I, Keith A. Rosing, hereby submit this original work as part of the requirements for the degree of: Master of Science in Nutrition.

It is entitled: The Feasibility of a Randomized Controlled Trial Investigating the Effects of Fish Oil - Eicosapentaenoic Acid (EPA) and Docosahexanoic Acid (DHA) - on Chronic Ventilator Patients in a Long-Term Acute Care Hospital (LTACH) Setting.

Student Signature: Keith A. Rosing

This work and its defense approved by:

Committee Chair: Dr. Sarah Couch
Dr. Kari Dunning

Approval of the electronic document:

I have reviewed the Thesis/Dissertation in its final electronic format and certify that it is an accurate copy of the document reviewed and approved by the committee.

Committee Chair signature: Dr. Sarah Couch
The Feasibility of a Randomized Controlled Trial Investigating the Effects of Fish Oil- Eicosapentaenoic Acid (EPA) and Docosahexanoic Acid (DHA)- on Chronic Ventilator Patients in a Long Term Acute Care Hospital (LTACH) Setting.

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by

Keith A. Rosing
B.S., University of Cincinnati, 2006
M.S., Wright State University, 2007

Committee Chair:
Sarah C. Couch, PhD, RD
Abstract

The Feasibility of a Randomized Controlled Trial Investigating the Effects of Fish Oil- Eicosapentaenoic Acid (EPA) and Docosahexanoic Acid (DHA) - on Chronic Ventilator Patients in a Long Term Acute Care Hospital (LTACH) Setting.

by

Keith Rosing

Objective: Assess the feasibility and efficacy of an ongoing randomized control trial currently in progress at the Drake Center in Cincinnati, Ohio, to determine the effects of EPA + DHA on inflammation, infections, weaning, length of stay and mortality among mechanically ventilated patients in a LTACH setting.

Subjects: Thus far, there have been three patients to complete the study. All three were females, two were Caucasian and one was African American. Their ages were 58, 42, and 53. All three were diagnosed with Respiratory Failure, though each one’s conditions stemmed from a different initiating event. In addition, the screening results for the first 32 patients screened for study enrollment are presented. Of these patients, 17(53%) were male, 15(47%) were female, and the average age of those patients screened who met the age criteria was 56.1± 5.99. Potential participants were assessed based on five inclusion criteria and eleven exclusion criteria. Overall, a history of ventricular tachycardia or atrial fibrillation was the most commonly indicated reason for exclusion (n=13)
followed by a treatment dosage of heparin or coumadin (n=11), and an aPTT>33.5s (n=10).

**Study Design:** Double-blind, randomized, placebo-controlled trial.

**Methods:** Study participants were randomized to either a study group or a placebo control group. Subjects randomized to the study group received 8g of fish oil per day in divided doses administered through enteral tube every 6 hours for 14 days. Saline was administered to those in the control group in the same manner. The primary and secondary measures being investigated in this study include the inflammatory markers IL-6, IL-8, and LTB₄, infectious events, weaning, length of stay, and mortality.

**Results:** The study records for the first three patients to complete the trial indicated that the study protocol was followed completely and appropriately. Each of the participants weaned successfully and were eventually discharged, but only one of the participants completed the study. Two dropped out upon experiencing a bad taste or bad odor that they attributed to the study medication even though only one of these participants was actually receiving fish oil. Analysis of the screening results indicated that among the males and females screened for study enrollment, males were excluded more often than females mostly due to a greater likelihood of having a history of ventricular tachycardia or ventricular fibrillation.
**Conclusion:** The results of this feasibility study suggest that the trial is going well and according to the research methodology mapped out previously by the researchers. There were a couple of areas that were identified as being of potential concern. These include the variation in initiating events observed among the first three participants, an all female enrollment, the enrollment of patients with a recent or current infection, and the significance of participant complaints concerning the taste/odor of the study medication.
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Introduction

Both observational and experimental evidence support the finding that lower rates of sudden death exist among individuals/cultures whose diets are heavily dependent on fish. Numerous studies have been conducted to date that have aimed at identifying the source of the protective benefits of fish. The results of these studies support the conclusion that the omega-3 polyunsaturated fatty acids (PUFAs) in fish oil are mainly responsible for the protective properties of this food. Furthermore, recent evidence suggests that omega-3 PUFA’s may reduce mortality, particularly from cardiovascular related sudden death, in part by reducing inflammation. The inflammatory process appears to be a critical step in the progression of atherosclerosis. Following these discoveries a considerable amount of additional research has been devoted to determining clinical applications of omega-3 PUFA’s, primarily from fish oils, in disease prevention. Specifically, such research has focused on the implications of its supplementation for individuals suffering from or at risk for cardiovascular disease, rheumatoid arthritis, inflammatory bowel diseases, and other diseases in which chronic inflammation represents an important step in the disease process.

For some conditions, inflammation represents not only a contributor to disease development but also a barrier to recovery. Respiratory failure represents one such condition, suggesting that the potential benefits of fish oil supplementation in these patients may be of clinical significance. This is the focus of an ongoing double-blind randomized placebo-controlled trial whose
initial findings will be presented herein following a review of the literature concerning n-3 PUFA supplementation. The purpose of presenting these initial findings is to assess the feasibility and efficacy of this ongoing clinical investigation.

**Literature Review**

**n-6 and n-3 Polyunsaturated Fatty Acids**

The enzymes 12- and 15-desaturase are required for the insertion of double bonds at the n-6 and n-3 positions of oleic acid, 18:1(n-9), to produce the omega-6 (n-6) and omega-3 (n-3) series polyunsaturated fatty acids (PUFAs), respectively.\(^1,2\) Humans require both types of PUFAs for the synthesis of lipid mediators and the production of membrane phospholipids, but are incapable of producing either type themselves. The two desaturases are present only in plants, making the consumption of Linoleic acid (LA, 18:2, n-6) and α-Linolenic acid (ALA, 18:3, n-3) in the diet necessary for proper cellular function and normal human health.\(^2\)

The average intake of n-6 PUFA in a healthy adult is 11-17 g/day.\(^3\) LA is found in corn oil, sunflower seed oil, and safflower oil, and is the primary source of n-6 PUFA in the diet.\(^1,2\) In the body it is readily converted to arachidonic acid (AA), 20:4(n-6). Arachidonic Acid is mostly obtained in the diet through the consumption of meat, and it is typically found to account for the rest of the dietary intake of n-6 PUFAs.\(^1\) The recommended dietary allowance (RDA) of n-3 PUFAs is 1.6 g/day for adult males and 1.1 g/day for adult females.\(^3\) ALA, found in soybean oil and canola oil, is the predominant plant-derived n-3 PUFA, and the
precursor for all other biologically significant n-3 PUFAs.1,2 However, eicosapentaenoic acid (EPA), 20:5(n-3) and docosahexanoic acid (DHA), 22:6 (n-3), found in fatty fish, such as salmon, are more significant sources of n-3 PUFAs in the diet.2

Once absorbed, precursor n-6 and n-3 PUFAs, e.g. LA and ALA, respectively, can undergo elongation and desaturation reactions in the endoplasmic reticulum to add carbon atoms two at a time, or double bonds one at a time.1,2 n-6 PUFAs are important to many different functions in the body, including signal transduction, the synthesis of cell mediators, and the regulation of gene expression. While both LA and AA contribute to the structural components of membranes, LA is especially important for the synthesis of the sphingolipids that prevent water loss from the skin. A deficiency in n-6 PUFAs can lead to growth cessation in young children, dermatitis, and an inability to heal wounds.4 The elongation products of ALA, EPA, and DHA, are antithrombotic and anti-inflammatory cell mediators. DHA also has special implications for retinal function and neuronal development. A deficiency in n-3 PUFAs is marked by a decrease in visual acuity, memory loss, diminished cognitive function, and peripheral neuropathy.1

Both types of essential PUFAs are not typically found to be deficient, especially in western society, yet a number of observational studies indicate that the relative concentration of the two may have serious long-term implications for both cardiovascular and overall health. Early indications that the level of n-3 and n-6 PUFAs in the diet may influence immunity and inflammation have come from
epidemiological studies of populations displaying strikingly low levels of inflammatory diseases, such as acute myocardial infarction, multiple sclerosis, and diabetes mellitus.\textsuperscript{1,5,6}

\textbf{Arachidonic Acid and Eicosanoid Synthesis}

Disturbances such as infection or tissue injury can trigger the initiation of a cascade of events resulting in inflammation. A number of cellular reactions including platelet aggregation, clot formation, vasodilatation, and leukocyte activation produce the characteristic wheal and flare response. The signaling and coordination of these cellular responses are in large part due to a class of inflammatory mediators called the eicosanoids that include, among others, the prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs).\textsuperscript{2} They are derived from n-3 and n-6 20-carbon PUFAs, the most common being AA, which plays a significant role as a major substrate for eicosanoid synthesis in the production and modulation of inflammation.

Following the occurrence of an initiating event, such as tissue injury, cellular phospholipase A\textsubscript{2} becomes activated and catalyzes the hydrolysis of intracellular membrane glycerophospholipids at the sn-2 position, releasing AA within the cytosol of the cell.\textsuperscript{1} Once free within the cell, AA reacts with one of the cyclooxygenases (COX-1, or -2), lipoxygenases (5-, 12-, or 15-LOX), or cytochrome P450 monooxygenases (CYPs).\textsuperscript{1} The products of the COX pathway are modulators of thromboregulatory, inflammatory, and chemotaxic responses,
whereas the products of the LOX pathway are modulators of vascular permeability, vasoconstriction, and bronchoconstriction.\textsuperscript{1}

Reaction with COX-1 produces the intermediate PGH\textsubscript{2}, which is converted to the series-2 PGs and thromboxane A\textsubscript{2} (TXA\textsubscript{2}) by the enzymes prostacyclin synthase, prostaglandin isomerase, and thromboxane synthase.\textsuperscript{1} The prostaglandin PGE\textsubscript{2} is the main pro-inflammatory eicosanoid responsible for a large portion of the inflammatory response by acting to induce fever, pain, vasodilatation, and vascular permeability.\textsuperscript{1,7} TXA\textsubscript{2} also promotes vascular permeability in addition to platelet aggregation, leukocyte adhesion, and both vaso- and bronchoconstriction.\textsuperscript{7} Prostaglandins tend to be formed in a cell-specific manner with monocytes and macrophages producing PGE\textsubscript{2} and PGF\textsubscript{2}, neutrophils producing PGE\textsubscript{2}, and mast cells producing PGD\textsubscript{2}.\textsuperscript{8}

The reaction of AA with 5-Lipoxygenase produces the series-4 leukotrienes (LTA\textsubscript{4}) by catalyzing the insertion of molecular oxygen, followed either by reaction with hydrolase to produce LTB\textsubscript{4}, or conversion to LTC\textsubscript{4}, LTD\textsubscript{4}, or LTE\textsubscript{4} by reaction with the enzyme LTC\textsubscript{4} synthase.\textsuperscript{1} Of the leukotrienes produced along the pathway, LTB\textsubscript{4} seems to have the greatest significance concerning inflammation. Its primary function is that of an inducer of inflammation by stimulating the release of lysosomal enzymes, the generation of reactive oxygen species, leukocyte adherence, leukocyte chemotaxis, and the production of IL-1, IL-6, and TNF-\textalpha.\textsuperscript{7} Like prostaglandins, leukotrienes are also found to be produced in a cell-specific manner, LTB\textsubscript{4} is produced mainly by
neutrophils and macrophages, while LTC₄, LTD₄, and LTE₄ are produced by eosinophils and mast cells.⁹

**n-3 PUFAs and Inflammatory Eicosanoid Production**

By monitoring cellular plasma fatty acid composition and dietary intake, a direct correlation has been demonstrated between an increased dietary intake of n-3 PUFAs, increased incorporation of n-3 PUFAs into cell membranes, and decreased incorporation of n-6 PUFAs.¹⁰ EPA has been shown to be incorporated into plasma and mononuclear cells (MNCs) in a linear dose response manner, and to be associated with a decrease in the production of PGE₂ by MNCs.¹¹ Upon increasing n-3 PUFA content in the diet, EPA and DHA begin to out compete and replace AA at the sn-2 positions of membrane phospholipids, thereby decreasing AA concentration in the tissues, and decreasing its availability as a substrate for the reactions of eicosanoid synthesis.¹⁰ In addition, as indicated by its low turnover rate, 10% to 35% that of AA, EPA’s occupation of the COX active site lasts much longer than that of AA, further reducing the frequency of reaction with AA.¹² Both the substitution of n-3 PUFAs at the sn-2 position of membrane phospholipids and their prolonged occupation of the COX active site, are effective mechanisms by which an increased consumption of n-3 PUFAs can decrease the synthesis of pro-inflammatory mediators from AA.

In addition, as a substrate for both COX and 5-LOX, EPA gives rise to eicosanoids of both altered structure and activity compared to those formed from
AA. The metabolization of EPA by COX-1 generates the 3-series PGs and TX instead of the 2-series PGs and TXA₂.\textsuperscript{1,2} Although they too are pro-inflammatory, a comparison of PGE₃ activity with that of PGE₂ indicates that the 3-series PG is far less potent of a stimulator of inflammation than the series-2 derivative.\textsuperscript{13} In comparison to PGE₂, PGE₃ displays no mitogenic activity, induces COX-2 translation at a level that is four times lower, and stimulates a level of IL-6 secretion that is more than 50% less.\textsuperscript{13} The 3-series TX, TXA₃, also reacts differently than its series-2 counterpart in that it has minimal platelet-aggregating and vasoconstricting activity, such that its synthesis is vasodilatory and inhibiting of platelet aggregation.\textsuperscript{32} Similarly, synthesis of the 5-series LT, LTB₅, rather than the four-series LT, LTB₄, produces a less inflammatory state in that LTB₅ is 10-100-fold less potent of an inducer of inflammation than LTB₄.\textsuperscript{8} Substitution of 3-series PGs for 2-series PGs, and 5-series LTs for 4-series LTs, has the overall effect of reducing inflammation.

Together, a reduction in the synthesis of pro-inflammatory mediators, along with the reduced potency of the alternative eicosanoids synthesized from n-3 PUFAs, lend support to the idea that n-3 PUFAs are anti-inflammatory. More recent findings further support this contention by having identified oxygenated products of EPA and DHA displaying potent anti-inflammatory and immunoregulatory properties.\textsuperscript{14} Those formed from EPA, termed E-series resolvins (resolution phase interaction products), or Rv-E₁, and those formed from DHA, the D-series resolvins (Rv-D₁), both, regulate the infiltration of polymorphonuclear neutrophils (PMNs) and inhibit key mediators of
inflammation, such as TNF-α, helping to resolve an inflammatory reaction.\textsuperscript{14} Their identification in human whole blood, lymphocytes, brain cells, and glial cells suggests that Rv-E1 and Rv-D1 are important neural protectors, Rv-D1 especially, earning the name, neuroprotectin.\textsuperscript{15} This molecule in particular, 10,17S-Docastriene (10,17S-DT), a DHA derivative, has been identified as a principle inhibitor of neuronal injury following the generation of lipid peroxides due to brain ischemia by inhibiting lymphocyte infiltration as well as proinflammatory gene expression.\textsuperscript{16} The generation of E- and D-series resolvins by COX-2, an enzyme that can be induced by IL-6 during an inflammatory reaction, is surprising given that it mostly produces proinflammatory mediators.\textsuperscript{17} The finding that 10,17S-DT may feedback inhibit COX-2 suggests that these lipid mediators serve a highly specialized role whose disruption may be integral to the development of certain of chronic inflammatory conditions.\textsuperscript{18}

The Regulation of Gene Expression by n-3 PUFAs

The effects of fatty acids on gene expression represent a direct control over gene function by essential fatty acids. The transcriptional effects of n-3 and n-6 PUFAs are exerted through regulation of the nuclear receptors, peroxisome proliferator activated receptors (PPARs), liver X receptors (LXRs), sterol regulatory element binding proteins (SREBPs), and nuclear factor κ B (NFκB). Nuclear receptors are ligand-activated transcription factors that directly or indirectly control several genes.\textsuperscript{4,7} Their structural organization generally involves an NH\textsubscript{2}-terminal region, containing a ligand-independent transcriptional
activation domain and a DNA-binding domain, as well as a carboxyl-terminal region containing a ligand-binding domain.\textsuperscript{7}

The first fatty acid receptor to be identified was PPAR\(\alpha\), which was found to regulate genes involved in glucose and lipid metabolism.\textsuperscript{1} Later, other members of the PPAR family (PPAR\(\alpha\), -\(\beta\), -\(\gamma1\), and -\(\gamma2\)) were also discovered, and have since been found to play important roles in cellular differentiation, cancer, insulin sensitization, atherosclerosis, and several metabolic diseases.\textsuperscript{1,7,19} Following ligand-activated dimerization of a PPAR with the retinoid X receptor (RXR) the resulting heterodimer translocates into the nucleus where the complex is able to bind to a PPAR responsive element (PPRE) in the regulatory region of a target gene to promote its expression.\textsuperscript{7,19} Both n-3 and n-6 PUFAs are natural ligands for the PPARs, as too are leukotrienes and prostaglandins.\textsuperscript{19} Although EPA and DHA are more potent activators of PPARs than n-6 PUFAs, the eicosanoids, LTB\(_4\) and PGJ\(_2\), are the most potent activators of PPAR\(\alpha\) and PPAR\(\gamma\), respectively.\textsuperscript{7}

The types of cells that most highly express the PPAR family are mixed depending on the specific PPAR type: PPAR\(\alpha\) is highly expressed in the vasculature, mostly by endothelial cells, smooth muscle cells, and macrophages, PPAR\(\beta/\delta\) are expressed ubiquitously, and PPAR\(\gamma\) is expressed at high levels by adipose tissue and skeletal muscle cells.\textsuperscript{19} Upon activation, PPAR\(\alpha\) modulates lipid metabolism by stimulating \(\beta\)-oxidation and lipoprotein lipase (LPL) activity in addition to inducing the transcription of genes for apolipoprotein A1 (apoA1) and A-II to increase the production of HDL.\textsuperscript{19} The overall effect of PPAR\(\alpha\) activation
is the reduction of triglycerides and free fatty acids and an increase in HDL. Consequently, this fatty acid receptor has important implications for the treatment of hyperlipidemia and the prevention of atherosclerosis.\textsuperscript{19} The effect of PPAR\_\gamma activation is an increased insulin sensitivity by increasing expression of the GLUT-4 transporter and phosphatidyl-3-kinase (P3K).\textsuperscript{19} In addition to the regulation of inflammation, PPARs inhibit nuclear factor $\kappa$ B (NF\_kB), a significant inducer of proinflammatory pathways.\textsuperscript{7}

The two LXR isoforms, LXR\_\alpha and LXR\_\beta, are involved in both fatty acid, and glucose homeostasis as well as cellular differentiation, apoptosis, and a number of other immune responses, but their primary function is the regulation of cholesterol metabolism that, like the expression of the LXR isoforms themselves, is cell-specific.\textsuperscript{1} While LXR\_\beta is expressed ubiquitously throughout the body, LXR\_\alpha is found only in specific tissues.\textsuperscript{1,20} It is expressed predominantly in the liver, but it is also found in the kidneys, intestines, adipose tissue, macrophages, spleen, and skeletal muscle. In the liver, LXR\_\alpha activation has mostly been found to cause the upregulation of Cytochrome P450 enzymes in support of hepatic bile acid synthesis, and in the intestines its activation supports cholesterol secretion by stimulating the production of the transport proteins, ABCG5 and ABCG8.\textsuperscript{20,21,82} LXR\_\alpha regulates the expression of genes involved in hepatic bile acid synthesis, and plays a major role in lipogenesis by regulating the genetic
expression of the sterol regulatory element binding protein-1c (SREBP-1c).\textsuperscript{20} Oxysterol binding induces the activation of LXR mediated gene expression of SREBP-1c as well as that of the reverse cholesterol transport proteins, ABCA1 and ABCG1.\textsuperscript{20} The result of LXR activation is to stimulate lipogenesis and cholesterol efflux. This role implicates LXRα activation, especially in macrophages, as an important safe guard against the development of foam cells known to contribute to atherosclerosis.\textsuperscript{21,22} Excessively high levels of intracellular cholesterol follows both an increased availability of LDL particles in the circulation and chronic inflammation that elevates the number and activity of phagocytic cells.\textsuperscript{21} In addition to preventing against foam cell development, the stimulated upregulation of fatty acid synthesis is thought to also help in reducing the total cholesterol level by buffering the concentration of free cholesterol through the formation of cholesterol esters.\textsuperscript{22} 

There are three SREBP isoforms encoded by two genes (SREBP-1a, -1c, and -2).\textsuperscript{1} They are members of the basic helix-loop-helix, leucine zipper (BHLHLZ) family of transcription factors.\textsuperscript{1} Each of the isoforms is synthesized as a precursor that undergoes a two-step cleavage by two different proteases at the amino-terminus following the signaled need for increased cellular cholesterol. SREBP-1c is present in most tissues, but is up-regulated in the liver, brain, white adipose tissue, adrenal glands, and macrophages.\textsuperscript{1} By binding to sterol regulatory elements (SREs) in the promoter of genes encoding enzymes such as acetyl-CoA carboxylase, fatty acid synthase, and approximately 30 others, SREBP-1c promotes the synthesis of fatty acids and cholesterol.\textsuperscript{20,22}
Increases in PUFA concentration, especially dietary fish oil, have been found to decrease the level of transcriptionally active SREBP-1c through a variety of mechanisms that involve changes in cholesterol homeostasis, gene expression, and the disruption of protein activation. At high levels of PUFAs, the rate of hydrolysis of sphingomyelin in the plasma membrane is elevated, reducing sphingomyelin availability and generating excessive amounts of both ceramide and phosphocholine. The reduction in the availability of sphingomyelin leads to an intracellular displacement of cholesterol that in turn causes a decrease in SREBP transcription, meanwhile the elevated levels of ceramide contribute further to the inhibition of SREBP by disrupting sphingolipid synthesis. A high PUFA concentration has also been found to inhibit binding of SREBP-1c to the SREs of target genes by interfering with the proteolytic cleavage of the transcription factor domain of the SREBP precursor.

The transcription factor, nuclear factor κ B (NFκB) plays an important role in various inflammatory signaling pathways by regulating the expression of a number of proinflammatory cytokines, chemokines, adhesion molecules, and inducible effector enzymes. It becomes activated following the binding of a surface toll-like receptor (TLR) to a small molecular motif on a pathogen’s surface called a Pathogen Associated Recognition Pattern (PARP). This triggers TLR-4 homodimerization and the initiation of a subsequent downstream signaling cascade ending in the degradation of IκB and activation of NFκB. n-3 PUFAs are thought to inhibit this pathway at the point of receptor-ligand interaction, IκB phosphorylation/degradation, and DNA binding. However, the
ability of PUFAs to inhibit at the level of DNA binding, through the interaction of EPA/DHA with PPARα/γ, is the only one of the three to have been demonstrated in vivo as well as in vitro. Elevated levels of n-3 PUFAs enhance transcription factor binding leading to up-regulation of lipid degradation and inhibition of lipid biosynthesis. This finding may have important implications for the treatment of patients with hypertriglyceridemia.

**Inflammation and Inflammatory Diseases**

In addition to supporting tissue repair following infection or injury, the inflammatory response also benefits the removal of pathogens and toxins. The initiation of cell signaling pathways in response to physiological stress such as injury or infection can lead to the rapid production of signaling molecules that are capable of propagating cell-to-cell communication within a short period of time. Following the initiation of inflammation, the movement of inflammatory cells to the site of infection/injury is accomplished with the help of the up-regulation of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on the surface of endothelial cells, allowing leukocyte binding and diapedesis. Arriving first, granulocytes, monocytes, and macrophages function in the killing of pathogens, clearing of cellular debris, and repair of tissues. Cellular activation of monocytes and macrophages upon interaction with an antigen, such as lipopolysaccharide (LPS), triggers the synthesis of proinflammatory cytokines, eicosanoids, and other mediators of inflammation.
Whereas controlled inflammation is beneficial, uncontrolled inflammation can be harmful, and is characterized by the hyperexpression of adhesion molecules, the appearance of soluble forms in the circulation, sequestration of leukocytes in unusual sites, and the over production of inflammatory mediators.\textsuperscript{2,25} Examples of uncontrolled inflammatory reactions are provided by a number of diseases and disorders including rheumatoid arthritis, ulcerative colitis, Crohn’s disease, cardiovascular disease, and acute respiratory distress syndrome.\textsuperscript{9}

Rheumatoid arthritis (RA) is characterized by joint inflammation that manifests as swelling, pain, functional impairment, morning stiffness, osteoporosis, and muscle wasting.\textsuperscript{25} In RA there is a dysregulated T helper 1-type response that promotes the production of inflammatory cytokines, such as TNF-\textit{\textalpha}, IL-1\textit{\textbeta}, IL-6, and IL-8.\textsuperscript{1,2,27} The synovial fluid of patients with active RA has been found to contain PGE\textsubscript{2}, LTB\textsubscript{4}, 5-hydroxyeicosatetraenoic acid, and platelet activating factor.\textsuperscript{27,28} In addition, the expression level of COX-2 as well as that of the adhesion molecules, E-selectin, ICAM-1, and VCAM-1 are up-regulated in the synovial fluid.\textsuperscript{25,27}

The inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn’s disease (CD), are chronic inflammatory diseases of the alimentary tract.\textsuperscript{29} In both UC and CD the underlying pathology is an elevated production of inflammatory cytokines and eicosanoids, specifically LTB\textsubscript{4}, in the intestinal mucosa.\textsuperscript{25,29} While UC mainly involves the mucosa of the colon, CD can occur in any part of the alimentary tract.\textsuperscript{25} The presence of elevated levels of
AA-derived eicosanoids, mainly LTB₄, in the intestinal mucosa of the area affected, suggests a role for the AA-derived lipid mediator in the underlying pathology.²⁹,³¹

For the majority of cardiovascular diseases, such as coronary artery disease and a high percentage of cases of heart failure and stroke, the underlying pathology is atherosclerosis brought on by a combination of risk factors that include dyslipidemia, hypertension, and hyperglycemia.³² At each stage of disease progression, inflammation is identified as a driving force contributing to both the etiology and severity of cellular damage. Monocyte recruitment, for instance, is considered a crucial event in the onset of atherosclerosis by contributing to foam cell generation and plaque formation.³² Other contributing events influenced by inflammation include endothelial dysfunction, LDL oxidation, cell proliferation, and thrombosis.³³ In addition, the inflammatory events of atherosclerosis may also play a significant role in the electrical and structural remodeling associated with atrial fibrillation, an important contributor to stroke, heart failure, and mortality for which available therapies often prove suboptimal.³⁴

Like cardiovascular disease, the development of acute respiratory distress syndrome (ARDS) is marked by the occurrence of distinct biological events despite there being great variation among initiating factors.³⁵ These events include the accumulation and sequestration of PMNs in the lungs, lung endothelium activation and injury, disruption of the alveolar epithelium, and fibroproliferation.³⁵ The accumulation and sequestration of PMNs in the lung
microvasculature is recognized as an early and classical characteristic of ARDS and is found to correlate strongly with, both, bronchiolar alveolar lavage fluid (BALF) levels and plasma levels of IL-8.\textsuperscript{36} Endothelial activation and injury are also fundamental characteristics of the ARDS that lead to increased pulmonary permeability associated with an increased inflammatory response and the development of a protein-rich pulmonary edema.\textsuperscript{37,38}

Damage to the alveolar epithelium in the lung contributes to ARDS pathophysiology by disrupting the alveolar-capillary barrier, further increasing pulmonary permeability, and causing alveolar flooding. During what is termed the fibroproliferative phase of ARDS, activated fibroblasts secrete a number of extracellular matrix proteins within the interstitium and form attachments to damaged basement membranes after migrating into the alveolar space.\textsuperscript{39} Described as being the result of disordered repair caused by severe tissue damage, the impairment of fibrinolytic activity and the enhancement of coagulation results in increased fibrin production and deposition.\textsuperscript{40} Fibrin contributes to the pathogenesis of ARDS patients by further increasing the vascular permeability, inhibiting surfactant functions, and providing a matrix for fibroblasts to migrate and produce collagen.\textsuperscript{41}

Studies of n-3 PUFAs in Rheumatoid Arthritis and Inflammatory Bowel Diseases

In a study conducted by Adam et al. (2003), sixty-eight patients with RA were instructed either to consume a typical western diet (WD) (n=34), having a high level of AA, or to begin eating an anti-inflammatory, modified lacto-
vegetarian diet (AID) containing less than 90 mg/day of Arachidonic Acid.\textsuperscript{42} Additionally, as part of their double-blind, crossover study, both groups were given fish oil tablets (30 mg/kg body weight) for three of the eight months of the study’s duration. The results from this study indicated that those who consumed an AID and were assigned to the placebo group experienced a 14\% decrease in the number of tender or swollen joints, while those who consumed a WD and were assigned to the placebo group experienced no change.\textsuperscript{42} Moreover, those consuming the AID and also receiving fish oil capsules in comparison with those consuming the WD and receiving fish oil capsules had a significantly reduced occurrence of tender (28\% vs. 11\%) and swollen (34\% vs. 22\%) joints (P<0.01) along with reduced levels of \textit{LTB}_4 and 11-dehydro-thromboxane B\textsubscript{2}, indicating inhibition of TXA\textsubscript{2} synthesis.\textsuperscript{42} Although some improvement was observed due to the anti-inflammatory diet alone, the authors found that the greatest benefit was observed among those consuming both an anti-inflammatory diet and the fish oil supplements. Their results make an argument for the importance of supplementation with n-3 PUFAs in addition to the consumption of an anti-inflammatory diet in decreasing the occurrence of RA symptoms.

A number of research trials have reported moderate benefits of n-3 PUFA supplementation among individuals with IBDs, though overall, support for such benefits in the literature is fairly weak. Reported benefits have included reduced production of \textit{LTB}_4, \textit{PGE}_2, and INF-\gamma, as well as improvements in gut mucosal histology and reduced rates of relapse.\textsuperscript{30} In a randomized, placebo-controlled trial conducted by Trebble et al. (2004), patients with CD were administered
either 2.7 g of EPA and DHA a day or a placebo containing olive oil for 24 weeks. They found that n-3 PUFA supplementation had significantly reduced the amount of AA (P=0.006), PGE₂ (P=0.042), and INF-γ (P=0.012) produced by peripheral blood mononuclear cells. While the study conducted by Trebble et al. did not investigate whether there were any clinical benefits other than a reduction in lab values, their results demonstrate that, theoretically, supplementation with n-3 PUFAs should benefit patients with CD. In a study conducted by Belluzzi et al. (1996), 78 CD patients with a high risk of relapse were given either 2.7 g n-3 PUFAs (n=39) or placebo (n=39) a day for one year and found that those receiving n-3 PUFAs had 41% fewer relapses (95%CI: 21%-61%, P<0.001) than those receiving the placebo. In a review of the research concerning the effects of n-3 PUFA supplementation in patients with IBDs, Calder (2008) concluded that although the evidence supportive of any benefits from n-3 PUFA supplementation in patients with IBDs is weak, the findings reported by Belluzzi et al. warrant additional investigation in this area. A number of studies conducted to date have reported clinical improvement in outcomes such as gut histology, clinical score, sigmoidoscope score, relapse, and reduced corticosteroid use, yet meta-analysis of these findings have yet to indicate a significant benefit due to n-3 PUFA supplementation in patients with IBDs.

**Studies of n-3 PUFAs in Cardiovascular Disease**

Since observing a lower rate of sudden death among cultures whose diets are heavily dependent on fish, there have been numerous studies conducted in
order to identify the source of the protective benefits. With many epidemiological and animal studies pointing toward n-3 PUFAs, several large intervention trials have been conducted to investigate the connection further. The Diet and Reinfarction Trial (DART), which investigated the ability of increased fish consumption to prevent a second myocardial infarction (MI), was one of the earliest. It involved 2,033 male survivors of MI, who were either instructed to consume fatty fish twice a week or not given any instruction. The trial lasted two years and reported finding a 29% reduction in all-cause mortality among the intervention group but found no decrease in the risk of MI.

Since the completion of DART, additional support for a role of n-3 PUFAs in reducing coronary heart disease (CHD) mortality was provided by the GISSI-Prevensione Trial, the largest prospective randomized controlled trial to investigate the effects of n-3 PUFAs on CHD mortality and sudden death. The study involved 11,324 patients with diagnosed CHD from 172 health centers across Italy who were randomized to one of four groups: those given 300 mg/d of vitamin E, those given 850 mg/d of n-3 PUFAs, those given both, and those given neither. After three and half years, the study reported finding a 20% decrease in all cause mortality, a 30% decrease in CHD mortality, and a 45% decrease in sudden death among those receiving only n-3 PUFAs. There was no reported effect of vitamin E supplementation, and because all patients were treated with aspirin, β-blockers, angiotensin-converting enzyme inhibitors and cholesterol-lowering medication as needed, the GISSI trial was able to demonstrate an effect of n-3 PUFAs independent of these therapies.
In a meta-analysis of randomized controlled trials studying the effects of n-3 PUFA intake in patients with coronary artery disease, Bucher and colleagues identified 11 trials published between 1966 and 1999 involving a total of 15,806 patients and reporting clinical endpoint data along with at least 6 months of follow-up data. Their results indicated that dietary and nondietary sources of n-3 PUFAs reduced overall mortality (RR=0.8; 95% CI: 0.7-0.9; P<0.001), mortality caused by MI (RR=0.7; 95% CI: 0.6-0.8, P<0.001), and sudden death (RR=0.7, 95% CI: 0.6-0.9; P<0.01), thus finding no significant difference between dietary and nondietary interventions. More recently, León and colleagues performed a systematic review and meta-analysis of the literature investigating the effect of fish oil on mortality and arrhythmias. They ultimately identified 12 randomized controlled trials that investigated fish oil as a dietary supplement in humans reporting arrhythmic end points of appropriate implantable cardiac defibrillator (ICD) intervention and sudden cardiac death. Altogether the studies involved a combined 32,779 patients. Their analyses indicated that fish oil was associated with a significant reduction in the number of deaths from cardiac causes, although no effect on arrhythmias or all cause mortality was found.

The reduction in morbidity and mortality observed following n-3 PUFA supplementation is hypothesized to be associated with the suppression of cardiac arrhythmia and the reduction of atherogenesis. The anti-arrhythmic properties of n-3 PUFAs are believed to be partly due to their ability to inhibit voltage-gated sodium channels, which in turn produces a larger relative refractory period, requiring a larger voltage for membrane depolarization, and
causing a reduction in heart rate. Additionally, the ability of n-3 PUFAs to maintain the integrity of L-type calcium channels is thought to be protective by preventing calcium overload during times of stress. The ATTICA study (2007), which involved 1514 men (18-87 years of age) and 1528 women (18-89 years of age) has provided new insight into the anti-arrhythmic properties of n-3 PUFAs. The study, which lasted two years and took place in Attica, Greece, found that those who consumed >300 g fish/wk had a mean heart-corrected QT duration (QTc) that was 13.6% lower than that of nonconsumers of fish. This is significant due to there being a strong correlation between heart rate and the QT interval, which is a measure of the amount of time required for repolarization of the ventricle myocardium. This is such that the QTc is a more relevant measure of arrhythmia risk.

The reduction of atherogenesis by n-3 PUFAs has often been attributed to the reduction of specific atherothrombotic risk factors, such as thrombosis, hyperlipidemia, and hypertension. The significance of haemostatic factor modulation by n-3 PUFA is supported by a number of epidemiological studies. Both, fibrinogen and factor VII have been shown by prospective studies to be independent predictors of cardiovascular events, and a number of other hemostatic molecules have also shown promise as prognostic indicators. The population-based cross-sectional study, atherosclerosis risk in communities (ARIC) study investigated whether dietary intake of n-3 PUFAs had an effect on the blood levels of coagulation proteins, including many implicated in the development of atherogenesis. The study's results indicated an inverse
association between fish consumption and the hemostatic risk factors: fibrinogen, FVIII, and von Willebrand Factor (vWF), suggesting that the anti-thrombotic properties of n-3 PUFAs contribute to the cardioprotective nature of these molecules. However, no association has been found in either the Coronary Artery Risk Development in Young Adults (CARDIA) study (1988) or the Optimal n-6/n-3 Ratio in the UK Fiet (OPTILIP) trial.

The ability of n-3 PUFAs to reduce plasma triglyceride levels is supported by a number of published studies. In patients with hypertriglyceridemia, 4 g/d of n-3 PUFAs were found to reduce serum triglyceride concentration by 25-30%, increase serum LDL concentration by 5-10%, and increase HDL concentration by 1-3% with no effect on total cholesterol. In a randomized controlled trial of 59 CHD patients receiving 10-40 mg/d of simvastatin and either 840 mg/d EPA plus DHA or a placebo for twelve months, those receiving n-3 PUFAs were found to have a 20-30% (p<0.005) decrease in serum triglyceride concentrations and a 30-40% (p<0.005) decrease in VLDL cholesterol levels, with no increase in LDL.

The effect of n-3 PUFAs on blood pressure (BP) has been demonstrated to be small, dose-dependent, and directly related to the magnitude of BP elevation. Prior meta-analyses have reported a reduction of 3.4/2.0 mm Hg following the administration of a median dose of 5.6 g/d n-3 PUFAs, and more recently, 2.1/1.6 following administration of a median dose of 3.7 g/d. Most recently, these findings have been supported by the International Study of Macro- and Micro- nutrients and Blood Pressure (INTERMAP), a cross-sectional,
epidemiological study of 4680 men and women, ages 40-59, from 17 population-based samples in China, Japan, United Kingdom, and the United States. The findings from INTERMAP indicate an inverse relationship between n-3 PUFA consumption and BP, even in normotensive individuals. The antihypertensive properties of n-3 PUFAs are thought to be due to the combined actions of EPA and DHA to increase systemic arterial compliance (SAC), an indicator of arterial health, and improve endothelial function through the release of NO.

Studies of n-3 PUFAs in Mechanically Ventilated Patients
In light of supportive findings from both animal and human studies for the anti-inflammatory benefits of n-3 PUFAs, Gadek and colleagues (1999) investigated the benefits of a high fat, low carbohydrate diet supplemented with EPA, DHA, GLA, and antioxidants among mechanically ventilated patients with ARDS. They evaluated 98 mechanically ventilated patients with ARDS receiving enteral nutrition in the intensive care units (ICUs) of five different U.S. hospitals. They measured arterial blood gases, recorded ventilator settings, collected bronchoalveolar lavage fluid (BALF), and monitored clinical outcomes at the time of study entry as well as on study days 4 and 7. The study group received an enteral nutrition formula that was both isocaloric and isonitrogenous to that received by the control group. The experimental formula was supplemented with 6.9±0.3 g/d EPA, 2.9±0.1 g/d DHA, 5.8±0.3 g/d GLA, and antioxidants including vitamin C (1127±49 mg), vitamin E (413±18 IU), and beta-carotene (6601±285 μg). Their results indicated that while both the control and
study groups had comparable total cell and neutrophil counts at baseline, a significant drop in each was observed on day 4 for patients in the study group that was maintained through day 7 compared to the control group (p=0.0083; p=0.0081). They also found a significant improvement in PaO2/FIO2 (p=0.001) among patients receiving the study diet, finding lower values for FIO2, PEEP, and minute volume on day 4 and day 7. In addition, the authors found improvements in the clinical outcomes of patients in the study group compared to the control group. Patients receiving the study diet required fewer days of ventilator support (11 vs. 16.3 days; p=0.011), spent fewer number of days in the ICU (12.8 vs. 17.5 days; p=0.016), and experienced a fewer percentage of new organ failures (8% vs. 28%; p=0.015). Their study was the first to demonstrate the potential clinical benefits of an enteral formula containing n-3 PUFAs in mechanically ventilated patients as well as the safety and tolerance of such a formula in this patient population.58

In a continuation of this study, Pacht and colleagues (2003) investigated the benefits of the study diet compared to the control as described above on inflammatory markers in a subset of patients at the Ohio State University Medical Center, who were enrolled in the study conducted by Gadek et al. In addition to those measures reported by Gadek et al., Pacht and colleagues collected data on BALF concentration of total protein, ceruloplasmin, transferrin, IL-8, IL-6, TNF-α, and LTB4 BALF at baseline as well as on study days 4 and 7. Their findings illustrate a direct correlation between improvements in inflammatory mediators and clinical outcomes. The authors found significant reductions in the BALF
concentrations of both ceruloplasmin and IL-8, as well as nonsignificant decreases in total protein, neutrophils, IL-6, TNF-α, and LTB₄ that mirrored a significant improvement in oxygenation among patients in the study group.⁵⁹

While their study lacked sufficient power to properly investigate these outcomes, the finding of a significant association between several inflammatory mediators and clinical outcomes in the study diet group compared to the control group adds considerable support for a role of n-3 PUFAs and antioxidant therapy in immunomodulation to improve clinical outcomes in patients on a ventilator.⁵⁹

In a study conducted by Pontes-Arruda et al. (2006), 165 mechanically ventilated ICU patients with sepsis or septic shock at a tertiary care center in Brazil were randomized to either a study group to receive enteral nutrition containing 4.9±0.14 g/d EPA, 2.2±0.06 g/d DHA, 4.6±0.13 g/d GLA, and elevated levels of antioxidants or a control group to receive an isocaloric, isonitrogenous formula for seven days.⁶⁰ In addition to monitoring weaning and respiratory parameters, the authors also collected patient information concerning mortality up to 28 days following study completion. Their findings indicated significant improvements in oxygenation status, weaning, and ICU discharge among patients in the study group, supporting the findings of previous reports. Their findings also illustrated a significant reduction in the 28 day mortality rate of patients in the study group compared to the control group (33% vs. 52%), with patients on the study diet experiencing an absolute reduction in mortality of 19.4% (p=.037) and a relative risk of death of 0.63 (95%CI: 0.39-1.00).⁶⁰
In a recent meta-analysis of these three studies by Pontes-Arruda and colleagues (2008) the combined results for 296 mechanically ventilated patients receiving an n-3 PUFA enteral nutrition formula were evaluated. The results of the meta-analysis indicated a 60% reduction in the risk of 28 day in hospital all-cause mortality among patients on the study diet (enteral formula supplemented with n-3 PUFA + antioxidants) compared to the control diet (an enteral formula, isonitrogenous and isocaloric to that of the study diet) (OR=0.40; 95% CI: 0.24-0.68; p=.001), as well as increased number of ventilator-free (mean increase=4.9 days; p<.0001) and ICU-free days (mean increase=4.3; p<.0001).

In addition, the authors report finding a significant reduction in the risk of new organ failure among those receiving the study diet (OR=0.17; 95%CI: 0.08-0.34; p<.0001), and significant improvements for a number of respiratory variables, including FIO₂, minute ventilation, tidal volume, and oxygenation status.

Over the past ten years there has been a considerable amount of research conducted to investigate the clinical significance of n-3 PUFA supplementation in patients with a wide range of conditions from CHD to cachexia. Research investigating the effects of n-3 PUFA supplementation in mechanically ventilated patients has been significant but is far from comprehensive. There are three areas in particular that require additional research to address gaps in the current literature. The first is that the majority of studies investigating n-3 PUFA supplementation in mechanically ventilated patients, including those discussed above, have used study and control diets high in fat (55%), a diet for which there is a poor understanding of its clinical
relevance. There is a need for additional research in which the study and control formulas contain a lower percentage of fat (29-44%). The use of high fat diets is a biologically plausible method of improving blood gas variables, however such diets also lead to increased n-6 PUFA intakes and may potentially skew study findings.

Second, the current understanding regarding the clinical utility of enteral nutrition supplemented with n-3 PUFAs has been largely dictated by the manufacturer of these formulas. The research studies discussed above by Gadek et al. (1999) and Pontes-Arruda et al. (2006) as well as a number of others were sponsored by the manufacturer of the enteral formulas used, and although the integrity of their findings is not being questioned, the lack of a more cost-effective alternative to their product is. The previously mentioned studies investigated the benefits of the two formulas Oxepa® and Pulmocare®, both of which are produced by Abbott Nutrition, formerly known as Ross, a division of Abbott, a global, broad-based health care company. The high cost of these products considerably limits their use in a health care setting. Research investigating the clinical feasibility and utility of a standard product with fish oil supplement is needed to identify cost-effective alternatives to these high priced formulas.

Lastly, there have been no studies conducted to date that have investigated the feasibility of an enteral formula supplemented with n-3 PUFAs in chronic, mechanically ventilated patients in a long term acute care hospital setting. This setting serves a considerable patient population that could
potentially benefit greatly from an increased n-3 PUFA intake. Based on the number of patients in 2005 to have required mechanical ventilation in nine U.S. states, it is estimated that the incidence rate of mechanical ventilation in the U.S. is 227.5 per 100,000 people per year and rising due to demographic trends. At $38,000 per admission, mechanically ventilated patients represent an expensive and significant health care concern that cost the U.S. $11.1 billion in 2005. Immunonutrition is recognized as a valid approach to reducing hospital stay and improving outcomes among mechanically ventilated patients. Nutritional support with an enteral formula having an elevated n-3 PUFA content and reduced n-6 PUFA content is thought to benefit ventilator weaning. Weaning outcome has been shown to be a modifiable risk factor that contributes to the development of poor health outcomes following mechanical ventilation. A significant percentage of the patient population at long-term acute care hospitals are admitted specifically for weaning from the ventilator. It seems appropriate then that research be conducted to address the feasibility of n-3 PUFA supplementation in this setting.

**Purpose**

This study was conducted to determine the feasibility of a planned double-blind, randomized, placebo-controlled trial to determine the effects of EPA + DHA on inflammation, infections, weaning, length of stay and mortality for mechanically ventilated patients in a LTACH setting. We expected that the
methodologies proposed for this investigation and described below would be practical and feasible in a LTACH setting.

**Methods**

*Study Design, Patient Identification, Enrollment, and Randomization*

The study design of this research trial was a randomized, double-blind, placebo controlled clinical trial. This thesis examined data on the first three participants who enrolled in the study and completed study related assessments. This thesis also evaluated inclusion and exclusion criteria for the planned study by examining persons screened for potential study inclusion. Reasons for exclusion were discussed.

Potential study participants were identified via daily prospective screening of all patients admitted to the Drake Center requiring mechanical ventilation. Initial eligibility was determined by the primary investigator (PI) or a trained research nurse coordinator upon chart review using a standardized screening tool. Following this initial review the Medical Director of Pulmonary Services at the Drake Center (Dr. Bauer) assured eligibility.

Inclusion and exclusion criteria are provided in Table 1 and the screening form used to assess patient eligibility is provided in the Appendix. The criteria for inclusion in the study were that the patient be admitted as an in-patient at the Drake Center, require positive-pressure ventilation, receive enteral tube feeding, be between the ages of 18 and 80 years of age, inclusive, and have a rapid shallow breathing index (RSBI) greater than 80. The RSBI was calculated by
dividing the respiratory rate by the tidal volume and was used to assess a patient’s readiness for extubation. An RSBI<105 breaths/min/L was first described by Yang and Tobin (1991) as a threshold value capable of predicting weaning failure and is considered an acceptable criteria for weaning to extubation. This initial threshold was based on a definition of weaning failure that included failure of a spontaneous breathing test that has since been recognized as an insignificant event. More recent studies, in defining weaning failure as simply extubation failure following a successful breathing trial, suggest that lower RSBI values offer a predictive advantages in assessing a patients readiness to be weaned. In studies conducted by Smina et al. (2003) and Upadya et al. (2005) RSBI values of 88 and 80, respectively, where found to be associated with extubation failure in comparison to the respective values: 60 and 50. An RSBI value greater than 80 was used in this study as an indicator that a patient was not yet ready to be weaned, and therefore appropriate for inclusion in this research trial.

Patients were excluded from the study for being pregnant, having a history of post-cardiac arrest with suspected anoxic brain injury, a platelet count < 30,000/µL, a history of ventricular tachycardia (VT) or atrial fibrillation (AF), HIV, metastatic cancer, or a history of organ transplantation. In addition, patients were excluded if they had received Oxepa fish oil in the last 14 days, had an activated partial thromboplastin time (aPTT) greater than 33.5s, or were currently receiving a treatment dose of heparin or coumadin. The aPTT is the amount of time, in seconds, that it takes for the patient’s plasma to clot after the addition of
Table 1. Inclusion and Exclusion Criteria for Study Entry

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<tbody>
<tr>
<td>Drake Center in patient</td>
<td>Receiving rh-APC for sepsis</td>
</tr>
<tr>
<td>Requiring positive-pressure mechanical ventilation</td>
<td>Diagnosed with HIV</td>
</tr>
<tr>
<td>Receiving enteral tube feeding</td>
<td>Metastatic cancer</td>
</tr>
<tr>
<td>Age &gt; 18 years</td>
<td>History of bone marrow, lung, liver, cardiac, kidney, or pancreas transplant</td>
</tr>
<tr>
<td>RSBI &gt;80</td>
<td>Receiving any Oxepa fish oil in the last 14 days</td>
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Once enrolled, participants could be terminated from the study for the following reasons: 1) platelet count <30,000/µL; 2) international normalized ratio (INR) >3.0; 3) enteral tube removal; 4) death. However, to assure subject safety, subjects would continue to be followed per the study protocol. Platelet and INR lab values were conservatively chosen based upon data from the phase III trials of recombinant human-activated protein C (rh-APC).\(^{62,63}\) Based on these trials, it was recommended that rh-APC not be given to patients with platelets<30,000 per microliter of blood or an INR>3.0, both of which are associated with an increased risk of severe bleeding.\(^{78}\) The INR was calculated by dividing the patient’s prothrombin time (PT) by that of a normal control group and raised to the power of an international sensitivity index determined by the manufacturer of the PT.
A normal platelet count is between 150,000 and 450,000 per microliter of blood and a value of 30,000/μL. With rh-APC, the infusion was stopped if platelets or INR reached these thresholds. The bleeding risk with fish oil is theoretical and has not been proven (unlike rh-APC). If an enrolled subject experienced these lab values, administration of fish oil or placebo would stop, however, the subject would continue to be in the study and be monitored per protocol. Thereafter, if platelets and INR returned to inclusion criteria values (platelets≥30,000 or INR≥3.0), administration of fish oil or placebo could resume with approval from the participant’s pulmonologist.

The Drake Pharmacy was responsible for randomizing each subject following an established protocol. Per protocol, the pharmacist was unblinded and was responsible for treatment assignments, formulations, and maintaining the list of codes revealing participant assignments. Patients were randomized in a 1:1 ratio to receive either enteral fish oil or placebo. Study drug delivery began within 6 hours of randomization.

**Materials and Supplies**

The fish oil product was supplied by Nordic Naturals as a sterile liquid in labeled 4-ounce bottles. It, along with the placebo, was prepared and packaged into opaque syringes by the Drake Center Pharmacy. One case of fish oil was obtained from the Nordic Natural Company (California). All fish oil used was from the same processing batch and was delivered in one single shipment. One vial of fish oil was tested by an independent lab prior to starting the study and it
was confirmed that the EPA and DHA concentration was approximately 350 mg and 250 mg per liter, respectively. The oil was also tested for contaminants including heavy metals, and was found to be contaminant free.

**Intervention and Standard Care**

Patients randomized to the treatment group received 8g of EPA+DHA per day in divided doses as 7.5mL of fish oil delivered enterally every 6 hours. Doses were administered at 12:00 AM, 6:00 AM, 12:00 PM, and 6:00 PM. This dose was chosen because it is similar to the amount of EPA that patients in the prior Oxepa® trial received per day. In the prior study conducted by Gadek et al. (1999) the study group received 6.9±0.3 g EPA and 2.9±0.1 g DHA per day. The saline placebo was given enterally in the same manner as the fish oil. Both were prepared and packaged by the Drake Pharmacy in opaque syringes.

The fish oil product received from Nordic Naturals, a producer of pharmaceutical grade fish oil, has a lemon scent and only a minimal fish smell. Flavor is irrelevant since the product was delivered through a feeding tube. Both the fish oil and placebo were packaged in tinted syringes to ensure visual resemblance and fish oil was rubbed on the outside of both the syringes containing placebo and those containing fish oil so that both syringe types would smell identical as well. Saline was chosen for the placebo because it is inert. Fish oil or placebo was administered until removal of the enteral tube, 48 hours of unassisted breathing, or 14 days after initiating the study, whichever occurred first. In patients who started to eat foods orally, fish oil or placebo would continue
to be given via the enteral tube (not orally) to minimize unintentional unblinding. If a patient’s feeding tube was no longer clinically indicated, it would be removed as per usual practice and fish oil administration would stop (an enteral tube would not be left in place for the sole purpose of continuing the study).

The pharmacy was responsible for storing, processing, and packaging fish oil and placebo for administration. The primary nurse caring for the patient was responsible for administering the syringe contents into the enteral tube using standard enteral procedures. All nursing staff were trained in study procedures, including the need for the subject and the nurse to remain blinded. The only non-blinded staff was the Pharmacist. The PI and other research staff remained blinded unless unblinding was needed for patient management or data safety monitoring. Brown opaque 10 cc syringes were used to maintain blinding of the patient and nurse.

All participants were provided standard LTAH care under Dr. Brauer’s direction. As per standard nutrition care at the Drake Center, feeding strategy was not protocolized in this study and was decided by Dr. Bauer and the staff nutritionists. The Drake Center does not use Oxepa as a standard enteral formula choice so use of this formula among usual care participants was not a confounder.

Data Collection

Completed screening forms were reviewed for the first 32 patients screened. Chart abstraction for demographic, laboratory, and physiologic data occurred at study entry, daily during the 14-day intervention, and weekly for the
remainder of the hospitalization. An example of the daily checklist used to monitor drug administration and continuation criteria is provided in the Appendix along with the study form used to collect data on respiratory status, Systemic Inflammatory Response Syndrome (SIRS) criteria, and infectious events. Dichotomous variables of wean (yes/no), the presence of SIRS criteria (yes/no), and infectious events were among the data collected on a daily basis. The four SIRS criteria assessed during the study included the presence (yes/no) of a white blood cell count greater than 12,000 cells/mm$^3$, body temperature greater than 38°C, heart rate greater than 90 beats per minute, and respiratory rate greater than 20 breaths per minute. Blood was drawn by a Drake phlebotomist as is standard procedure, and blood samples for study days 1, 5, and 7 were then processed by the Drake Laboratory and stored in a Sub Zero freezer to minimize sample degradation. The Drake Lab is accredited by the College of American Pathologists and is CLIA certified. The samples were batched and sent to Case Western Reserve University Inflammatory Mediator Core Services to be tested for C reactive protein, IL-6, and IL-8. Each sample will be evaluated for an individual analyte in duplicate plus a standard curve and internal controls were run to assure quality control, however the results of this evaluation have not yet been obtained and so will not be presented as part of this thesis.

Data Analysis

Ultimately, the primary endpoints of the larger, planned study are blood concentrations of C reactive protein, IL-6 and IL-8. However, these variables will
not be assessed for this thesis as the results of lab testing on the blood draws have not yet been received. Instead, with regard to the inflammatory markers, this report will consider simply whether blood draws were properly collected. Secondary endpoints to be assessed in the larger, planned study as well as in this thesis for the first three participants include infectious events defined by finding blood stream infections; ventilator acquired pneumonia; hospital acquired pneumonia; and other infections (e.g. urinary track infections, cdiff). In addition, a dichotomous variable of wean (yes, no); numerical variables of length of free ventilator (days) and length of total ventilator (days); a count variable of numbers of on/off the ventilator, and a numerical variable of length of stay (days) in hospital are included in this report as well. In addition, completed screening forms were analyzed to determine the frequency in which the patient population met or did not meet various inclusion/exclusion criteria.

As part of the final analysis for this thesis, numerical variables were summarized by mean (standard deviation) and/or median (range); and categorical and/or binary variables were summarized by frequency (or rate, in %) and/or odds. Odds ratios (OR) were calculated using the equation \((a/b)/(c/d)\) in reference to a typical two by two table of disease status vs. exposure variable. The equation used to calculate the 95% confidence interval (CI) for the OR was \(\ln(OR) = \ln(OR) \pm 1.96 (1/a + 1/b + 1/c + 1/d)^{0.5}\). The mean 95%CI was calculated using the equation, \(\mu \pm (t_{n-1,1-\alpha/2})(SE_x)\), where \(\mu\) is the mean, \(t_{n-1,1-\alpha/2}\) is the t-statistic specific to the sample size and \(\alpha\) value, and \(SE_x\) is the standard error.
For all calculated statistical measurements presented in this thesis, an \( \alpha \)-value of 0.05 was used.

**Results**

The results presented herein are for the first three participants randomized in the trial and the first 32 patients screened for eligibility. Review of the first three patients’ medical records and study trial logs indicated that the study protocol was carried out as planned and described in “Methods.” Additionally, all study outcomes were collected as planned indicating that methodologies employed for this clinical trial were feasible in a LTACH setting. Importantly, the lab results for the EPA+DHA study solution indicated that its composition was 69.60% n-3 PUFAs, 35.89% EPA, and 24.27% DHA, which is very close to the values expected (Table 2).

<table>
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<th>Fatty Acid</th>
<th>% of Fat</th>
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<tr>
<td>( \alpha )-Linolenic acid</td>
<td>0.74</td>
</tr>
<tr>
<td>Stearidonic acid</td>
<td>2.75</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>0.90</td>
</tr>
<tr>
<td>Gadoleic acid</td>
<td>3.24</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>1.37</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>35.89</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>3.72</td>
</tr>
<tr>
<td>Erucic acid</td>
<td>1.41</td>
</tr>
<tr>
<td>Docosapentaenoic acid</td>
<td>4.57</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>24.27</td>
</tr>
</tbody>
</table>
Results for Participant A

Patient A was a fifty-seven year old white female diagnosed with acute respiratory failure stemming from a case of *Pseudomonas* pneumonia. Her medical history states that she has diabetes type II and is a smoker with chronic obstructive pulmonary disease (COPD) and coronary artery disease (CAD). She was found to meet all of the inclusion criteria, and was enrolled in the study on 01/01/2009. After enrolling in the study, she spent a total of three days on the ventilator before weaning, and remained in the study for nine days after coming off of ventilator support (Table 3). She remained in the study until 01/12/2009 when she dropped out of the study complaining of a bad taste, which she attributed to the study treatment. Over the course of the study, she was found to have a white blood cell (WBC) count greater than 12,000 cells/mm$^3$ only once, and that was on the second day of the study. Normally, individuals in a non-inflammatory state have WBC count levels between 5,000 and 10,000 cells/mm$^3$. Her temperature was consistently reported to be below 38ºC and her heart rate was never indicated to have been above 90 beats per minute (bpm). Her respiratory rate (RR) however was consistently found to be above 20 breaths per minute (BPM) every day except for two. SIRS is a condition that is diagnosed on the basis of whether at least two of four criteria are present. As described in the methods section, these criteria include a HR>90 bpm, a RR>20 BPM, a WBC count> 12,000 cells/mm$^3$, and a temperature >38ºC. Over the course of the study duration, Participant A met the diagnostic criteria for SIRS only on study day 2 when both her RR and WBC count were found to be above
the indicated values. While a participant in the trial she was treated for two episodes of infection that occurred prior to her enrollment in the trial, a *Pseudomonas aeruginosus* sputum infection diagnosed on 12/30/2008 and a urinary tract yeast infection diagnosed on 12/30/2008, which were each classified as an “Other Infection.” Both infections were indicated to have been resolved on 01/05/2009. The total number of days she spent in the hospital was 22. Pharmacy records indicated that she had been randomized to the study group and had been receiving the EPA+DHA solution.

**Results for Participant B**

Patient B was a forty-two year-old white female diagnosed with ARDS with respiratory failure stemming from a case of Emphysematous polynephritis with *Actinomyces israelii* septicemia. Her medical history states that she has diabetes type II and is a smoker. She was found to meet all of the inclusion criteria, and was enrolled in the study on 01/03/2009. After enrolling in the study she spent a total of two days on the ventilator before weaning, and remained in the study for ten days after coming off of ventilator support (Table 3). She remained in the study until 01/14/2009 when she dropped out of the study complaining of a bad odor, which she attributed to the study treatment. Over the course of the study she was never found to have a WBC count greater than 12,000. Her temperature was consistently reported to be below 38°C, and her heart rate was above 90 bpm every day that she was enrolled. Her RR was found to be above 20 BPM for the first three study days but not afterward. Participant B met the
diagnostic criteria for SIRS on study days 1 through 3 by having both a HR and RR above normal values. While a participant in the trial she experienced no new infectious events. The total number of days she spent in the hospital was 29. Pharmacy records indicated that she had been randomized to the control group and had been receiving the placebo.

Results for Participant C

Patient C was a fifty-three year-old African American female diagnosed with respiratory failure stemming from an intracranial hemorrhage. Her medical history stated that she has diabetes type II. She was found to meet all of the inclusion criteria, and was enrolled in the study on 01/07/2009. After enrolling in the study she spent a total of nine days on the ventilator before weaning, and remained in the study for five days after coming off of ventilator support (Table 3). She remained in the study until 01/21/2009 when she completed the 14-day study period. Over the course of the study she was never found to have a WBC count greater than 12,000, and her temperature was consistently reported to be below 38ºC except for on study day 1. Her heart rate was never above 90 bpm, but her RR was found to be above 20 BPM on days 1, 5, 6, 7, 9, and 13. Participant C did not meet the diagnostic criteria for SIRS on any day while enrolled in the research trial. While a participant in the trial she did not experience any infectious events, however her chart did indicate that she began treatment for a Staphylococcus aureus infection on 12/27/2008 and for ventilator pneumonia on 01/05/2009. No indication could be found as to if or when these
infectious events were resolved. The total number of days she spent in the hospital was 50. Pharmacy records indicated that she was randomized to the study group and had been receiving the EPA+DHA solution.

Table 3. Results for Participants A, B, and C

<table>
<thead>
<tr>
<th>Participant</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Acute Respiratory Failure</td>
<td>Respiratory Failure</td>
<td>Respiratory Failure</td>
</tr>
<tr>
<td>Initiating Event</td>
<td>Pneumonia</td>
<td>Pylonephritis</td>
<td>Intracranial Hemorrhage</td>
</tr>
<tr>
<td>Weaned (Yes/No)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Days on Ventilator</td>
<td>3</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Days off Ventilator</td>
<td>9</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Days in Study</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Length of Stay (Days)</td>
<td>22</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>Randomization</td>
<td>Study Group</td>
<td>Control Group</td>
<td>Study Group</td>
</tr>
</tbody>
</table>

**Results of Individual Screening Data**

To date, of the 32 patients screened for possible inclusion in this clinical trial (excluding the three patients enrolled in the study), the average age of all patients screened, whose age had been recorded (N=29), was 56.1 years (range, 19-79; 95% CI: 50.11-62.09). The date of birth was recorded appropriately for every patient screened except for three, whose date of birth was not indicated or inaccurate. Seventeen (53%) of the patients screened were male and fifteen (47%) were female, 27 (84%) were Caucasian and four (16%) were African-American. The average age of all males screened for whom date of birth was reported accurately (n=15) was 55.93 ±8.92 (range, 19-78; 95% CI: 47.01-64.85), and the average age of all females screened, for whom age has appropriately recorded, (n=14) was 56.28±8.25 (range, 18-79; 95% CI: 48.03-64.53). There were 10 patients who did not meet the first four inclusion criteria: of
these, seven did not require positive-pressure ventilation, one did not require enteral tube feeding, and two did not require either positive-pressure ventilation or enteral tube feeding (Table 4).

Of the 22 patients who were not indicated to have not met the first four criteria for inclusion, four were excluded solely due to a history of VT or AF, three for having an aPTT > 33.5s, and one for receiving a treatment dose of heparin or coumadin (Table 4). In addition, three patients were excluded for both having an aPTT > 33.5s and receiving a treatment dose of heparin or coumadin, and three others were excluded for both having a history of VT or AF and receiving a treatment dose of heparin or coumadin. One patient was excluded for having an aPTT > 33.5s, a history of VT or AF, and receiving a treatment dose of heparin or coumadin. One patient was excluded for having an aPTT > 33.5s and a history of VT or AF, and another patient had an aPTT > 33.5s, a history of VT or AF, and was HIV positive. Two patients were found to not have an RSBI > 80, two declined enrollment, and one was found to be scheduled to have his/her enteral tube taken out too soon to participate.

Overall, there were 13 patients screened who had a history of VT or AF, 11 who were receiving a treatment dose of heparin or coumadin, 10 with an aPTT > 33.5s, and one patient each was found to be post-cardiac arrest with suspected anoxic brain injury, HIV positive, or diagnosed with metastatic cancer (Table 4). A history of VT or AF was solely responsible for the exclusion of the most patients, followed by an aPTT > 33.5s, and receiving a treatment dose of heparin or coumadin.
Table 4. Screening Results for Males and Females (N=32)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs:</td>
<td>56.1±5.99 (N=29)</td>
</tr>
<tr>
<td>Gender, n (%):</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (53%)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (47%)</td>
</tr>
<tr>
<td>Did Not Meet First Four Inclusion Criteria: (N=10)</td>
<td></td>
</tr>
<tr>
<td>No Positive-Pressure Ventilation (PPV)</td>
<td>7</td>
</tr>
<tr>
<td>No enteral tube feed</td>
<td>1</td>
</tr>
<tr>
<td>No PPV or tube feed</td>
<td>2</td>
</tr>
<tr>
<td>Met First Four Inclusion Criteria but Not Enrolled: (22)</td>
<td></td>
</tr>
<tr>
<td>Hx of Ventricular Tachycardia or Fibrillation</td>
<td>4</td>
</tr>
<tr>
<td>aPTT &gt;33.5s</td>
<td>3</td>
</tr>
<tr>
<td>Receiving a Trt Dose of Heparin or Coumadin</td>
<td>1</td>
</tr>
<tr>
<td>aPTT &gt;33.5s and a Hx Vent Tach/Fib</td>
<td>1</td>
</tr>
<tr>
<td>aPTT &gt;33.5s &amp; Received Trt Dose of H/C</td>
<td>3</td>
</tr>
<tr>
<td>Hx of Vent Tach/Fib &amp; Received Trt Dose of H/C</td>
<td>3</td>
</tr>
<tr>
<td>aPTT &gt;33.5s, Hx Vent Tach/Fib, &amp; Trt Dose H/C</td>
<td>1</td>
</tr>
<tr>
<td>aPTT &gt;33.5s, Hx Vent Tach/Fib, &amp; HIV Positive</td>
<td>1</td>
</tr>
<tr>
<td>RSBI &lt;80</td>
<td>2</td>
</tr>
<tr>
<td>Declined Enrollment</td>
<td>2</td>
</tr>
<tr>
<td>NG Tube removed</td>
<td>1</td>
</tr>
<tr>
<td>Frequency of Individual Exclusion Criteria:</td>
<td></td>
</tr>
<tr>
<td>Hx of Ventricular Tachycardia or Fibrillation</td>
<td>13</td>
</tr>
<tr>
<td>Receiving a Trt Dose of Heparin or Coumadin</td>
<td>11</td>
</tr>
<tr>
<td>Post Cardiac Arrest with Suspected Brain Injury</td>
<td>1</td>
</tr>
<tr>
<td>HIV Positive</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic Cancer</td>
<td>1</td>
</tr>
</tbody>
</table>

Of the 17 males who were screened, 13 met at least the first four inclusion criteria. Of these, four were excluded solely for having a history of VT or AF, four were excluded for having a history of VT or AF in combination with one or more of the other exclusion criteria, and three were excluded for having an aPTT>33.5s and receiving a treatment dose of heparin or coumadin (Table 5). One patient was eligible for the study but was scheduled to have his feeding tube removed within 24 hours of screening and one patient’s RSBI was less than 80. Males were more likely than females to have a history of VT or AF, however this
finding did not reach significance (OR=3.09; 95% CI: 0.69 to 13.69). None of the male patients screened were excluded solely for having an aPTT>33.5s.

Table 5. Screening Results for Males (N=17)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>(N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs:</td>
<td>55.93±8.92</td>
</tr>
<tr>
<td>Did Not First Four Meet Inclusion Criteria:</td>
<td>(N=4)</td>
</tr>
<tr>
<td>No Positive-Pressure Ventilation (PPV)</td>
<td>3</td>
</tr>
<tr>
<td>No PPV or tube feed</td>
<td>1</td>
</tr>
<tr>
<td>Met First Four Inclusion Criteria but Not Enrolled:</td>
<td>(N=13)</td>
</tr>
<tr>
<td>Hx of Ventricular Tachycardia or Fibrillation</td>
<td>4</td>
</tr>
<tr>
<td>aPTT &gt;33.5s</td>
<td>0</td>
</tr>
<tr>
<td>Receiving a Trt Dose of Heparin or Coumadin</td>
<td>0</td>
</tr>
<tr>
<td>aPTT &gt;33.5s &amp; Received Trt Dose of H/C</td>
<td>3</td>
</tr>
<tr>
<td>Hx of Vent Tach/Fib &amp; Received Trt Dose of H/C</td>
<td>2</td>
</tr>
<tr>
<td>aPTT &gt;33.5s &amp; Hx Vent Tach/Fib</td>
<td>1</td>
</tr>
<tr>
<td>aPTT &gt;33.5s, Hx Vent Tach/Fib, &amp; HIV Positive</td>
<td>1</td>
</tr>
<tr>
<td>RSBI &lt;80</td>
<td>1</td>
</tr>
<tr>
<td>NG Tube removed</td>
<td>1</td>
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</tbody>
</table>

Of the 15 females screened, nine met at least the first four exclusion criteria. Of these, three were excluded solely for having an aPTT >33.5s, one solely for receiving a treatment dose of heparin or coumadin, and one for not having an RSBI >80 (Table 6). One patient was excluded for having a history of VT or AF and receiving a treatment dose of heparin or coumadin, and another patient had a history of VT or AF, an aPTT >33.5s, in addition to receiving a treatment dose of heparin or coumadin. Two patients were eligible for the study but declined enrollment. Females were found to be less likely than males to have an aPTT>33.5s, however this relationship was weak and not statistically significant (OR=0.67; 95%CI: 0.14 to 3.03). None of the female patients screened were excluded solely for having a history of VT or AF.
### Table 6. Screening Results for Females (N=15)

<table>
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<tr>
<th>Characteristic</th>
<th>N=14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs:</td>
<td>56.28±8.25</td>
</tr>
<tr>
<td>Did Not Meet Inclusion Criteria:</td>
<td>N=6</td>
</tr>
<tr>
<td>No Positive-Pressure Ventilation (PPV)</td>
<td>4</td>
</tr>
<tr>
<td>No tube feed</td>
<td>1</td>
</tr>
<tr>
<td>No PPV or tube feed</td>
<td>1</td>
</tr>
<tr>
<td>Met First Four Inclusion Criteria but Not Enrolled:</td>
<td>N=9</td>
</tr>
<tr>
<td>Hx of Ventricular Tachycardia or Fibrillation</td>
<td>0</td>
</tr>
<tr>
<td>aPTT &gt;33.5s</td>
<td>3</td>
</tr>
<tr>
<td>Receiving a Trt Dose of Heparin or Coumadin</td>
<td>1</td>
</tr>
<tr>
<td>Hx of Vent Tach/Fib &amp; Received Trt Dose of H/C</td>
<td>1</td>
</tr>
<tr>
<td>aPTT &gt;33.5s, Hx VentTach/F, &amp; Trt Dose of H/C</td>
<td>1</td>
</tr>
<tr>
<td>RSBI &lt;80</td>
<td>1</td>
</tr>
<tr>
<td>Declined Enrollment</td>
<td>2</td>
</tr>
</tbody>
</table>

**Discussion**

The discussion to follow pertains to the first three study participants randomized to a planned randomized clinical trial currently being conducted at the Drake Center, a long-term acute care hospital (LTACH) facility in Cincinnati, Ohio. The aim of the study is to investigate the effects of enteral fish oil (EPA+DHA) supplementation on inflammatory markers, infection, weaning, and clinical outcomes among a population of mechanically ventilated patients. This being the first study to investigate the benefits of such an intervention in this patient population, the feasibility and efficacy of the intervention are unknown.

Each of the endpoints being investigated in this research were collected and are reported here except for the inflammatory markers, which are being sent as batches of fours to be processed. An assessment of the collection and storage of these specimens indicated that they were all collected appropriately, were drawn at the correct times, and were stored under suitable conditions as described in the methods section. Upon review of daily checklists and other trial
logs it was found that all trial procedures and protocols were consistently followed.

Though unable to draw many specific conclusions regarding study outcomes for the first three study participants, the cases do illustrate some interesting feasibility issues pertaining to the planned trial. While the three participants had similar diagnoses, their ARDS stemmed from initiating events that differed significantly from one another. How this will ultimately influence the study’s outcome is not entirely clear. Previous research involving patients with ARDS encountering similar variation in their patient populations have previously suggested that such conditions pose a considerable challenge to the interpretation of study results. With there being a number of potential sources of variation encountered in this study that may be difficult or impossible to take into consideration or control for, such as manufacturer variation in enteral formula, patient’s socioeconomic status, family history, and genetic variation, any opportunity to reduce this variation may have important implications for the elucidation of a clinically significant relationship.

Another interesting point to be made concerning the first three participants is the fact that all three enrollees were female despite there having been more males screened. Moreover, of the six patients who met the study criteria, but were not enrolled for various reasons, only one was male. Analysis of patient screening records indicated that males and females were often excluded for different reasons. Males were more likely than females to be excluded solely for having a history of ventricular tachycardia or ventricular fibrillation, and females
were more likely than males to be excluded solely for having an \( \text{aPTT} > 33.5 \text{s} \).

Overall, though not statistically significant, males were found to be three times more likely than females to have a history of VT or AF and 1.5 times more likely to have an \( \text{aPTT} > 33.5 \text{s} \). These initial results suggest that for this patient population there may be a difference between males and females regarding their suitability for the intervention. Rates of CHD are typically higher for men than they are for women, and from these initial results it seems possible that this trend extends to male patients in this population such that a history of VT or AF is much more common in men than women. This is one potential explanation for the observed differences between men and women in the screening data, however more investigation is needed in order for a better understanding of any potential relationship to be elucidated.

The decision to exclude patients with a history of VT was based on the findings of Raitt et al. (2005) and Brouwer et al. (2006), in which both agreed there to be little or no benefit of n-3 PUFA supplementation in patients with implantable cardioverter defibrillators (ICDs).\(^7^2,^7^3\) The study by Raitt et al. involved 200 patients from six U.S. medical centers, who had recently received an ICD following a documented episode of ventricular fibrillation (VF) or VT. Participants were given either 1.8 g of fish oil (40% EPA and 30% DHA) or a placebo (olive oil) and were followed for a median of 718 days (range, 20-828).\(^7^2\) Though not statistically significant, the study found that ICD therapy for VT or VF was more common among the group that received fish oil than among those who received the placebo. This was interpreted by the authors to suggest that fish oil
may have proarrhythmic effects in individuals with an ICD and a history of VT or VF.  In the study conducted by Brouwer et al. (2006) 546 patients with an ICD and a history of VF or VT from 26 European cardiology clinics were given either a placebo (sunflower oil) or 2 g of fish oil (961 mg n-3 PUFA; 464 mg of EPA, 335 mg of DHA, and 162 mg of other n-3 PUFAs) a day. The results of this study, did not support of the conclusion made by Rait et al. but also failed to find a statistically significant benefit of n-3 PUFA supplementation among patients with an ICD and a history of VT/VF. However, they did report finding a statistically insignificant trend toward a longer event-free survival in the fish oil group compared to the placebo group (P=0.13). These two studies along with a third and similar study by Leaf et al. (2005) that reported data from the Fatty Acid Antiarrhythmia Trial (FAAT) were recently reviewed in a meta-analysis by Jenkins et al (2008). FAAT included 402 patients with an ICD, who were given either fish oil containing 2.6 g/d of EPA+DHA or a placebo containing olive oil. The study’s results indicated that the group taking fish oil had 32% fewer fatal arrhythmias than those receiving the placebo (RR=0.62; 95% CI, 0.39-0.97; P=0.037). In addition, the meta-analysis performed by Jenkins et al. using data from these three articles reported an overall RR of 0.93 (95% CI, 0.70-1.24; p=0.63) and significant heterogeneity (p=0.04) in patient outcomes among individuals with ICDs following supplementation with n-3 PUFAs. These findings support the general conclusion that although some patients are likely to benefit from supplementation with DHA and EPA, there is a reasonable possibility that others may not, and the potential
for fish oil to be harmful for certain individuals has not been sufficiently resolved in the literature. The lack of certainty found in the literature justifies the exclusion of patients with a history of VT from this study, especially in such a high risk population as that which is being investigated in this research trial.

It is encouraging that none of the first three patients experienced any serious adverse events and that all three eventually weaned from the ventilator. None of the three experienced any new infections while enrolled in the study, and all were eventually discharged. It is notable however that Participants A and C each experienced two infectious events prior to their enrollment in the study. In the case of Participant A, these infections were not resolved until four days after entry, and for Participant C it could not be determined when or if these prior infections were resolved. Prior infections are likely to affect participants’ response to fish oil supplementation. Participants entering the study with an infection or having recently resolved an infection are more likely to have elevated levels of inflammatory markers, meet SIRS criteria, have a more severe condition, and differ in their responsiveness to fish oil supplementation compared to participants entering the study with no prior infection. Although Participant A’s prior infectious events were noted on the study data log along with the date of resolution, neither one of Participant C’s prior infections or their outcomes were recorded. In order to account for the potential effects due to recent or current infections among study participants, it is necessary that they be accounted for and their date of resolution recorded. If left unaddressed this issue may be
responsible for there being some questions left unanswered regarding the utility of n-3 PUFA supplementation in this health care setting.

Secondary to concerns for the potential affect of recent or current infectious events on study outcomes is the potential affect that antibiotics or other medications may have on outcomes. A participant entering the study with an infection seems more likely to be prescribed antibiotics than a participant who is infection free. A participant receiving antibiotics could reasonably be expected to be less susceptible to developing new infectious events than participants who are not receiving antibiotics. There is a possibility then that patients entering the study with an infection might have a decreased risk of developing new infections than participants who enter the study without any recent or current infections. It may be advantageous to account for this potential source of variation if it is determined that a difference in risk exists, but if this population represents a sizable portion of the patients treated at the Drake Center then doing so might also limit the study’s relevance to a significant segment of the patient population in this setting.

Two of the three patients did complain about either a bad taste or a bad odor, which they attributed to the study drug. Study participants should perhaps be asked to comment further on such complaints to determine whether or not this represents a significant concern and the best way for it to be addressed. Being that the complaints were made by one patient who was receiving the study medication and one who was not, further inquiry may be helpful in determining the reasons for similar concerns in the future. If participants think they are
receiving fish oil, they may be more inclined to report a bad odor or taste than if they were completely unaware of which study group they have been randomized.

In rubbing fish oil on the outside of syringes containing both the fish oil solution and the placebo, study participants may be more likely to draw the conclusion that they are in the study group than they otherwise would. This could potentially also make participants more likely to complain of a bad taste or odor. Participants might be less likely to attribute these perceptions to being in the study if they are not given a reason to suspect that they are being administered syringes containing the fish oil solution. If participants’ suspicions concerning their randomization are found to contribute to the process whereby they come to attribute a bad taste or odor to the research trial, then it might be more effective to develop another means of disguising syringe contents.

There are many complications associated with prolonged ventilation, such as ventilator induced lung injury, ventilator associated pneumonia, nosocomial infection, hyponutrition, sepsis, and cardiac failure that act only to further impede and possibly prevent patient recovery while also elevating health care costs.76 Extubation at the first possible day is a continuing goal in the treatment of mechanically ventilated patients. In addition to improving ventilation techniques this goal requires improved weaning protocols, better methods of identifying patients ready to wean to unassisted breathing, as well as more effective support therapies, of which nutritional support is an area with considerable potential to contribute to the improvement of patient outcomes.
Conclusion

The results of this feasibility study suggest that the current trial investigating the effects of EPA and DHA at Drake Center is going well and according to the research methodology mapped out previously. However, there were a couple of areas that were identified as being of potential concern. These include the variation in initiating events observed among the first three participants, an all female enrollment, the enrollment of patients with a recent or current infection, and the significance of participant complaints concerning the taste/odor of the study medication.
References


Appendix
Screening Form

Patient ID: ________________________________
Screener: ________________________________
Date: __________
Time: __________

**Inclusion Criteria**

<table>
<thead>
<tr>
<th>Drake Center in-patient:</th>
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<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requiring positive-pressure mechanical ventilation 24hrs/day:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiving enteral tube feeding:</td>
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<td></td>
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<tr>
<td>Age 18-80 years, inclusive:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSBI &gt;80:</td>
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**Exclusion Criteria**

<table>
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<tr>
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<th>No</th>
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</thead>
<tbody>
<tr>
<td>Pregnant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count &lt; 30,000, active bleeding, or INR&gt;3.0:</td>
<td></td>
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</tr>
<tr>
<td>aPTT &gt; 33.5 s:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of Ventricular Tachycardia or Atrial Fibrillation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiving rh-APC for sepsis:</td>
<td></td>
<td></td>
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<tr>
<td>Diagnosed with HIV:</td>
<td></td>
<td></td>
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<tr>
<td>Metastatic cancer:</td>
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<tr>
<td>History of bone marrow, lung, liver, cardiac, kidney, or pancreas transplant:</td>
<td></td>
<td></td>
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<tr>
<td>Received Oxepa fish oil in the last 14 days:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiving treatment dosage of Heparin or Coumadin:</td>
<td></td>
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</tr>
</tbody>
</table>

**Demographics:**

Date of Birth: _______________
Sex: _______________
Ethnicity: _______________
Primary Diagnosis: _______________
Initiating Event: _______________

**Medical History:**

CHF/COPD: Y/N
Smoking: Y/N
Diabetes: Y/N

Enrolled (Y/N): __________
Daily Checklist

Day:____  Patient ID:___________________
Date:_____

**Drug Administration:**

<table>
<thead>
<tr>
<th>Dose Time:</th>
<th>12:00AM</th>
<th>6:00AM</th>
<th>12:00PM</th>
<th>6:00PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initials: (to be completed by licensed nurse)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Continuation Criteria:**

| Date/Time/Initials: |         |        |         |        |
| Atrial Fibrillation or VTach (Y/N): |         |        |         |        |
| DVT/PD (Y/N):       |         |        |         |        |
| Active Bleeding (Y/N): |         |        |         |        |
| Platelet Count < 30,000 (Y/N): |         |        |         |        |
| INR >3.0 (Y/N):     |         |        |         |        |
| aPTT >33.5 (Y/N):   |         |        |         |        |
| Rate Irregular (Y/N): |         |        |         |        |
| Treatment dose Heparin or Coumadin (Y/N): |         |        |         |        |

*Contact PI, Mary Kaplan (Pager#: 269-5537), if answered Yes to any of the above do not give dose.*

<table>
<thead>
<tr>
<th>Sign Name</th>
<th>Initials</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

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Respiratory Status, SIRS, Infectious Events

Patient ID: ______________

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
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</tbody>
</table>

**Respiratory Status:**

To be completed by RT

- Date:
- Time:
- Initials:
- On Vent (Y/N):
- Hours Off Vent:

**SIRS:**

To be completed by study nurse

- WBC > 12,000: (Y/N)
- Temperature > 38°C: (Y/N)
- HR > 90 BPM (Y/N):
- RR > 20 BPM or PaCO2 < 4.3 kPa (Y/N):

**Infectious Events:**

To be completed by study nurse

- Date/Time/Initials:
- New Infectious Event (Y/N):
- Origin:
- Date:
- Date Resolved:

<table>
<thead>
<tr>
<th>Origin</th>
<th>Sign Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood Stream Infections</td>
</tr>
<tr>
<td>2</td>
<td>Vent Aquired Pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>Hospital Aquired Pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>Other Infections</td>
</tr>
</tbody>
</table>

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