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I, Jessica C Harvey, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
The Effects of Fish Oil (EPA+DHA) on Chronic Ventilator Patients in a Long Term Acute Care Setting: A Randomized Control Trial

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University of Cincinnati
The Effects of Fish Oil (EPA+DHA) on Chronic Ventilator Patients in a Long Term Acute Care Setting: A Randomized Control Trial

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Master of Science in Nutrition in the Department of Nutritional Science of the College of Allied Health Sciences

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Abstract

**Purpose:** The aim of this study was to determine whether patients in a long term acute care setting who received an enteral supplement containing EPA+DHA, would have a shorter weaning time from the ventilator, a decrease in length of hospital stay, decreased inflammatory markers, and number of infections from baseline to post-treatment compared to patients who received a placebo of normal saline solution.

**Methods:** Nine participants who required mechanical ventilation and enteral nutrition support in a long term acute care hospital were randomized to either receive the treatment fish oil (n=5) or the placebo saline solution (n=4). Participants in the treatment group were given 8g fish oil per day through the enteral feeding tube for 14 days. Subjects randomized to the control group received a blinded saline solution for 14 days. All enteral supplement formulations were created by a certified pharmacist.

**Results:** There were no significant differences between the treatment and control groups in regards to time to weaning, percent of days on the ventilator, or length of hospital stay. Inflammatory markers did not significantly decrease in either group from baseline to post-treatment. There was a trend towards a lower rate of infection in the treatment group compared to the control group, however these results were not significant.

**Conclusion:** The results of this randomized double blind clinical trial suggest that adding fish oil (EPA/DHA) to enteral formulas does not result in reduced weaning time for patients on a ventilator, decreased length of hospital stay, or markers of inflammation. Additional research needs to be conducted with a larger group of participants to determine whether a high dose of EPA +DHA from fish oil can decrease inflammation in this specific population.
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Introduction- Content of the Review

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), are common disease states of patients who require ventilatory support in the intensive care unit setting (1). ALI and ARDS are acute life-threatening forms of hypoxic respiratory failure due to persistent pulmonary and systemic inflammation (2). Although many advances have been made in the pathophysiology of respiratory failure diseases, new developments in nutritional interventions to improved clinical outcomes in these patients have yet to be fully investigated and completely understood.

Mechanically ventilated patients often suffer from respiratory distress, which is frequently caused by an inflammatory condition. These patients are generally are unable to meet the increased caloric needs from trauma and inflammation eating solely by mouth, therefore nutrition support therapy in this group of patients is extremely important (3). The polyunsaturated fatty acids, linoleic acid, an omega-6 fatty acid, and alpha linolenic acid, an omega-3 fatty acid, are the dietary elements supplemented into many enteral formulas given enterally to critically ill patients. Specifically, the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from fish oil have been shown to have anti-inflammatory effects in humans (1).

Recently, several prospective, randomized, controlled studies have been conducted and have shown that the administration of an enteral nutrition formula rich in omega-3 fatty acids and elevated amounts of antioxidant vitamins to critically ill patients with lung injury and impaired oxygenation status produced significant improvements in oxygenation and lung compliance, and decreased length of ventilation and ICU stay (1, 4-6). Also, several studies have recently been conducted which confirm the beneficial effects of the addition of fish oil, gamma-linolenic acid as borage oil, and elevated antioxidants added to enteral nutrition formulas on
clinical outcomes of patients on a ventilator such as more ventilator-free days, more ICU free
days, improved oxygenation levels, and a lower incidence of reduced new organ failure (4-6).
These specialized enteral nutrition formulas composed of the anti-inflammatory fatty acids and
elevated levels of antioxidants have empirically been shown to be beneficial in the clinical
management of patients needing ventilatory support in the intensive care unit setting.

Although several randomized double blind studies have been conducted in patients
suffering from acute lung injury and requiring ventilatory support in an intensive care setting, to
our knowledge, there have been no studies which investigate the effects of an enteral formula
enriched with fish oils given to patients who require chronic ventilatory support in the long term
acute care hospital (LTACH). Therefore, the current study focuses on critically ill patients
requiring mechanical ventilation in the LTACH setting. Previous studies conducted in this
specific population examined the effect of the addition of a combination of fish oil, borage oil,
and elevated antioxidants in a standard enteral formula. Presently, it is unknown whether the
improved clinical outcomes are from the combination of EPA, DHA, gamma-linolenic acid, and
antioxidants, or are improved because of only one of these components. This study specifically
focuses on the anti-inflammatory effects of only the addition of an elevated dose of EPA+DHA
added to standard enteral formula (containing none of these fatty acids) compared to a standard
enteral formula containing a placebo (normal saline) in mechanically ventilated patients in a
LTACH.

Part 2: Metabolism of long chain polyunsaturated essential fatty acids

Essential fatty acids are defined as polyunsaturated fatty acids which are necessary for
growth and normal physiological function, such as cellular uptake and molecular translocation;
however they cannot be synthesized in the body (7). These fatty acids must be obtained through the diet since they cannot be completely made in the body. The present Western diet contains a much higher ratio of omega-6 fatty acids compared to omega-3 fatty acids. In order to maintain good health and prevent fatty acid deficiency, a moderate amount of both essential fatty acids should come from the diet. Current recommendations suggest higher ratio of omega 3-polyunsaturated fatty acids to omega-6 fatty acids. (8)

Linoleic acid (LA, 18:2) is an omega-6 polyunsaturated fatty acid consumed in the diet. Linoleic acid is metabolized by Δ6 desaturase to form gamma-linolenic acid (GLA, 18:3) in the liver. GLA is abundant in borage oil and human milk and can be rapidly metabolized to longer chain metabolites. GLA is elongated to form di-homo-gamma-linolenic acid (DGLA, 20:3). DGLA is incorporated into tissue and immune cell phospholipids and is a precursor to arachidonic acid. This conversion takes place through the action of via Δ5 desaturase. It is also converted to the 1-series prostaglandins, PGE1, which promotes pulmonary vasodilatation, and is shown to have anti-inflammatory effects (8).

Arachidonic acid (20:4) is the most abundant of the omega-6 polyunsaturated fatty acids and is synthesized from linoleic acid, which is found in animal products such as meat, dairy, and egg yolk. Arachidonic acid is a precursor for the synthesis of prostaglandins and thromboxanes, which are eicosanoid pro-inflammatory mediators.

Unlike the long chain metabolites of linoleic acid, those derived from the omega-3 precursor, alpha-linolenic acid (ALA 18:3), must be obtained completely from the diet. ALA, present in canola and sunflower oil, is the main precursor to docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5), which are synthesized from ALA by a sequence of chain elongation and desaturation steps, specifically by Δ5 and Δ6 desaturases.
EPA is an important fatty acid in the omega-3 family and is present in high concentration in fish oils. It is the predominant precursor to resolvins and the prostaglandin 3-series, which have anti-inflammatory effects (9). EPA and DGLA compete with arachidonic acid for eicosanoid synthesis in order to create more of the anti-inflammatory-derived eicosanoids (2).

The typical modern diet contains a higher ratio of omega-6 fatty acids to omega-3 fatty acids. Because of this disproportion, alpha-linolenic acid can only be partially transformed to EPA. Therefore, EPA and arachidonic acid must compete over enzymatic incorporation into phospholipids and transformation into eicosanoids (1). The eicosanoids derived from the omega-3 fatty acids have less biological potency for inducing cellular responses than the arachidonic-derived eicosanoids; therefore the omega-3 fatty acids are usually associated with decreased inflammatory responses (10). This lower cellular response creates a decreased amount of the anti-inflammatory prostaglandins, leukotrienes, and thromboxanes produced from EPA, which are less potent than their arachidonic acid-derived proinflammatory equivalents (1). Therefore, the ratio of omega-6 to omega-3 fatty acids in the diet must favor the omega-3 fatty acids in order to potentially reduce the inflammatory state at the cellular and systemic level.

DHA is another omega-3 fatty acid derived from fish oil and is most often the end point of alpha-linolenic acid metabolism in animal tissues. This long chain omega-3 fatty acid is shown to inhibit the prostaglandin synthase cyclooxygenase enzymes from being produced (11). Also, via the action of lipoxygenases, it is the precursor to resolvins and protectins, which have anti-inflammatory and immuno-regulatory actions (9).

Resolvins or - resolution phase interactive products- are mediators which perform biological functions relevant for the resolution of inflammation (12). These mediators are formed from the di-oxygenation of EPA or DHA. Resolvin E₁, produced from EPA, was identified in the resolution phase of inflammation. The E-series resolvins have been shown to have potent anti-
inflammatory actions through neutrophil, macrophage, dendritic and T-cell pathways (9). Resolvin produced from DHA are known as D-series resolvin. Protectin, or neuroprotectin, D₁ and the aspirin-triggered resolvin block T cell and polymorphonuclear neutrophil migration, promote T cell apoptosis, decrease TNF-α and INF-γ secretion, reduce airway inflammation, and exert neuroprotective action during ischemia-reperfusion injury (13). These functions are associated with the anti-inflammatory response; therefore, these resolvin may explain some of the anti-inflammatory actions of omega-3 fatty acids (9).

Each series of resolvin is defined by its unique structure, anti-inflammatory properties, and cellular protective actions (13). The discovery of resolvin and protectin produced from DHA has shown that DHA is at least as important as EPA as an anti-inflammatory agent (9). Both of these anti-inflammatory mediators are produced from the omega-3 precursor ALA. In the diet, EPA and DHA are derived primarily preformed from fish oil, and their effects on lipid mediators of inflammation are continually being investigated.

Part 3: The role of lipid inflammatory markers in the pathophysiology of critical illness

Inflammation is the process of a biological response of a tissue to injury and is the body’s defense mechanism which protects the immune system from infection. In order to restore homeostasis at the damaged site, macrophages initiate pathogen killing which is characterized by redness, swelling, heat, pain, and loss of function (9). The inflammatory response begins the immunologic process of the elimination of invading pathogens and toxins and to repair damaged tissue (14). Although inflammation is a normal biological response to injury, when it occurs in an uncontrolled manner, it can cause excessive damage to host tissues and can create an inflammatory disease state (14). The inflammatory response is a defense mechanism for many
acute and chronic conditions and is characterized by the activation of inflammatory cytokines, arachidonic acid-derived eicosanoids, adhesion molecules, and inflammatory mediators (15).

A family of bioactive mediated polyunsaturated fatty acids, known as eicosanoids, are signaling agents generated from the 20 carbon polyunsaturated fatty acids and are key mediators and regulators of inflammation (9). Eicosanoids are synthesized from the principal precursor of the polyunsaturated omega-6 fatty acids, arachidonic acid, and are the mediators and regulators of inflammation. Arachidonic acid is the major substrate for eicosanoid synthesis because of its abundance in inflammatory cells. Excessive eicosanoid production is present in the inflammatory state and modulates the intensity and duration of the inflammatory response (11).

The eicosanoids are important intracellular signaling agents which are synthesized by three families of enzymes, the cyclooxygenases (COXs), epoxygenases, and lipoxygenases (LOs). The most dominant family of enzymes involved in eicosanoid production is the COX enzymes, which initiate the formation of prostaglandins, thromboxanes, and prostacyclin (16). The COX enzyme is present in two specific isoforms: COX-1 and COX-2. COX-1 is known to be present in most of the tissues in the body and is specifically found in the lining of the stomach. COX-2 is induced in inflammatory cells and is responsible for the increased production of prostaglandins (11). The COX enzymes also metabolize arachidonic acid which forms prostanoids, prostaglandin E2 and thromboxane A2 (8). The amount of eicosanoids synthesized is dependent on the availability of arachidonic acid, the activity of phospholipase A2, and the activity of COX and LO enzymes (17).

Prostaglandins activate the pro-inflammatory response and are produced when white blood cells rush to the site where tissues are damaged. The specific eicosanoid prostaglandin E2 (PGE2) has several pro-inflammatory effects in the body such as inducing fever, increasing vascular permeability and vasodilatation, eliciting pain, and increasing the production of IL-6,
which is an important mediator of the acute phase response (17). PGE₂ is present in high
concentrations at the site of inflammation, produced by macrophages and endothelial cells (18),
and is a potent inhibitor of the pro-inflammatory cytokines TNF-alpha and IL-1. Inflammation
has been associated with the production of PGE₂ from the upregulation of COX-2 inhibitors,
which increase the amount of PGE₂ in the cells.

Another eicosanoid derived from arachidonic acid with a prominent role in inflammation
is leukotriene B₄ (LTB₄). LTB₄ is produced from leukocytes in response to inflammatory
mediators. Leukotrienes are synthesized from arachidonic acid by the 5-lipoxygenase pathway
which gives rise to hydroxyl and hydroperoxy derivatives and the 4-series leukotrienes,
specifically LTB₄ (17). This pro-inflammatory leukotriene has been shown to increase vascular
permeability, initiate leukocyte chemotaxis, enhance local blood flow, intensify the generation of
reactive oxygen species, and enhance the production of TNF- alpha, interleukin-1, and
interleukin-6, which all have pro-inflammatory effects (11).

Thromboxane is another type of eicosanoid produced from arachidonic acid by the COX-
1 pathway. The COX-1 enzyme pathway is essential for thromboxane formation in blood
platelets, and for maintaining the integrity of the gastrointestinal epithelium (17). Thromboxane
A₂ is a proinflammatory eicosanoid, which facilitates platelet aggregation, leukocyte adhesion,
and induces bronchoconstriction (18). TXA₂ is produced by macrophages, neutrophils, and
platelets in the inflammatory response.

In comparison to the arachidonic-derived 2 and 4 series eicosanoids, metabolism of EPA
and DHA by the COX enzymes and 5-LO enzymes forms EPA-derived eicosanoids, which are
prostanoids and leukotrienes of the 3 and 5 series. The major physiologic actions of TXA₃,
PGE₃, and LTB₅ are anti-inflammatory by inhibiting platelet aggregation, chemotaxis, and cell
adhesion (8). The EPA-derived eicosanoids are much less potent than the arachidonic-derived
eicosanoids. As a result, as the relative amount of omega-3 fatty acids increases, more
prostaglandins of the 3 series and leukotrienes of the 5 series are produced, which can enhance
the anti-inflammatory effects from EPA and DHA (19).

Part 4: Anti-inflammatory effects of EPA+DHA

DHA and EPA are the two essential omega-3 fatty acids predominately found in fish oil.
Ingestion of fish oil leads to an elevated cellular concentration of these omega-3 fatty acids in the
body. Omega-3 polyunsaturated fatty acids aid in reducing inflammation in the lungs due to the
reduction of arachidonic acid in the inflammatory cell membrane. Chronic supplementation of
the omega-3 fatty acids as alpha linolenic acid (ALA) increases the amount of EPA in humans
however; this conversion is not viable during critical illness because the enzymatic conversion of
ALA to EPA is inhibited by stress hormones (8). Therefore, critically ill septic patients are not
able to effectively convert the dietary ALA to EPA in a large enough quantity to displace the
arachidonic acid from the cell membrane phospholipids (8). For this reason, enteral formulas
supplemented with EPA and DHA may be beneficial to critically ill patients with ongoing acute
or chronic inflammatory processes in order to control the inflammatory response.

Modulation of the inflammatory response by the omega-3 fatty acids can be attributed to
the use of the same metabolic pathways and enzymes that metabolize EPA or DHA instead of
arachidonic acid (12). These polyunsaturated fatty acids replace arachidonic acid in cell
membranes and inhibit the conversion of arachidonic acid to the eicosanoids prostaglandin E₂,
thromboxane A₂, and leukotriene B₄. Therefore, both EPA and DHA produce beneficial effects
through alterations in membrane structure and function and gene transcription, which down-
regulate the inflammatory response and improve immune function (12).
Increased consumption of the long chain omega-3 polyunsaturated fatty acids results in an increased proportion of these fatty acids in inflammatory cell membrane phospholipids at the expense of arachidonic acid (17). As a consequence, arachidonic acid is converted to prostacyclin, which inhibits platelet aggregation, and to a weaker form of thromboxane, which promotes platelet aggregation and is a vasoconstrictor (17). EPA inhibits arachidonic acid from phospholipids by phospholipase A2 and also inhibits the oxygenation of arachidonic acid by COX enzymes (11). Because of the reduction of arachidonic acid in the cell membranes, the omega-3 polyunsaturated fatty acids can aid in reducing inflammation in the lungs (8). The amount of arachidonic acid is decreased by the consumption of omega-3 fatty acids, therefore the substrate is unavailable for the production of eicosanoids which in turn leads to a decrease in the inflammatory response.

EPA decreases leukotriene production which induces inflammation, leukocyte adherence, and proliferation at the site of injury. By reducing blood viscosity and increasing red blood cell membrane fluidity, EPA improves oxygen supply to tissues (9). EPA can favorably modulate proinflammatory eicosanoid production from arachidonic acid (2). EPA is a substrate for the COX and 5-LOX pathway enzymes and is metabolized to the 3 series prostaglandins, which use linolenic acid as the fatty acid base, and to the 5 series leukotrienes. EPA is a potential COX substrate for the synthesis of PGE3 which has little efficient inflammatory activity. EPA is also a 5-lipoxygenase substrate which can lead to the formation of LTB5 which also has little inflammatory effect (20).

DHA aids in decreasing inflammation by suppressing the COX-2 enzymes and blocking interleukin-1 and tumour necrosis factor- alpha (TNF-α) (7). Fish oil and DHA have been shown to reduce the level of thromboxane and increase prostacyclin levels which can lead to enhanced tissue perfusion and oxygen delivery due to vasodilatation and decreased blood viscosity (7).
DHA is also known to be crucial to fetal brain development and functionality (7). Because of their proposed usefulness in promoting oxygen delivery and vasodilatation, the essential fatty acids EPA and DHA derived from fish oil have been increasingly more prevalent in numerous human and animal studies which confirm their anti-inflammatory effects.

The combination of the EPA, from fish oil, plus gamma-linolenic acid (GLA), from borage oil, supplementation was derived from several studies which used pig and rodent models of sepsis-induced ARDS (21-23). The main hypothesis of these studies was the combined benefit of EPA and GLA could reduce the severity of inflammatory injury by altering the availability of arachidonic acid in the tissue and immune cell phospholipids. Therefore, this combination of fatty acids might favorably alter the inflammatory response while promoting vasodilatation and improved oxygen delivery (2). As an example, one of the studies, conducted by Mancuso et al (21), investigated the anti-inflammatory effects of EPA, as fish oil, and GLA, as borage oil, in rats with endotoxic-induced ARDS. These researchers examined the effects of a diet enriched with EPA and GLA on lung permeability, intrapulmonary eicosanoid biosynthesis, and pulmonary neutrophil accumulation in the rats (21). The results showed that when compared to an omega-6 polyunsaturated enriched diet composed of corn oil, the diets supplemented with EPA or EPA+GLA were protective against increased pulmonary microvascular protein permeability and hypotension in the rats (21). Also observed was a reduced synthesis of the 2- and 4-series arachidonic acid derived mediators accompanied by a decrease in the amount of arachidonic acid and an increase in the amount of EPA and DGLA in the cell membranes (21). This study indicated that changes in lung fatty acid phospholipids composition with EPA and EPA +GLA- supplemented diets were associated with a suppressed synthesis of proinflammatory 2-series prostaglandins and 4-series leukotrienes (8). Therefore, the results of this study
suggested that the increased synthesis of EPA could be related to the anti-inflammatory effects on the rats.

Long chain omega-3 fatty acids from fish oil decrease the production of the inflammatory eicosanoids and adhesion molecules by decreasing overall arachidonic acid availability and by forming anti-inflammatory resolvins (17). Therefore in general, the omega-3 fatty acids diminish inflammatory and adverse vascular responses because of their specific effects on cytokines and eicosanoid production (10). Long chain omega-3 fatty acids are potentially useful anti-inflammatory agents and may produce benefits in specific disease states associated with inflammation and vascular pathology (10), such as in critically ill patients on mechanical ventilators.

Part 5: Immunonutrition in Ventilator Dependent Patients

In mechanically ventilated critically ill patients, malnutrition is a serious concern. Malnutrition is associated with impaired immune function, impaired ventilatory drive, and weakened respiratory muscles which lead to prolonged ventilatory dependence (24). Nutrition support therapy with recommendations to prevent malnutrition and specific nutrient deficiencies is essential in critically ill patients. Although it is agreed by medical professionals that enteral nutrition support is crucial for ventilator dependent patients, there is still debate as to which specific nutrients should be supplemented in enteral formula in order to improve clinical and ventilator dependent outcomes.

The supplementation of immune-enhancing nutrients, otherwise known as immunonutrition, in enteral formulas for critically ill patients has been debated and improved over the past several years. Most recent advances in this area have focused on nutrition therapy to attenuate the metabolic response to stress, to prevent oxidative cellular injury, and to aid in bolstering the immune response (25). Although professionals agree that early enteral feeding,
appropriate macronutrient delivery, and glycemic control are crucial for feeding (25), there is
debate as to which specific additional pharmaconutrients could lead to improved ventilator
dependent outcomes in this population in the critical care setting.

The enteral formula, Oxepa, is a low-carbohydrate, high-fat, calorically dense enteral
nutrition product which was designed for the dietary management of critically ill patients
requiring mechanical ventilation for respiratory support. This product is supplemented with EPA,
GLA, and elevated levels of antioxidants compared to a standard enteral formula. Positive results
from animal studies supplementing enteral formulas with the addition of EPA, GLA, and
elevated antioxidants (21) led to the initiation of human clinical trials which assessed the effects
of using the product Oxepa on ventilated patients. In several studies, this product was compared
to a standard enteral formula, Pulmocare, which contains a similar caloric value as Oxepa;
however this product is not supplemented with the EPA, GLA, or elevated antioxidants, in order
to compare and determine the effect on mechanically ventilated patients.

In 1999, Gadek and colleagues (4) conducted a study which evaluated whether an enteral
formula supplemented with EPA, GLA, and elevated antioxidants would reduce pulmonary
inflammation and improve gas exchange and clinical outcomes compared with a standard control
diet given to patients with acute respiratory distress syndrome (ARDS). In this prospective,
double-blinded, randomized, mulitcentered trial, 146 patients with illnesses associated with
ARDS were recruited from various intensive care units. Subjects were randomized to either the
control diet which was a high-fat, low-carbohydrate, omega-3 fatty acid free enteral nutrition
formula, or received the experimental diet which was isocaloric and isonitrogenous to the control
diet, however was supplemented with fatty acids (EPA from fish oil, GLA from borage oil), and
a higher level of antioxidants. The results of this study found that patients in the experimental
group showed a reduction in days on ventilator support (11 vs. 16.3 days; \( p = .011 \)), decreased
length of stay in the intensive care unit (12.8 vs. 17.5 days; \( p = .016 \)), and decreased mortality (12 vs. 19; \( p = .31 \)) as compared with the control group (4). This study confirmed the beneficial effects of an enteral diet supplemented with EPA+GLA and elevated antioxidants on clinical outcomes for critically ill ventilator dependent patients.

In 2006, Singer et al (5) conducted a randomized, single-center unblinded controlled study to determine the effect of an enteral diet enriched with EPA, GLA, and antioxidants on the respiratory profile and outcomes of patients with acute lung injury (ALI) (5). Ninety five patients with ALI were randomized to either a control or experimental enteral diet for 14 days. The control group received the enteral formula, Pulmocare, which is a high-fat, low carbohydrate formula. The experimental group received Oxepa which, in comparison with Pulmocare, only differs in the lipid composition with a higher amount of EPA, GLA, and antioxidants. The results of this study found that gas exchange parameters and oxygenation were improved in the EPA+GLA study group (5). Also, the experimental group had a significantly shorter length of time on ventilation than the control group (5). This study confirmed the benefits of a diet enriched with fish oil and borage oil on respiratory improvement and shorter length of time on ventilation compared to a control group (5).

More recently, Pontes-Arruda et al (6) conducted a similar study in 2006 examining the effects of an enteral diet enriched with EPA, GLA, and elevated levels of antioxidants in patients with severe sepsis requiring mechanical ventilation (6). This double blinded, randomized, single-center, controlled study recruited 165 ventilator dependent patients from intensive care unit settings. Subjects were randomized to either receive a high-fat, low-carbohydrate enteral formula or to receive the experimental diet which was enriched with EPA, GLA, and elevated levels of antioxidants. Similar results as the previous studies were found. The experimental diet
was associated with decreased intensive care unit stay, a lower rate of mortality, improved oxygenation, and more ventilator-free days as compared to the control diet (6).

A meta-analysis comparing the above mentioned published studies and others concluded that when ARDS, ALI, and septic patients requiring mechanical ventilation were given an enteral diet enriched with EPA, GLA, and antioxidants, there were significant improvements in oxygenation and clinical outcomes compared to a control diet (2). Based on these results, the product Oxepa is clinically shown to modulate the inflammatory response in critically ill, mechanically ventilated patients (26). Clinical trials outlined in this meta-analysis have concluded that patients given the enteral formula Oxepa compared to a standard enteral formula have more ventilator free days (4-6), more ICU-free days (4,5,27), reduced new organ failures (4,6), and reduced 28-day mortality rates which suggests Oxepa formula can improve clinical outcomes (6).

In 2003, the Canadian Clinical Practice Guidelines for Nutrition Support in Mechanically Ventilated, Critically Ill Adult Patients summarized the current literature and developed clinical practice guidelines for nutrition support. It was recommended that the use of enteral formulas with fish oils, borage oils, and antioxidants should be considered in patients with ARDS (28). Therefore, the committee recommended products containing the combination of fish oil, borage oil, and antioxidants, and not fish oil alone (28). However, based on current literature, the independent beneficial effect of fish oil could not be distinguished from borage oil and elevated antioxidants.

As mentioned above, the enteral formula Oxepa has been shown to improve clinical outcomes in mechanically ventilated patients. Although this formula has been proven to have beneficial effects for this population, the high cost of Oxepa limits its use in the health care setting. Costs of specialty immunity formulas are much higher than standard formulas. The
immunity formulas typically range in 2.5 to 3.5 times the cost of standard formulas. Justification of the higher expense of immunity formulas in the LTACH setting for ventilator patients has not been established in the literature. There have been no studies to our knowledge which have been done that show more cost effective nutrition approaches to reducing inflammation. Therefore, use of a lower cost standard enteral formula with a fish oil (EPA+DHA) supplement may be more cost effective. There is a need for more studies in this area.

Part 6: What gap in the literature does this study attempt to fill?

Recent studies have been conducted to examine the effect of a standard enteral formula supplemented with omega-3 fatty acids and antioxidants compared to a standard control formula in patients with acute respiratory distress syndrome and acute lung injury (4-6). These studies have been specifically designed for critically ill patients in the intensive care unit. Our current study specifically focuses on mechanically ventilated patients in a long term acute care hospital compared to an intensive care unit setting. LTACH ventilated patients may not have an active diagnosis of ALI or ARDS and may experience longer weaning times due to a variety of respiratory conditions. Supplementation of essential nutrients which may modulate the inflammatory response have not been studied in a LTACH setting. In order to determine whether these pharmaconutrients, EPA, DHA, GLA, and antioxidants, can improve clinical outcomes, mortality rates, length of stay, and improve mechanical ventilator dependence, studies need to be conducted in long term health care hospitals, in addition to critical care units, to create the most thorough interpretation of the results.

Recent studies have concentrated on acute lung diseases, such as acute lung injury or acute respiratory distress syndrome. To our knowledge, there have been no studies conducted in chronically ventilated patients to determine the effect of fish oil added to an enteral formula on
ventilator weaning time and other clinical outcomes. It is important to determine whether the supplementation of EPA+DHA to an enteral formula in chronically ventilated patients yields the same results as in acutely ventilated patients. Significant findings regarding this for those requiring long term ventilation could help improve the weaning process.

The specific aims of the current study are as follows:

**Specific Aim 1:** To determine the effect of enterally delivered EPA+DHA (treatment) versus enterally delivered placebo (control) on time to wean off a ventilator and length of hospital stay in ventilated patients in a LTACH. We hypothesize that time on a ventilator and length of hospital stay will be shorter in participants randomized to the EPA+ DHA treatment group compared to the control group.

**Specific Aim 2:** To determine the effect of enterally delivered EPA+DHA versus enterally delivered placebo on inflammatory markers (IL-6, IL-8, and LTB₄) in ventilated patients in a LTACH. We hypothesize that inflammatory markers will show greater improvement in participants randomized to the EPA+ DHA treatment group compared to the control group.

**Specific Aim 3:** To determine the effect of enterally delivered EPA+DHA versus enterally delivered placebo on number of infectious events defined as number of blood stream infections, ventilator acquired pneumonia, hospital acquired pneumonia, and other infections. We hypothesize the number of infectious events will be less in participants randomized to the EPA+ DHA treatment group compared to the control group.
### Methods

#### Study Design:

This randomized, double-blind, placebo controlled trial was designed to examine the effects of enterally delivered EPA+DHA on weaning, inflammatory markers, occurrence of infections, and length of hospital stay on mechanically ventilated patients in a LTACH setting. This thesis project focused on the data collected from the first nine subjects who were enrolled in this study whether or not they completed the 14 days of intervention in its entirety.

#### Patient Identification:

All potential study participants were identified by a daily prospective screening tool, which was used to assess all newly admitted patients on mechanical ventilators to the Drake Rehabilitation Center. By using a standard screening tool, the primary investigator (PI) or a trained research nurse coordinator determined initial eligibility of the subjects for this study. After initial review, the Medical Director of Pulmonary Services at the Drake Center, Dr. Bauer, confirmed eligibility.

#### Patient Enrollment:

Inclusion and exclusion criteria are provided in Table 1 and the screening form used to determine eligibility is included in Appendix A. The inclusion criteria were as follows: admitted as an in-patient at the Drake Center, required positive-pressure mechanical ventilation 24-hours a day, received enteral tube feeding, between the ages of 18 and 80 years, inclusive, and had a rapid shallow breathing index (RSBI) greater than 80.

Patients were excluded from this study if they were pregnant, suffered from a post-cardiac arrest with suspected significant anoxic brain injury, had a history of ventricular...
tachycardia (VT) or atrial fibrillation (AF), HIV, metastatic cancer, or a history of bone marrow, lung, liver, cardiac, kidney, or pancreas transplantation. In addition, patients were excluded if they were receiving recombinant human-activated protein C (rh-APC) for sepsis, had a platelet count < 30,000/µL, active bleeding, or an international normalized ratio (INR) > 3.0. If the patient had received Oxepa enteral formula in the last 14 days, was receiving a treatment dosage of Heparin or Coumadin, or had an activated partial thromboplastin time (aPTT) greater than 33.5s.

Subjects who were alert and oriented and matched the inclusion criteria were approved regarding potential participation. Some potential participants at the Drake Center were sedated and unable to provide consent to the study, therefore a legally authorized representative or next of kin was asked to provide consent for the patient. The PI or nurse coordinator explained the purpose of the study, answered any questions the patient or family had regarding the study protocol, and left the informed consent documents with the eligible patient or representative to review privately. If the eligible patient was interested in participating, the PI or nurse coordinator returned and obtained informed consent.

Once enrolled and randomized, subjects were terminated from the study for any of the following reasons: 1) platelet count <30,000 µL, 2) international normalized ratio > 3.0, 3) activated partial thromboplastin time (aPTT) > 33.5s, 4) enteral tube removal, or 5) death. Institutional Review Board approval was obtained from the Drake Center Institutional Review Board. All subjects signed informed consent prior to participation.

**Randomization:**

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

**Materials and Supplies:**

Formulation, Packaging, and Labeling:

The fish oil product was supplied by Nordic Naturals, high concentration ProOmega as a sterile liquid in labeled four ounce bottles. The fish oil and placebo were prepared and packaged into opaque syringes by the Drake Center Pharmacy. One case of fish oil was obtained from the Nordic Natural Company in California. All fish oil given was from the same processing batch and was delivered in one single shipment. One vial of fish oil was tested by an independent lab, Bodycote Testing Group, Portland Oregon, prior to starting the study to confirm that EPA and DHA concentration was approximately 350 mg and 250 mg per liter, respectively. The fish oil was tested for contaminants, specifically heavy metals, and was found to be contaminant free.

The lab results for the EPA+DHA solution indicate that its composition is 69.6% n-3 PUFAs, 35.89% EPA, and 24.27% DHA. The breakdown of the fatty acid profile of the solution is shown in Table 1.

**Table 1: Fatty Acid Profile for the EPA+DHA Solution**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>% of fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric Acid</td>
<td>0.00</td>
</tr>
<tr>
<td>Myritic acid</td>
<td>0.7</td>
</tr>
<tr>
<td>Myristoleic acid</td>
<td>0.00</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>3.67</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>C 16:2</td>
<td>.37</td>
</tr>
<tr>
<td>C 16.3</td>
<td>.41</td>
</tr>
<tr>
<td>C 16:4</td>
<td>0.48</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>3.88</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>8.94</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.35</td>
</tr>
<tr>
<td>A-Linolenic acid</td>
<td>0.74</td>
</tr>
<tr>
<td>Stearidonic acid</td>
<td>2.75</td>
</tr>
<tr>
<td>Arachidic 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>

**Intervention:**

Subjects randomized to the treatment group received 8 grams of fish oil per day in divided doses of 7.5mL delivered through the enteral tube every six hours. Doses were administered by study nurses at 12:00AM, 6:00AM, 12:00PM, and 6:00PM. In a prior study conducted by Gadek et al. in 1999, the study group received 6.9±0.3 g EPA and 2.9±0.1 g DHA per day (4). Therefore, the dose for this study was chosen because it was similar to the amount of EPA that patients in the prior Oxepa® trial received per day (4). The saline solution placebo was given in the same manner as the fish oil.

The fish oil product supplied by Nordic Naturals had a lemon scent and only a minimal fish smell. Flavor of the fish oil was deemed irrelevant because the product was delivered
through an enteral feeding tube. Both the fish oil and the saline solution were packaged in opaque syringes to ensure visual resemblance. Both syringes were prepared with fish oil rubbed on the outside so both syringes smelled identical. Saline was chosen for the placebo because it is inert. The fish oil or placebo was given to the subject until death, removal of the enteral tube, unassisted breathing, or 14 days after initiating the study, whichever occurred first. For patients who did not require enteral nutrition after the study began, the fish oil or placebo was continued 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 was removed as per usual practice and fish oil administration was stopped (an enteral tube would not be left in place for the sole purpose of continuing the study).

The Drake pharmacy was responsible for storing, processing, and packaging the 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. Nursing staff was trained on 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 the nurse.

Co-interventions:

All subjects were provided standard long term acute hospital care under Dr. Bauer’s direction. As is usual care, feeding strategy was not protocolized in this study and was decided by Dr. Bauer and the staff nutritionists. Placebo was delivered along with the usual care enteral
formulation as previously described. The Drake Center does not use Oxepa® as a standard enteral formula therefore this formula was not a confounder in this study.

**Data Collection:**

Each study participant was assigned a specific identification number to be used for all patient data. Links to the patient name and identifiers were maintained and stored in files on computers protected by password and in locked office cabinets. Chart abstraction for demographic, laboratory, and physiologic data occurred at study entry, daily until the intervention was discontinued, and weekly for the remainder of the hospitalization.

The primary endpoints of this study were blood concentrations of IL-6, IL-8, and LTB4. These measures were extracted from blood drawn on day 1 (baseline), between days 2-5, and between days 7-8 of the 14 day study period. Secondary endpoints for the first 9 subjects were assessed in this thesis and included infectious events defined by presence of blood stream infections; ventilator acquired pneumonia; hospital acquired pneumonia; and other infections (e.g. urinary tract infections, cdiff, etc.).

All blood draws were collected by lab phlebotomists who were blinded to treatment assignment. Blood was transported and processed by the Drake Center lab. Aliquots of serum were stored in a subzero freezer at Drake Center lab. Batch samples were packaged on dry ice by research personnel and sent to Case Western Reserve University where inflammatory markers were assessed by trained lab technicians at the Inflammatory Mediator Core Services laboratory.

Nurses trained in study procedures and blinded to treatment assignments collected other primary endpoint data from chart review including: wean (yes/no); numerical variables of length of free ventilator days and length of total ventilator days; a count variable of numbers of days
on/off the ventilator, and a numerical variable of length of stay (days) in the hospital. All data was recorded on study assessment forms that were included in participant charts at bedside.

**Data Analysis**

Numerical variables for this thesis were summarized by mean (standard deviation) and/or median (range); and categorical and/or binary variables were summarized by frequencies. Unpaired t-tests were used to compare treatment and control groups for continuous variables. Chi-square procedures were used to determine differences between groups for categorical variables.

Statistical analyses were performed with SAS software (version 9.2, SAS Institute, Cary, North Carolina). P values <0.05 were considered to be statistically significant.
Results

Baseline Demographics and Comparison of Study Groups:

As shown in Table 2, a total of nine participants met the eligibility criteria for this randomized double blind clinical trial. Five of the eligible patients were randomized to receive the experimental fish oil (EPA+DHA) solution. Four eligible participants received the control saline solution. At baseline (before treatment), there were no significant differences in demographic characteristics between participants in the treatment and control groups. In both groups, there were more females than males and more Caucasians than African Americans or Asians. The primary initiating event in the experimental group was intracranial hemorrhage (n=2/5), while the control group experienced varying initiating events. The primary diagnosis for all patients eligible was respiratory failure in which every patient required mechanical ventilation.

Table 2. Participant Characteristics: Treatment versus Control

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Age (years) mean ± S.D</td>
<td>55.6 ± 3.2</td>
<td>53.0 ± 7.7</td>
</tr>
<tr>
<td>Gender, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Race, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>African American</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Initiating Event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pylonephritis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intracranial Hemorrhage</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal Aortic Aneurism</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Coronary Artery Bypass Surgery</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Motor Vehicle Trauma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intravascular Hemorrhage</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dissecting Aortic Aneurysm</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Enteral Formulas:

Participants in both groups were prescribed a variety of enteral formulas as indicated in Table 3. As discussed in the methods section, Oxepa® was the only enteral formula noted as an exclusion factor for eligibility in the study. At the initiation of this RCT, Oxepa and Impact were the only two enteral formulas on the market that contained DHA/EPA. Patients were excluded from the study if they were receiving either formula for nutrition support because of the potential for confounding the study outcomes.

All participants were prescribed an enteral formula based on individualized nutrient needs and to accommodate the specific nutrient requirements associated with the stress of the initiating event prior to randomization. Only one study participant had the enteral formula discontinued by the physician by day 3 of the study. Despite the patient not requiring enteral nutrition, the treatment continued to be given to the patient via the enteral tube to minimize unintentional blinding. The enteral formula prescribed before randomization by each patient’s attending physician was not changed during the course of the trial.

According to the formularies used for enteral nutrition support for each participant enrolled in this study, none contain additional EPA/DHA. As shown in Table 3, some participants in both the treatment and control groups were receiving formulas which contained the precursor of EPA/DHA, alpha-linolenic acid, ranging in amounts from 3.5 g/L to 1.72 g/L. This omega-3 fatty acid metabolizes to EPA/DHA at a rate of 12% conversion efficiency in healthy subjects\textsuperscript{31}. 
Table 3: Enteral Formula Type

<table>
<thead>
<tr>
<th>Enteral Formula Type</th>
<th>Kcal/mL of enteral formula</th>
<th>g/L Alpha-Linolenic Acid in each formula</th>
<th># in Treatment Group on formula</th>
<th># in Control Group on formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>1.5</td>
<td>3.5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>High Fiber</td>
<td>1.2</td>
<td>2.7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Standard High Residue</td>
<td>1.2</td>
<td>2.7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2 kcal/ml</td>
<td>2.0</td>
<td>1.72</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Specific brand names of enteral formulas not included

Weaning/Recovery Indicators:

There was no difference between the experimental and control participants for the frequency of occurrence of weaning from the ventilator (Table 4). There was a trend (p<.10) for differences between groups for the mean number of days in the study on a ventilator and the percent of days in the study on the ventilator. The control group had ~ 3.8 fewer days in the study on a ventilator, which equated to about 40% fewer days in the study on a ventilator. There were no significant differences between groups for total length of stay in the hospital (Table 4).

Table 4. Weaning/Recovery Indicators: Treatment versus Control

<table>
<thead>
<tr>
<th>Weaning/Recovery Indicators</th>
<th>Treatment Group</th>
<th>Control Group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaned, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>4</td>
<td>0.15</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mean number of days in the study on a ventilator&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6 +/- 4.2</td>
<td>3.8 +/- 3.8*</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean number of days in the study&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.4 +/- 3.5</td>
<td>12 +/- 2.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Percent of days in the study on a ventilator, % (mean ± SD)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.7 ± 31.17</td>
<td>31.39 ± 15.27*</td>
<td>0.06</td>
</tr>
<tr>
<td>Total length of stay in the hospital, days (mean ± SD)</td>
<td>34.6 ± 11.69</td>
<td>42.50 ± 38.14</td>
<td>0.67</td>
</tr>
</tbody>
</table>

<sup>a</sup> = maximum number of days in the study on a ventilator is 14 days; 2 participants in the treatment group dropped out due to intolerance to the enteral supplement (disagreeable smell); 2 participants in the control group dropped out, one patient due to intolerance to the placebo (disagreeable smell) and one patient expired.

<sup>b</sup> = maximum number of days in the study is 14 days

<sup>c</sup> = percent of days in the study on a ventilator was calculated as number of days in the study on a ventilator/number of days in the study

* p<=.10
The maximum number of days in the study was 14 days, however some participants in both groups dropped out of the study before completion of the 14 days. In the treatment group, one subject only received the treatment for 12 days due to intolerance to the supplement, and one subject only received three days of the treatment before dropping out of the study due to intolerance as well. In the control group, one subject received 12 days of the placebo control and then dropped out of the study due to intolerance, and one subject expired after eight days of receiving the placebo for reasons unrelated to the study.

**Inflammatory Indicators: Treatment vs Control:**

Inflammation may be one of the underlying reasons why patients remain on the ventilator for a longer period of time, and one of the hypothetical nutrient benefits of EPA/DHA is to decrease inflammation. For this reason, group differences in change in inflammatory markers were examined over the length of the trial. IL-8, LTB4, and IL-6 were chosen as markers of inflammation as these were previously shown to be elevated in patients with an increased inflammatory response due to trauma or events requiring mechanical ventilation (Table 5). In this trial, difficulties with sample dilution for IL-6 resulted in 47% of values being below the detectible level. For samples with values outside the detectible range, the lowest detectible limit (e.g., 1.22 pg/ml) was used in the calculation of means. Group means were compared for markers using unpaired t-tests. The results showed no significant differences between groups for absolute values of IL6, IL8 or LTB4 at baseline, day 2-5, day 7-8, or changes in IL-6, IL8 or LTB4 from baseline to day 2-5 and day 7-8.

**Table 5. Inflammatory Indicators: Treatment versus Control**

<table>
<thead>
<tr>
<th>Inflammatory Measures</th>
<th>Treatment Group</th>
<th>Control Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious events, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>0.29</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>White blood cell count &gt;20,000, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>-----</td>
<td>----</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>IL6, pg/ml (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.89 ± 1.74</td>
<td>8.01 ± 6.65</td>
<td></td>
</tr>
<tr>
<td>Day 2-5</td>
<td>3.07 ± 2.27</td>
<td>3.85 ± 1.44</td>
<td></td>
</tr>
<tr>
<td>Day 7-8</td>
<td>2.59 ± 2.63</td>
<td>4.88 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>Δ Day 2-5 – baseline</td>
<td>0.19 ± 3.59</td>
<td>-4.16 ± 6.72</td>
<td></td>
</tr>
<tr>
<td>Δ Day 7-8 – baseline</td>
<td>-0.34 ± 4.16</td>
<td>-4.67 ± 7.98</td>
<td></td>
</tr>
<tr>
<td>IL8, pg/ml (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>25.39 ± 17.75</td>
<td>28.84 ± 16.63</td>
<td></td>
</tr>
<tr>
<td>Day 2-5</td>
<td>27.19 ± 10.32</td>
<td>37.52 ± 22.47</td>
<td></td>
</tr>
<tr>
<td>Day 7-8</td>
<td>17.66 ± 3.83</td>
<td>41.13 ± 30.56</td>
<td></td>
</tr>
<tr>
<td>Δ Day 2-5 – baseline</td>
<td>1.81 ± 24.02</td>
<td>8.67 ± 8.08</td>
<td></td>
</tr>
<tr>
<td>Δ Day 7-8 – baseline</td>
<td>-8.51 ± 24.12</td>
<td>9.11 ± 15.14</td>
<td></td>
</tr>
<tr>
<td>LTB4, pg/ml (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>121.64 ± 72.08</td>
<td>115.0 ± 45.23</td>
<td></td>
</tr>
<tr>
<td>Day 2-5</td>
<td>131.40 ± 23.90</td>
<td>108.5 ± 31.84</td>
<td></td>
</tr>
<tr>
<td>Day 7-8</td>
<td>80.05 ± 55.69</td>
<td>102.67 ± 15.88</td>
<td></td>
</tr>
<tr>
<td>Δ Day 2-5 – baseline</td>
<td>9.76 ± 73.77</td>
<td>-6.50 ± 36.58</td>
<td></td>
</tr>
<tr>
<td>Δ Day 7-8 – baseline</td>
<td>-32.00 ± 95.46</td>
<td>-25.00 ± 38.19</td>
<td></td>
</tr>
</tbody>
</table>

- Negative values indicate a reduction in marker and reduced inflammation
- Day 7 sample is missing for one participant in the treatment group and one participant in the control group; missing values were excluded from mean derivations.
- 47% of the values for IL6 were below the detectible range (possible dilution problem); the lower end of the detectible range (1.22 pg/ml) was used for samples with values below the detectible range.

Patients in a non-inflammatory state have normal white blood cell count levels between 5,000-10,000 cells/mm. A trauma, infection, or inflammatory response can lead to an increase in the white blood cell count. In this study, a white blood cell count >20,000 was considered to be indicative of presence of an infection. Our findings show no significant differences between groups for occurrences of infections during the study (Table 5). Infectious events in this study were characterized as frequency of blood stream infections, ventilator acquired pneumonia, hospital acquired pneumonia, and other infections such as C. Diff or urinary tract infections. No patients randomized to the control group developed an infectious event during the study. One patient in the treatment group did begin the study with an infection which was acquired before the study began, and resolved on day three.
Discussion

In this double blinded randomized control trial, we investigated the anti-inflammatory effects of an enteral formula supplemented with a dose of EPA+DHA compared to an enterally delivered placebo saline solution in mechanically ventilated patients in a LTACH. The aim of this study was to investigate the effects of supplemental fish oil on inflammatory markers, weaning off the ventilator, length of hospital stay, and number of infectious events in patients in the LTACH setting. We tested the hypothesis that the patients given the fish oil supplementation would have improved outcomes (inflammatory markers, weaning from the ventilator, and infections) compared to patients randomized to the placebo group.

Our results suggest that there was no significant difference between those receiving the fish oil supplement and those receiving the placebo for length of stay in the hospital, number of days in the study on a ventilator, or change in concentration of the inflammatory markers IL-6, IL-8, and LTB4 during the study period.

Explanation of Results

There is evidence in the literature confirming the anti-inflammatory effects of omega-3 fatty acids. These studies consistently show that the additions of GLA, antioxidants, and omega-3 fatty acids to enteral formulas produced more ventilator free days, more ICU free days, and a total decreased length of stay in the hospital. For this reason we expected that the enteral formulas supplemented with a large dose of EPA+DHA versus the placebo used in this study would have these same therapeutic benefits for patients on mechanical ventilation in the LTACH setting.
In particular, we expected that the results would indicate that the control group would have a higher percentage of days on the ventilator than the treatment group. However, this hypothesis was not confirmed as evidenced by our results. In fact, our results from weaning/recovery indicators showed that the control group had a trend for lower percentage of days in the study on a ventilator than the treatment group. These unexpected findings could not be explained by the number of patients who dropped out of the study since there was no difference between groups for mean number of days in the study. It should be noted that individuals in both groups had poor tolerance to the supplement, whether it was the fish oil or the placebo due to the strong odor of the product or the placebo (fish oil was rubbed on the enteral feeding tube before delivery). This led to 2 participants dropping out of the treatment group and 1 from the control group. Another participant expired at day 8 of the trial for causes unrelated to the study.

In regards to the IL-6 marker of inflammation, the baseline values differs greatly between the treatment (2.89 ± 1.74) and the control groups (8.01 ± 6.65). As indicated by the lab, reference ranges for IL-6 between 3.63-2650 pg/ml were considered detectible. However, nearly 50% of our participant samples for IL6 were below the detectible range. This likely indicates a problem with sample dilution. Mean values for all subjects for IL8 and LTB4 were also on the lower end of normal, which suggests that overall measurement of inflammatory markers in this study may have been affected by over diluting of blood samples in processing. In addition, samples were run in two batches and the second batch overall had higher concentrations of all inflammatory markers. In hindsight, having extra blood samples available for re- analysis of each inflammatory marker by another laboratory would have strengthened our ability to draw more meaningful conclusions from the results of these tests.
In this trial, a dose of EPA/DHA was given that was quite a bit larger than previous trials. It may be that this dose (8 grams/day) compared to the 3-4 grams normally recommended to provide anti-inflammatory effects and that has been used in previous studies\(^4\)\(^6\) negated any therapeutic potential of the fish oils on weaning time. Additionally, no benefits were observed on inflammatory markers or number of infectious events, e.g., markers of inflammation did not decrease more in the treatment group compared to the control group over the course of the trial. Further, there was no difference in infectious events between groups. Future studies may need to be conducted to determine the optimal dose of fish oils to produce beneficial effects on weaning time, inflammatory markers and number of infectious events. Importantly, this study was greatly underpowered to detect differences between groups for the outcomes of this trial. Future studies would need to include an adequate sample size to detect group differences for variables of interest.

Although our results for total length of stay in the hospital were not lower in the treatment group than the control group and were not statistically significant, there are meaningful clinical implications that can be extracted from this data. The average length of stay in a long term acute care hospital is 25 days (32); however the mean number of days for patients in both the treatment and control groups was greater than this, which may indicate more significant injuries/trauma in the population studied. The average cost of stay in a LTACH is between $2500-$3000/day (32). Therefore a decrease in length of stay by an average of 8 days in the treatment group compared to the control group may not have been statistically significant, but a cost savings of $20,000 per patient has definite clinical relevance and value. This finding if reproduced in a larger population, with a more rigorous study design could justify the higher cost of a specialty formula or clinical use of a EPA/DHA supplement.
Challenges and Limitations:

For this pilot study, 10 patients were to be selected with the ultimate goal of enrolling 100 chronic ventilator patients with 50 patients in the treatment and control groups. Due to the difficulty of enrolling patients who matched the eligibility criteria, this number was difficult to obtain.

This study also had a high non-completer rate due to the intolerance to both the fish oil supplement and the fish oil rubbed on the placebo. Although the fish oil was to be made with a lemon scent with only a minimal fish smell, the high dose provided was distasteful to over 1/3 of study participants. These 2 factors likely contributed to the low sample size obtained and reduced our ability to find group differences in the proposed outcomes of this trial.

Another limitation to the study was enteral formula selection. Before this study protocol was implemented, the enteral formulas Oxepa and Impact were the only known formulas on the market with added omega-3 fatty acids. Therefore, patients requiring these formulas for nutrition support were excluded from participating in the trial. While the trial was in progress, several large nutrition companies began to supplement alpha-linolenic acid into their formulas. Although the enteral formulas that patients were prescribed in the current study did not contain additional EPA/DHA, many of the formulas had a significant amount of alpha-linolenic acid which is a precursor to EPA/DHA and metabolizes to EPA/DHA at a rate of 12% conversion efficiency. Due to the varying amount of alpha-linolenic acid within the different formulas (3.5-0 g/L), it is unknown whether these levels may have had an impact on the study outcomes and potentially confounded the results.

A previous medical history of COPD or CHF were items on the screening form, however these conditions were not considered exclusion criteria. Smoking history was also part of the
screen, but was not a reason for exclusion from the study. It is unknown whether patients with these diagnoses or a previous smoking history would wean off a ventilator at a slower rate compared to participants who did not have this history. In this study, if these two factors would have been a part of the exclusion criteria, five of the participants (4 from treatment group and 1 from control group) would not have qualified for the current study. If this study were to be completed again, it may be beneficial to exclude all individuals who have a previous medical history of COPD or who have a smoking history. Although the primary diagnosis for all patients was respiratory failure, it is unclear how the different initiating events between patients could influence the study outcomes. If we were to include the participants with a previous history of COPD or smoking, it may be beneficial to stratify the individuals with these diagnoses.

Future directions:

If this study was to be conducted in the future, there are several revisions that should be investigated. The fish oil dose used in this study of 8 g/day was a very large dose compared to prior studies. The fish oil dose should be more consistent with previous studies, such as the Gadek and Arruda studies (3-4 grams/day) in order to have more comparable results. If this study were to be repeated in the future with a lower dose of fish oil, exclusion for anticoagulation (aPTT) would not need to be included. In a summary of reports on the effects of omega 3 fatty acids on bleeding complications by Harris, W., in 2006 (33), it was concluded that doses of 1-4g/day do not cause clinically significant bleeding even in patients taking antiplatelet or antithrombotic medications. In a future study with a decreased dose of 4gm/day omega 3 fatty acids this exclusion may no longer be of concern. Subject enrollment would be more easily facilitated to obtain a subject number and increase the power of the results.
During this study, there were two different groups of pulmonologists weaning patients from the ventilator in the acute care hospital where the study took place. Both groups may have had different approaches to weaning. Although these differences may not have affected the outcomes of this trial, if this study were to be conducted again, it would be beneficial for all physicians to follow the same protocol for weaning.

The inflammatory markers that were chosen as indicators of inflammation did not give the most accurate results for decreased inflammation due to dilution problems, particularly with the IL-6 marker. The outcomes should focus on the specific inflammatory marker that is the most sensitive to fish oil, which is still being investigated. Also, in a future study, processing of the blood samples in a single batch rather than divided batches would limit possible variations in assay analysis.

Conclusion:

The results of this randomized double blind trial suggest that adding fish oil (EPA/DHA) to enteral formulas at a dose of 8 g/day does not result in reducing time to wean, length of hospital stay or markers of inflammation in patients managed with mechanical ventilation in a LTACH compared to placebo. There were several methodological problems in the conduct of this study that may have reduced our ability to draw meaningful conclusions from the results. Future trials in this area should focus on the following: consistent fish oil dosing consistent with previous studies (3-4 grams/day), focus on the most sensitive markers of inflammation which specifically effect fish oil, exclude or subcategorize individuals with a past history of COPD or smoking, and improve non-completer ratio by better disguising the smell and taste of treatment.
References


