatment group or in the total sample in this study. However, regardless of treatment group, greater baseline plasma EPA+DHA concentrations significantly predicted reductions in depressive symptoms over 12 weeks of CR, though the contribution of baseline plasma EPA+DHA concentrations to those changes was small and likely one of many explanatory factors. Greater plasma ω-3 FA concentrations may indicate resilience to the progression of depressive symptoms in CAD patients or the likely antidepressant benefits of CR. As mentioned, plasma ω-3 FA concentrations are 4-fold more variable than erythrocyte ω-3 FA fractions within patients. The discrepancy between the predictive roles of erythrocyte fractions and plasma ω-3 FA concentrations for depressive symptoms may be due to this variability. While erythrocyte ω-3 FA fractions may reflect long-term ω-3 FA intake and/or long-term ω-3 FA metabolism, plasma ω-3 FA concentrations may reflect the available concentrations of ω-3 FAs for biological processes such as metabolism into anti-inflammatory mediators, fatty acid pools available to the brain, or other potentially relevant processes in CAD and/or depressive symptoms (Kidd, 2007, Levant, 2013). Accordingly, greater baseline plasma EPA+DHA concentrations may indicate a greater availability of EPA and DHA in circulation for use in active processes to resolve pro-inflammatory and neurotoxic
conditions, such as those thought to underlie depressive symptoms in CAD (Leonard and Maes, 2012, Moylan et al., 2012). In keeping with this, plasma EPA concentrations have been shown to correlate significantly with EPA concentrations in the cerebrospinal fluid (Freund Levi et al., 2014), implicating plasma ω-3 FAs in the uptake and metabolism of EPA by the brain during periods of inflammatory activity (Chen et al., 2011a), which has been shown to be neuroprotective (Orr et al., 2013a). Given those findings, the lack of ω-3 FA treatment efficacy observed in this study is interesting and invites investigation of factors affecting or predicting treatment efficacy.

Aside from baseline plasma EPA+DHA concentrations, younger age predicted greater reductions in HAM-D total scores over 12 weeks in the total group. In line with Study 1 which found that younger patients demonstrated greater depressive symptom severity at baseline, this finding suggests that younger CAD patients may experience more reactive and remediable depressive symptoms than older CAD patients.

In summary, 1.9 g/day (1.2 g EPA + 0.6 g DHA) ω-3 FA treatment was not efficacious for reducing depressive symptoms over 12 weeks in CAD patients participating in CR, despite increasing plasma EPA and DHA concentrations. However, a predictive association between greater baseline plasma EPA+DHA concentrations and reductions in depressive symptoms over 12 weeks was observed regardless of treatment. These findings suggest that EPA and DHA are relevant to depressive symptoms in CAD, but that EPA- and DHA-enriched supplements may not target those mechanisms despite increasing plasma EPA and DHA concentrations. Pre-treatment biomarkers predicting efficacy in CAD patients would be clinically helpful.

Investigating pre-treatment lipid peroxidation as a predictor of ω-3 FA treatment response [Study 3] revealed that higher baseline LPH concentrations, a marker of early-stage lipid
peroxidation, predicted worsening of depressive symptoms, particularly depressive mood symptoms, in ω-3 FA treated patients. Presently, this study is the first to investigate baseline lipid peroxidation markers as a predictor of ω-3 FA treatment efficacy for depressive symptoms in CAD patients or other populations. However, previous studies have investigated immune system biomarkers as a predictor of antidepressant treatments. Aberrant inflammatory activity at baseline, which is closely related to oxidative stress activity (Maes et al., 2011), has been shown to differentially predict response to SSRIs and anti-inflammatory therapies. For example: while elevated baseline concentrations of circulating inflammatory markers such as CRP, IL-6, and TNF may not be associated with response to sertraline (Bot et al., 2011) or may predict non-response to escitalopram (TNF) (Eller et al., 2008), they appear to predict the antidepressant efficacy of the TNF-antagonist infliximab (Raison et al., 2013) and exercise interventions (Rethorst et al., 2012), which have anti-inflammatory effects (Swardfager et al., 2012).

In light of that evidence, the findings from this study may be interpreted in different ways. As LPH represents the early stages of lipid peroxidation, which can progress to the formation of HNE and other late-stage markers such as malondialdehyde and 8-isoprostane (Moore and Roberts, 1998), greater LPH concentrations at baseline might indicate less conversion of LPH to late-stage markers, due to intact antioxidant defenses or other factors. Accordingly, greater baseline LPH concentrations might indicate a subgroup of CAD patients for whom oxidative stress is not associated with their depressive symptoms, and for whom an anti-inflammatory and anti-oxidant treatment such as ω-3 FAs might be inefficacious. While no correlation between baseline HNE concentrations and ω-3 FA treatment efficacy was observed, it is possible that LPH conversion to late-stage markers was distributed across HNE, malondialdehyde, and 8-isoprostane, therefore diluting the potential for an association between depressive symptoms and
HNE. Investigation of relationships between those late-stage markers and ω-3 FA treatment and/or CR may clarify their roles as predictors of depressive symptom changes.

Alternatively, greater baseline LPH concentrations might indicate active oxidative stress pathophysiology, regardless of the concentrations of HNE or other late-stage markers, which may be formed after long-term elevations of LPH and exhaustion of the antioxidant defenses. As described, ω-3 FAs are highly vulnerable to free radical-mediated, non-enzymatic oxidative damage. Enzymatic metabolism and anti-inflammatory effects of ω-3 FA treatment may therefore be diminished in CAD patients with high baseline oxidative stress activity, rendering treatment inefficacious for depressive symptoms in that subgroup. This hypothesis is supported by a recent animal study demonstrating that ω-3 FA-derived resolvin species mediate antidepressant-like effects in rats post-myocardial infarction (Gilbert et al., 2014). Thus, oxidative stress-induced shifts in ω-3 FA metabolism may underlie variability in antidepressant efficacy. Future studies measuring malondialdehyde, 8-isoprostane, and relevant antioxidant species such as glutathione peroxidase, which protects LPH from transitioning to late-stage markers, may help to clarify the interpretation of these study findings.

Interestingly, this study found that greater baseline LPH concentrations were particularly associated with worsening depressive symptoms in a subgroup of ω-3 FA treated patients meeting depression criteria at baseline. Though depression has been consistently associated with greater circulating concentrations of lipid peroxidation markers (Palta et al., 2014), previous meta-analyses have suggested that ω-3 FA treatment may be most efficacious in those with greater baseline depressive symptom severity (Sublette et al., 2011, Martins et al., 2012, Grosso et al., 2014), which seemingly contradicts the findings from the present study. Additional studies may clarify this discrepancy and better characterize the role of lipid peroxidation markers as pre-treatment predictors of response to ω-3 FAs.
After adjustment for the predictive effects of baseline LPH concentrations, greater baseline plasma EPA+DHA concentrations remained a trend level predictor of reductions in depressive symptoms over 12 weeks in ω-3 FA treated patients. This finding suggests that ω-3 FA availability in plasma may have a separate, overarching relationship with the course of depressive symptoms over time in CAD patients, independently of the relationship between lipid peroxidation and ω-3 FA treatment. As such, ω-3 FA availability in plasma may provide resilience to the effects of oxidative stress on depressive symptoms during ω-3 FA treatment.

Further study of the relationships between serum LPH concentrations, plasma EPA+DHA concentrations, and depressive symptom changes over CR may elucidate the role of each blood marker as a potential predictor and/or mechanistic component of depressive symptom changes.

Collectively, the conducted studies suggest that lower EPA+DHA to AA ratios, indicating reduced anti-inflammatory ω-3 FAs available to balance pro-inflammatory AA mediators, were associated with greater cross-sectional depressive symptom severity among CAD patients, but that increasing EPA and DHA concentrations through 12 weeks of ω-3 FA supplements (1.2 g/day EPA + 0.6 g/d DHA) was not associated with a statistically significant reduction in depressive symptoms compared to placebo. However, ω-3 FA treatment efficacy for depressive symptoms in CAD may be related to lipid peroxidation, of which greater pre-treatment LPH concentrations predict smaller reductions in depressive symptoms, particularly depressive mood symptoms, in ω-3 FA treated patients.

As CR is an exercise intervention that has been shown to produce anti-inflammatory effects (Swardfager et al., 2012) and antidepressant efficacy (Rutledge et al., 2013, Yohannes et al., 2010), greater baseline LPH concentrations might have been expected to predict antidepressant benefits from exercise in CR among placebo using CAD patients, as other immune system makers have previously shown (Rethorst et al., 2012). However, that was not observed.
Furthermore, changes in fitness were not associated with changes in any depressive symptom outcome over 12 weeks in either treatment group. Those findings further indicate that the observed lack of ω-3 FA treatment efficacy for depressive symptoms overall may be a legitimate finding, and not a potentially efficacious treatment clouded by the antidepressant efficacy of CR. Importantly, the lack of antidepressant efficacy of CR is a consistent problem in CAD and is in contrast to the meta-analytic evidence supporting the use of exercise interventions for reducing depressive symptoms (Herring et al., 2012, Rimer et al., 2012, Bridle et al., 2012, Krogh et al., 2011). Thus, our study findings highlight the potential relevance of ω-3 FAs and oxidative stress in the mechanisms and management of depressive symptoms in CAD patients participating in CR, who are in need of novel efficacious and effective antidepressant interventions.

4.2 Limitations

Study 1 was limited by its cross-sectional design, which precluded the investigation of the stability of the relationship between EPA+DHA to AA ratios in erythrocyte phospholipid fractions and depressive symptoms in CAD over time. The cross-sectional design also precluded the determination of causality in that relationship, as it is possible that reduced EPA+DHA to AA ratios in erythrocyte PI and SM fractions are a consequence and not a cause of depressive symptoms, or vice-versa.

Inferences from Study 2 were limited by low power to detect significant ω-3 FA treatment efficacy for depressive symptoms in CAD given the observed treatment effect size. Given the wide range of depressive symptom changes over time, a sample size in the hundreds of patients would have increased the observed power. However, post-hoc power calculations and effect size estimates suggest that treatment efficacy would likely not be clinically meaningful even in an
adequately powered study. Study 2 may have also been limited by floor effects of HAM-D and BDI-II measurements in patients who were not depressed or who were experiencing only mild depressive symptom severity. This may have limited the detection of antidepressant efficacy in those groups. However, the lack of significant treatment efficacy in a subgroup of patients who met DSM-IV depression criteria at baseline suggests that efficacy may not have been present regardless of limitations of these depressive symptom measures. Finally, EPA+DHA to AA ratios in erythrocyte phospholipid fractions were not reassessed at week 12 and, therefore, increases in those fractions with ω-3 FA treatment, as well as the correlation between those increases and ω-3 FA treatment efficacy for depressive symptoms could not be determined. However, changes in plasma ω-3 FAs have been shown to correlate strongly with changes in erythrocyte ω-3 FAs after supplementation (Harris et al., 2004) and were deemed to be appropriate surrogate measures in the present studies.

Study 3 was limited by a small sample size, which may have precluded the detection of a significant treatment X LPH X time interaction on change in depressive symptoms over 12 weeks. As a result, the potentially treatment-modifying effects of baseline oxidative stress had to be investigated in each treatment group individually and could not be directly compared. Investigating each treatment group individually also meant that fewer covariates could be included in each model, limiting the ability to address potential confounders. However, the lack of a significant treatment X LPH X time interaction in this study might have been the result of the nature of the association between LPH and ω-3 FA treatment, rather than low sample power. Specifically: this study found that greater baseline serum LPH concentrations predicted worsening of depressive symptoms over 12 weeks in the ω-3 FA treatment group, but had no significant effect on depressive symptoms in either direction in the placebo group. The LPH X treatment X time interaction would have been strengthened by opposing relationships between
LPH and depressive symptom change between the two treatment groups; however, clinically, the lack of a relationship between LPH and depressive symptom change in the placebo group is of no consequence. Therefore, the predictive association between baseline serum LPH concentrations and change in depressive symptoms over 12 weeks in the ω-3 FA treatment group might be a clinically meaningful finding, despite the lack of a significant LPH X treatment X time interaction.

Studies 1 and 2 were limited by the exclusion of CAD patients who had recently began or changed the use of antidepressant pharmacotherapy. For some of those patients, a change in antidepressant dose might have been a necessary adjustment to accommodate the metabolism of other cardiac medications. For some others, a change in dose or initiation of antidepressant therapy might have been the result of emerging or worsening depressive symptoms. Although excluding those patients may have protected the studies from the potentially confounding effects of modified antidepressant therapy on the detection of reductions in depressive symptom severity, it may have also excluded a subgroup of patients exemplifying the clinical problem that the present studies were attempting to investigate: the onset or worsening of depressive symptoms post-CAD. However, few CAD patients were excluded from the studies for recent changes in antidepressant pharmacotherapy (n=2) and this limitation likely did not affect the study findings.

The generalizability of all study findings was limited to CAD patients. Therefore, inferences cannot necessarily be made about the study findings in those without CAD. Furthermore, the subgroup of CAD patients who participate in CR is not necessarily representative of all CAD patients. Compared to CAD patients who do not attend CR, those who do attend CR have been shown to be of higher socioeconomic status, if depressed, they tend to demonstrate milder depressive symptom severity, and they may be generally more engaged in the health behaviour
changes necessary post-CAD (Martin et al., 2012, Alter et al., 2014). As such, the
generalizability of all study findings to all CAD patients may be limited. However, as many
study findings were significant in the CR sample, it may be expected that those findings would
be more, not less, pronounced if non-CR attending CAD patients were included.

Ultimately, the present studies were designed to address gaps in knowledge regarding the
relationships between ω-3 FAs and the presence and management of depressive symptoms in
CAD patients participating in CR. The availability of literature on the management of
depressive symptoms in the general adult population minimizes the relevance of the lack of
generalizability of these study findings.

4.3 Recommendations for Future Studies

These results add clarity to the relationships between ω-3 FAs, depressive symptoms in CAD
patients, and oxidative stress; however, confirmatory studies are needed. Specifically,
replication of the relationship between erythrocyte EPA+DHA to AA ratios and depressive
symptom severity in CAD patients entering CR is warranted in light of the potential
implications of different phospholipid fractions in membranes and circulations. Such future
studies ought to include relevant covariates such as cardiopulmonary fitness (VO₂ peak fraction)
and cognition in the analysis of those relationships, as was performed in Study 1, given their
previously documented relationships with depressive symptoms in CAD (Swardfager et al.,
2008, McDermott and Ebmeier, 2009, Ravnkilde et al., 2002). Confirmation of EPA+DHA to
AA ratios in erythrocyte PI and SM fractions as a correlate of depressive symptoms in CAD
would increase the evidence base for the balance between pro- and anti-inflammatory ω-3 FAs
and ω-6 FAs as one factor underlying mechanisms of depressive symptoms in CAD, and may
support it as a treatment target.
Although additional RCTs using EPA-enriched ω-3 FA supplements would help clarify their efficacy for treating depressive symptoms in CAD, it may be prudent to prioritize studies that can develop a better understanding of supplement metabolism that is relevant to antidepressant efficacy. The present studies measured plasma EPA and DHA concentrations as a measure of supplement use, which indicated that plasma ω-3 FA concentrations (and likely erythrocyte fractions, given their consistent associations) were increased with supplement use. However, those concentrations do not necessarily indicate changes in plasma ω-3 FA profile that are relevant to depressive symptom reductions, despite a cross-sectional link between ω-3 FA deficits and increased depressive symptom severity. Investigating the range of ω-3 FA metabolites, such as resolvins, protectins, and CYP eicosanoid species may identify mediators that are more closely related to depressive symptom changes in CAD. This approach may generate a biological profile of ω-3 FA metabolism that is relevant to depressive symptoms, which may then be tested in blood samples to confirm appropriate “target” engagement of supplements during RCTs. Once a biological profile of target engagement can be identified, investigation of other pathophysiological pathways which may influence that profile in depressed CAD patients may then be explored in order to identify barriers to treatment efficacy. Collectively, these approaches may ensure that future RCTs can be designed to assess whether the ω-3 FA supplements are being metabolized in a manner that is relevant to their potential antidepressant efficacy.

Beyond an ω-3 FA metabolic profile, further exploration of the lipid peroxidation pathways that are relevant to depressive symptoms, CAD, and ω-3 FAs is warranted. Study 3 provided a first-step in exploring lipid peroxidation stages related to ω-3 FA metabolism. As described in the Introduction, LPH is an early stage in lipid peroxidation, which can be neutralized by antioxidant defenses. If oxidative stress overwhelms the antioxidant defenses, LPH may be
converted to later-stage lipid peroxidation products such as HNE, malondialdehyde, or isoprostane species. Exploring how those later-stage species may be related to ω-3 FA supplement efficacy for depressive symptoms, or the presence of depressive symptoms in CAD generally may help to characterize the role of oxidative stress in disease pathophysiology. It may also be informative to explore ω-3 FA-derived lipid peroxidation byproducts such as HHEs, which may indicate the degree of ω-3 FA supplement peroxidation in the plasma during an RCT. Furthermore, exploring the enzymatic and oxidative pathways involved in the conversion of LPH to late-stage lipid peroxidation species, and whether particular pathways are more or less relevant to depressive symptoms or ω-3 FAs may help to understand how lipid peroxidation pathways interact with other genetic and protein vulnerability factors which may underlie depressive symptoms or lack of treatment efficacy.

There have been at least 44 RCTs, comprising over 7,500 treated patients, which have investigated ω-3 FA antidepressant efficacy across a range of co-morbidities, depressive symptom severities, concomitant medications, ages, and ω-3 FA formulations (Grosso et al., 2014). This collection of RCTs provides a rich database from which to explore the biological and clinical factors which may influence ω-3 FA antidepressant efficacy, with the potential to provide meaningful evidence for best formulations, populations, and predictive biomarkers for likely benefits. As such, it may be worthwhile to explore the feasibility of a consortium of ω-3 FA and depression investigators, which may drive the development of the proposed future directions and provide much needed evidence that can inform the design of future RCTS.

The potential antidepressant efficacy of ω-3 FA supplements observed in previous RCTs supports its continued investigation. Beyond a potential role in managing depressive symptoms, ω-3 FA supplements are commonly used over-the-counter by CAD patients in order to experience their potential cardiovascular benefits (Lee et al., 2009). As such, it is clinically
important to understand the mechanisms by which these supplements are metabolized and how disease pathophysiology in CAD or other populations may affect their health benefits.

4.4 Conclusions

These results suggest that lower EPA+DHA to AA ratios in erythrocyte PI and SM phospholipid fractions are associated with increased depressive symptom severity in stable CAD patients, post-ACS, with particular relevance to increased depressive mood symptoms. They also suggest that greater baseline plasma EPA+DHA concentrations predict greater reductions in depressive symptoms during CR, but that increasing EPA and DHA concentrations through 1.9 g/day ω-3 FA supplements did not result in lower depressive symptom severity overall or in specific symptom clusters. Finally, they suggest that greater pre-treatment concentrations of lipid peroxidation markers predicted smaller depressive symptom reductions with ω-3 FA treatment, with particular relevance to depressive mood symptoms. These studies collectively investigate the relationships between ω-3 FAs and depressive symptoms in CAD patients accounting for a novel mechanistic variable, lipid peroxidation, enabling us to explore a potential factor in treatment variability. These findings support ω-3 FA deficits as a factor in the presence of depressive symptoms and suggest that ω-3 FA treatment may be efficacious in certain patient subgroups. The heterogeneity in clinical presentation and likely heterogeneity in biological underpinnings of depressive symptoms in CAD may necessitate the use of pre-treatment biomarkers that can predict response to antidepressant interventions. To this end, pre-treatment lipid peroxidation markers may be a relevant predictor of response to ω-3 FA treatment and, as such, provide a potential signal for future research in this area. In conclusion, these findings provide insight into the role of ω-3 FAs, both in circulation and in supplement form, in the pathophysiology of depressive symptoms in CAD. As depressive symptoms are associated with poor cardiovascular and rehabilitation outcomes, reduced quality of life, and significant costs to
the healthcare system, the modest efficacy and generally poor effectiveness of currently available antidepressant interventions in those with CAD calls for further research into alternative antidepressant interventions for CAD patients. These studies therefore provide knowledge that addresses clinically important gaps in the present understanding of depressive symptom etiology and management in CAD patients.


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Appendices

Appendix A. CAROTID Research Ethics Board 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 C. Hébert  MD PhD FCFP
Chair, Research Ethics Board
May 27, 2010

Dr Paul Oh
TRI - Runsey Centre (Cardio)
347 Rumsey Road
Toronto, ON., M4H 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,

Toronto Rehabilitation Institute
Chair, Research Ethics Board
[ ] Paul Oh MD, MSc, FRCPC, FACP
[ ] Ann [Teckers BEd, BA, MA, PhD (ABD)
[ ] Toronto Rehabilitation Institute
[ ] Vice 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/unauthorized 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
May 28, 2012

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

Dear Dr. LaCoutre,

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.

CAROTID: CAD Randomized Omega-3 Trial In Depression

Patient Information and Consent

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 (2 1/3 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.
Side Effect | Frequency | Severity | Long Term Impact
--- | --- | --- | ---
 | Expected (30-100%) | Likely (10-30%) | Less Likely (1-10%) | Rare (0-1%)
Nausea | X | X | X
Diarrhea | X | X | X
Abdominal pain | X | X | X
Runny nose | X | X | X
Upper respiratory tract infection | X | X | X
Dyspepsia | X | X | X
Skin abnormalities | X | X | X
Fish flavoured burps | X | X | X

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

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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:

You have the right to have this form and all information concerning this study explained to you. By signing this consent form, you do not give up any of your legal rights. 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.

You have the right to access, review and request changes to your personal information (i.e. address, date of birth).

You have the right to be informed of the results of this study once the entire study is complete.
CAROTID: CAD Randomized Omega-3 Trial In Depression

**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. Adverse Events during Study 2.

Table E1. Adverse Event Total Score (out of 42) at Each Study Visit in the ω-3 FA and Placebo Groups in Study 2.

<table>
<thead>
<tr>
<th>Visit</th>
<th>ω-3 FAs (n=41)</th>
<th>Placebo (n=45)</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.4 (3.8)</td>
<td>3.6 (3.9)</td>
<td>1.14</td>
<td>.29</td>
</tr>
<tr>
<td>Week 4</td>
<td>5.7 (4.9)</td>
<td>4.1 (4.1)</td>
<td>3.53</td>
<td>.06</td>
</tr>
<tr>
<td>Week 8</td>
<td>5.7 (4.9)</td>
<td>5.4 (4.8)</td>
<td>0.10</td>
<td>.76</td>
</tr>
<tr>
<td>Week 12</td>
<td>5.6 (4.6)</td>
<td>5.6 (5.3)</td>
<td>0.01</td>
<td>.94</td>
</tr>
</tbody>
</table>

Abbreviations: ω-3 FAs, omega-3 fatty acid treatment; SD, standard deviation

Adverse events were measured using a 14-item scale with each item ranging from 0 (“none”) to 3 (“a lot”) of a given symptom, up to a maximum score of 42. The symptoms most frequently reported were pain, nasopharyngitis, and fatigue. There were no differences in the number or severity of adverse events experienced by patients in either group.