Prospective, Randomized, Multi-center Trial of Initial Trophic Enteral Feeding Followed by Advancement to Full-Calorie Enteral Feeding vs. Early Advancement to Full-Calorie Enteral Feeding in Patients with Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS)

and

Prospective, Randomized, Blinded, Placebo-controlled, Multi-center Trial of Omega-3 Fatty Acid, Gamma-Linolenic Acid, and Anti-Oxidant Supplementation in the Management of Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS)

ARDS Clinical Network
ARDS Network Study # 07
Protocol Version II

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ABBREVIATIONS

AA = Arachidonic Acid
ALI = Acute Lung Injury
ARDS = Acute Respiratory Distress Syndrome
BMI = Body Mass Index
BUN = Blood Urea Nitrogen
CHF = Congestive Heart Failure
CPAP = Continuous Positive Airway Pressure
CPR = Cardiopulmonary resuscitation
CT = Computed Tomography
DBP = Diastolic Blood Pressure
DGLA = Dihomo-γ-Linolenic Acid
DHA = Docosahexaenoic Acid
DSMB = Data Safety Monitoring Board
EPA = Eicosapentaenoic Acid
FACTT = Fluid and Catheter Treatment Trial
FiO_2 = Fraction of Inspired Oxygen
GCS = Glasgow Coma Scale
GLA = Gamma-Linolenic Acid
GRV = Gastric Residual Volume
Home = Type of residence immediately prior to study hospitalization
ICU = Intensive Care Unit
IgA = Immunoglobulin A
IL-1 = Interleukin 1
IL-6 = Interleukin 6
IL-8 = Interleukin 8
IL-10 = Interleukin 10
IMV = Intermittent Mechanical Ventilation
INR = International Normalized Ratio
IVRS = Interactive Voice Response System
LTB_4 = Leukotriene B_4
LTB_5 = Leukotriene B_5
mBW = measured body weight
n-3 FA = omega-3 Fatty Acids
NAC = N-acetylcysteine
NHLBI = National Heart Lung and Blood Institute
OR = Odds Ratio
$PaCO_2$ = Partial pressure of arterial carbon dioxide
PAI -1 = Plasminogen Activator Inhibitor 1
PaO_2 = Partial pressure of arterial oxygen
PAP = Pulmonary Artery Pressure
PB = Barometric Pressure
PBW = Predicted Body Weight
PCP = *Pneumocystis carinii* pneumonia
PEEP = Positive End-Expiratory Pressure
PEG = Percutaneous Endoscopic Gastrostomy
PGD_2 = Prostaglandin D_2
PGE_2 = Prostaglandin E_2
PGI_2 = Prostaglandin I_2
PIN = Personal Identification Number
PPAR = Peroxisome Proliferator-activated Receptor
Pplat = Plateau pressure
PS = Pressure Support Ventilation
PUFA = Polyunsaturated Fatty Acids
ROS = Reactive Oxygen Species
SBP = Systolic Blood Pressure
SBT = Spontaneous Breathing Trial
SpO_2 = Oxygen Saturation
TNF = Tumor Necrosis Factor
TPN = Total Parenteral Nutrition
TxA_2 = Thromboxane A_2
TxA_3 = Thromboxane A_3
VAP = Ventilator-associated Pneumonia
VFD = Ventilator-free Days
WBC = White Blood Cell

Part I

Study Summary

- **Titles:** Prospective, Randomized, Multi-center Trial of Initial Trophic Enteral Feeding Followed by Advancement to Full-calorie Enteral Feeding vs. Early Advancement to Full-calorie Enteral Feeding in Patients with Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS)

  and

  Prospective, Randomized, Blinded, Placebo-controlled, Multi-center Trial of Omega-3 Fatty Acid, Gamma-Linolenic Acid, and Anti-Oxidant Supplementation in the Management of Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS)

- **Objectives:**
  1. To assess the safety and efficacy of initial trophic enteral feeding followed by advancement to full-calorie enteral feeding vs. initial advancement to full-calorie enteral feeding management strategies in reducing mortality and morbidity in patients with ALI or ARDS
  2. To assess the safety and efficacy of omega-3 fatty acid, gamma-linolenic acid, and anti-oxidant supplementation in reducing mortality and morbidity in patients with ALI or ARDS

- **Hypotheses:**
  1. Initial trophic feeding followed by full-calorie enteral feeding will improve clinical outcomes (specifically increase the number of ventilator-free days to day 28 and decrease the 60-day, hospital mortality) in patients with ALI or ARDS by reducing systemic inflammation and the number of feeding complications as compared to early, full-calorie enteral feeding.
  2. Omega-3 Fatty Acid, Gamma-linolenic acid (GLA), and anti-oxidant supplementation, as compared to placebo, will improve clinical outcomes (specifically increase the number of ventilator-free days to day 28 and decrease the 60-day, hospital mortality) in patients with ALI or ARDS by attenuating systemic inflammation.

- **Study Design:** Multi-center, prospective, randomized, controlled clinical trials. Patients will be randomized into each of the two trials simultaneously (factorial design).
  1. A maximum of 1000 patients will be enrolled.
  2. Patients randomized to trophic enteral feeds will receive trophic feeding rates (10 cc / hr) for 144 hours prior to being advanced to full-calorie feeding rates which will continue for the duration of mechanical ventilation up to study day 28.
  3. Patients randomized to full-calorie enteral feeds will be advanced to full-calorie feeding rates on initiation of feeding and will continue to receive full-calorie feeds for the duration of mechanical ventilation up to study day 28.

4. Patients will be treated with n-3 fatty acids, GLA, and anti-oxidants or indistinguishable placebo every 12 hours for the shorter of 21 days or the duration of mechanical ventilation.
5. Patients will be followed to the earlier of 60 days or hospital discharge. In addition, vital status will be ascertained at 90 days.

- **Sample Size/Interim Monitoring:**

1 This study uses a 2 x 2 factorial design comparing the use of initial trophic enteral feeds followed by advancement to full-calorie enteral feeds versus initial full-calorie feeds and comparing treatment with n-3 fatty acid and anti-oxidant supplementation with placebo in patients with ALI or ARDS. The trial will accrue a maximum of 1000 patients (about 250 patients in each of four groups) providing about 500 patients treated initially with trophic enteral feeds to be compared against about 500 patients treated initially with full-calorie enteral feeds and about 500 patients treated with n-3 fatty acid, GLA, and anti-oxidant supplementation against about 500 patients treated with placebo. This provides 90 % power to detect an absolute difference of 2.25 ventilator-free days assuming a mean of 14 and standard deviation of 10.5 ventilator-free days (data from FACTT study) using a two sided p = 0.05 significance level.

2 The principal analysis will be intent-to-treat, based upon randomization assignment.

3 Trial progress will be evaluated by an independent Data and Safety Monitoring Board to determine if the study should stop for futility or efficacy. Interim analyses will be conducted after enrollment of approximately 100, 250, 500, and 750 patients. Either comparison may be stopped independently if the difference between the numbers of ventilator-free days for the two treatments is greater than the O'Brien-Fleming boundary. A Pocock boundary will be utilized to monitor for an interaction between the two comparisons.

4 The DSMB will also monitor the trial for feasibility. Feasibility parameters will include accrual, the ability to follow the enteral nutrition and ventilator protocols, separation of the enteral feeding groups based on volume delivered data, and the frequency of missed doses of the omega-3 fatty acid / GLA / anti-oxidant or placebo supplementation. If any of these parameters indicate that the trial is not feasible, the trial will be modified or terminated.

5 The trial will also be monitored by the DSMB for safety. Each comparison will be evaluated separately for safety parameters using mortality, vital sign and laboratory data, and adverse event reporting. If any of these parameters indicate to the DSMB that one or more of the interventions are not safe, the comparison of that intervention will be modified or terminated.

- **Inclusion Criteria**

Patients will be eligible for inclusion if they meet all of the below criteria. Criteria 1-3 must all be present within a 24-hour time period:

Acute onset (defined below) of:

1. PaO_2 / FiO_2 ≤ 300 (intubated). If altitude > 1000m, then PaO_2 / FiO_2 ≤ 300 x (PB/760)

2. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph. The infiltrates may be patchy, diffuse, homogeneous, or asymmetric

3. Requirement for positive pressure ventilation via endotracheal tube, and

4. No clinical evidence of left -sided cardiac failure to account for bilateral pulmonary infiltrates.

5. Intention of primary medical team to enterally feed the patient

   The 48-hour enrollment time window begins when criteria 1-3 are met. If a patient meets the first three inclusion criteria but has a PAOP (Pulmonary Arterial Wedge Pressure) greater than 18 mmHg, then the first four criteria must persist for more than 12 hours after the PAOP has declined to ≤ 18 mmHg, and still be within the 48-hour enrollment window.

   "Acute onset" is defined as follows: the duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must be ≤ 28 days at the time of randomization. Opacities considered "consistent with pulmonary edema" include any opacities not fully explained by mass, atelectasis, or effusion or opacities known to be chronic (greater than 28 days). Vascular redistribution, indistinct vessels, and indistinct heart borders alone are not considered "consistent with pulmonary edema" and thus would not count as qualifying opacities for this study.

- **Exclusion Criteria**

1. Age younger than 13 years
2. Greater than 48 hours since all inclusion criteria met
3. Neuromuscular disease that impairs ability to ventilate without assistance, such as cervical spinal cord injury at level C5 or higher, amyotrophic lateral sclerosis, Guillain-Barré Syndrome, or myasthenia gravis (see Appendix B)
4. Pregnant or breast-feeding
5. Severe chronic respiratory disease (see Appendix B for detailed exclusion criteria).
6. Burns greater than 40% total body surface area
7. Malignancy or other irreversible disease or condition for which 6-month mortality is estimated to be greater than 50% (see Appendix B).
8. Allogeneic bone marrow transplant in the last 5 years
9. Patient, surrogate, or physician not committed to full support (Exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest).
10. Severe chronic liver disease (Child-Pugh Score of 11-15)
11. Diffuse alveolar hemorrhage from vasculitis.
12. Morbid obesity (> 1kg/cm body weight)
13. No consent/inability to obtain consent

14. Unwillingness or inability to utilize the ARDS network 6 ml / kg PBW ventilation protocol
15. Moribund patient not expected to survive 24 hours
16. No intent to obtain central venous access for monitoring intravascular pressures.
17. > 72 hours since mechanical ventilation initiated
18. Refractory shock (See Appendix B)
19. Unable to obtain enteral access
20. Presence of partial or complete mechanical bowel obstruction, or ischemia, or infarction
21. Current TPN use or intent to use TPN within 7 days
22. Severe malnutrition with BMI < 18.5 or loss of > 30% total body weight in the previous 6 months
23. Laparotomy expected within 7 days
24. Unable to raise head of bed 30-45 degrees
25. Short-bowel syndrome or absence of gastrointestinal tract
26. Presence of high-output (> 500 cc/day) enterocutaneous fistula
27. INR > 5.0 or platelet count < 30,000 / mm^3 or history of bleeding disorder
28. Intracranial hemorrhage within the previous month
29. Allergy to enteral formula, n-3 fatty acids, gamma-linolenic acid, vitamin E, vitamin C, beta-carotene, taurine, or L-carnitine
30. Requirement for, or physician insistence on, enteral formula supplemented with omega-3 fatty acids (ex: Oxepa®, Impact®) or providing omega-3 fatty acid, GLA, or anti-oxidant supplementation

- **Enrollment and Study Initiation Time Window:** All patients must be randomized within 48 hours of meeting inclusion criteria and within 72 hours of initiating mechanical ventilation. The first three inclusion criteria may be met at either the Network or referring hospital. Following randomization, the low tidal volume protocol for mechanical ventilation must be initiated within one hour (if not already being utilized). Enteral feeds and the enteral feeding protocol must be initiated within 6 hours of randomization. The first dose of n-3 fatty acid / GLA / anti-oxidant supplementation or placebo must be administered within 6 hours of randomization.

- **Efficacy:** Primary efficacy variable is ventilator-free days to study day 28. Ventilator free days (VFDs): the number of days after initiating unassisted breathing to day 28 after randomization, assuming a patient survives for at least two consecutive calendar days after initiating unassisted breathing and remains free of assisted breathing. This is a composite endpoint reflecting days free of mechanical ventilation to day 28 and mortality. Patients who die before day 28 have zero VFDs.

- **Secondary Efficacy Variables:**

    1. The secondary efficacy variable is mortality before discharge home, with unassisted breathing to day 60. Patients alive in hospital at day 60 will be considered to have survived.

2. Mortality before hospital discharge home, with unassisted breathing, to day 90. Patients alive in hospital to day 90 will considered to have survived.
3. Number of ICU-free days at 28 days after randomization.
4. Organ-failure free days to study day 28 (renal, hepatic, central nervous system, hematologic, cardiovascular)
5. Incidence of Ventilator-associated pneumonia

Several other efficacy variables will also be analyzed, as outlined in the protocol.

Part II

Study Description

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Protocol for the NIH ARDS Network

1 Background

The following background sections discuss biochemical effects which many hypothesize as possible mechanisms for the results seen in the phase II data presented. The purpose of this study, however, is to determine the effects on clinical outcomes of the proposed interventions. These changes in clinical outcomes may be the result of the commonly hypothesized mechanisms or may result from other biochemical and/or clinical effects. Many of the proposed secondary outcomes are not meant to definitively establish the underlying mechanisms, but instead will explore biochemical endpoints to provide additional support or generate other hypotheses of how the interventions may result in different clinical outcomes.

1.1 Inflammation in ALI / ARDS

Early ALI/ARDS is pathologically characterized by neutrophilic lung inflammation, increased vascular permeability edema (Bernard, 2005; Ware, 2000) and intra-vascular and alveolar fibrin deposition (Idell, 2003;Abraham, 2000). Abundant evidence indicates the cytokines (e.g. tumor necrosis factor (TNF), and interleukin 8 (IL-8)) and the pro-inflammatory and pro-thrombotic fatty acid derivatives of cyclooxygenase (e.g. TxA_2) and 5- lipoxygenase (e.g. LTB_4) enzyme systems are mediators in the early phase of ALI/ARDS (Caironi, 2005; Gust, 1999; The Acute Respiratory Distress Syndrome Network, 2000). The ARDS network lower tidal volume ventilation trial produced significant clinical benefits, at least in part by reducing the inflammatory cytokine response (Parsons, 2005; The Acute Respiratory Distress Syndrome Network, 2000). It has also been recognized that ALI/ARDS, like severe sepsis, includes an exuberant pro-coagulant response in which fibrin is deposited in small vessels and alveoli (Abraham, 2000; Bernard, 2001; Idell, 2003; Idell, 1989).

1.2 Enteral Nutrition in Critical Illness

Experimental and clinical studies have shown that enteral nutrition has benefits over parenteral nutrition in the critically ill patient. Enteral nutrition has been reported to decrease intestinal bacterial translocation (Runyon, 1994; Wildhaber, 2005), reduce infection rates (Grahm, 1989; Kalfarentzos, 1997; Kudsk, 1992; Moore, F.A., 1992; Moore, F.A., 1989) and preserve gastrointestinal mucosal structure and function (Groos, 1996; Hadfield, 1995) as compared to parenteral nutrition. Clinical studies have shown that these findings translate into better outcomes (Gramlich, 2004; Kalfarentzos, 1997; Kudsk, 1992; Moore, F.A., 1992; Moore, F.A., 1989; Peter, 2005; Taylor, S.J., 1999; Windsor, 1998). However, there is no single standard for enteral nutrition and controversy continues to exist about most aspects of enteral feeding in the critically ill patient.

1.3 Timing of Enteral Nutrition

Recent observational data suggests enteral feeding within 48 hours of initiation of mechanical ventilation is associated with a shorter hospital length of stay and a reduction in mortality in patients with ARDS (Artinian, 2006; Stapleton, 2005). Clinical studies in critically ill surgical patients have reported that beginning enteral feeding early in the ICU and rapidly achieving full-calorie enteral feeding rates decreases infectious complications (Grahm, 1989), shortens hospital stay, decreases hypermetabolism and improves outcomes (Grahm, 1989; Gramlich, 2004; Moore, E.E., 1986; Moore, F.A., 1992; Taylor, S.J., 1999). Unfortunately, these trials were done in narrow sub-populations of critically ill surgical patients, were often not blinded or controlled, did not account for all the enrolled patients, included patients who were not mechanically ventilated, or were confounded by the use of supplemental parenteral nutrition. In addition, the benefits reported in these trials were often not consistently observed. Despite these limitations, these findings have resulted in a recent level II recommendation from the Canadian Clinical Practice Guidelines to initiate enteral feeds within 24-48 hours of ICU admission in all critically ill patients (Heyland, 2003). However, it is difficult to be confident of the findings or extrapolate the results of these studies to the majority of critically ill patients, especially those mechanically ventilated in the medical intensive care unit. Marik and Zaloga (Marik, 2001) performed a meta-analysis of randomized controlled trials that compared enteral feeding initiated earlier or later than 36 hours of hospital admission or surgery in trauma, head-injured, post-operative, burn, and medical intensive care patients. Their analysis showed a significantly lower risk of infection and shortened length of hospital stay in patients who received early enteral nutrition. However, interpretation was limited because of heterogeneity between studies, and none of the studies of medical ICU patients met the quality criteria for inclusion. No significant difference was found in mortality, although vital status data were available for just 40% of the studies.

Furthermore, a large retrospective database review recently found a lower mortality rate in critically ill, non-surgical patients who were fed within 48 hours of initiation of mechanical ventilation compared to those fed after 48 hours (Artinian, 2006). After controlling for all known confounders, the authors found that early enteral feeding was associated with a 20% decrease in ICU mortality and 25% decrease in hospital mortality, despite being independently associated with an increased risk of ventilator-associated pneumonia. Unfortunately, the retrospective nature of the study only allows determination of an association and not a cause and effect relationship.

To further complicate the picture, other clinical studies have shown no benefit to early initiation of enteral nutrition (Eyer, 1993; Ibrahim, 2002; Peck, 2004), and some even a trend towards increased number of infections with early enteral nutrition (Eyer, 1993; Ibrahim, 2002). A quasi-randomized, controlled trial by Ibrahim and colleagues found that early goal enteral feedings in mechanically ventilated medical patients had no effect on mortality, but increased the incidence of ventilator-associated pneumonia, length of ventilation and ICU stay (Ibrahim, 2002). This has caused some investigators to suggest that it is safe and possibly preferable to delay feeding for up to 1 to 2 weeks (GuidelinesGuidelines for the use of parenteral and enteral nutrition in adult and pediatric patients, 2002; Koretz, 1995). Unfortunately, these negative studies are also flawed with enrolling relatively small numbers of patients, lacking randomization, only analyzing a subset of the enrolled patients, or utilizing bolus-feeding techniques, which may increase the risk of aspiration.

Despite some consensus guideline recommendations on the acceptability of delaying enteral feeds (Cerra, 1997; Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients, 2002; Koretz, 1995), numerous surveys demonstrate clinician acceptance of the importance of early enteral feeding. Most clinicians report a practice of starting enteral nutrition early in the disease course for critically ill patients. Surveys of actual clinical practice, however, demonstrate that this is rarely the case. In most critically ill patients, enteral nutrition is not initiated for 2-4 days after intubation or ICU admission and many times, enteral feeds are advanced slowly to full-calorie rates over another couple of days (Barr, 2004; De Jonghe, 2001; Heyland, 2004; Heyland, 2003; Preiser, 1999; Rice, 2005). Similar practice occurs within the ARDS network sites. In the recently completed FACTT (National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network, 2006) study, only 17% of patients were receiving enteral feeds on day 2 and 20% on day 3. For patients still mechanically ventilated on day 7, only 30% were receiving enteral nutrition.

1.4 Volume of Enteral Nutrition and Trophic Feeds

In addition to timing, the optimal volume of enteral feedings is also debated. Animal studies demonstrate a trophic effect of low-volume enteral feeding on the intestinal epithelial border. Trophic feeds are generally defined as a small volume of enteral nutrition insufficient for the patients nutritional needs (usually < 25% of daily nutritional needs), but producing some positive gastrointestinal or systemic benefit (Sondheimer, 2004). Compared to enteral feeding abstinence, trophic feedings maintain intestinal microvilli height and structure, stimulate intestinal secretion of brush border enzymes, endogenous peptides, secretory IgA and bile salts, preserve epithelial cell tight junctions, increase intestinal motility and promote intestinal blood flow (Buchman, 1995; Groos, 1996; Hernandez, 1999). These local effects reduce systemic inflammation by helping prevent translocation of bacteria or bacterial products across the intestinal epithelial barrier and into the circulation (MacFie, 2006). In very low-birth weight infants, minimal enteral nutrition resulted in improved intestinal function and fewer septic complications, ventilator days, and hospital length of stay compared to parenteral nutrition with intestinal abstinence (McClure, 1999; McClure, 2000; McClure, 2002). Despite advocating for early enteral feeds, the Canadian Clinical Practice Guidelines admit the scarcity of data available regarding the optimal volume of early enteral feeds renders making any recommendation impossible (Heyland, 2003). Although the exact volume required to confer these effects in adult humans remains unknown, observational studies in mechanically ventilated patients (many of which did not have ARDS) have found that moderate volumes of feedings are associated with improved clinical outcomes, including lower risk of bloodstream infection (Rubinson, 2004) and lower mortality (Haddad, 2004). Other similarly designed studies have found that low volume feedings are associated with improved outcomes (Dickerson, 2002) in similar populations of critically ill patients. Furthermore, surveys of clinical practice suggest that only 55-75% of daily calories are administered to critically ill patients, even with the use of rigorous protocols (Barr, 2004; De Jonghe, 2001; Heyland, 2004; Heyland, 2003; Rice, 2005; Spain, 1999).

A phase II study comparing early trophic versus early full-calorie enteral feedings in patients requiring mechanical ventilation for at least 72 hours is currently ongoing. Although patients with acute lung injury are included, the study is not restricted to this population. In fact, of the first 100 patients, only 22% had acute lung injury. As a phase II study, the trial is powered to detect differences in biochemical endpoints and large differences in gastrointestinal intolerances, with planned enrollment of 200 patients. The study is progressing well, and an interim analysis evaluating safety, feasibility and separation of treatment arms has been conducted after the first 100 patients have been enrolled. This analysis found that administering trophic and full-calorie feeding rates are both feasible and safe. Patients randomized to the trophic arm received 220 ± 139 cc of enteral feedings per day compared to 950 ± 305 cc for the full-calorie group ($P<0.001$). These represent 15% and 64% of calculated target feeding rates, respectively. The full-calorie group reaches goal feeding rates on average in 11 hours, with 75% reaching goal rates within 15 hours. Only 4% of the group never reached full-calorie feeding rates. No safety concerns were seen in either group.

The data from these first 100 patients demonstrate that conducting this proposed study is both feasible and safe and have been extremely helpful in informing the proposed ARDS Network design. The final results of this phase II study, however, are unlikely to significantly alter practice or the need for a large, phase III study with important clinical outcomes as endpoints in patients with acute lung injury for many reasons. Like most single center studies, this study is powered to investigate mechanisms (i.e. effect of trophic and full-calorie enteral feedings on systemic inflammation) and is underpowered to detect significant differences in clinically relevant endpoints, such as mortality. This is especially true for patients with acute lung injury, which represent a subset of the population enrolled in the trial. In addition, administration of enteral feeding volumes in mechanically ventilated patients is widely variable in clinical practice without rigorous data supporting one practice over another. Lacking adequate statistical power to investigate clinical outcomes, the phase II study results will contribute to the argument for one practice, but are unlikely to definitively answer the clinical question. Regardless of which arm of the phase II study ultimately results in better biochemical endpoints, clinicians will desire data on the effects of that feeding practice on important clinical outcomes. Although biochemical endpoints help delineate mechanisms, well-designed, multi-center trials investigating the effects of different volumes of enteral nutrition on clinically important outcomes are needed to direct the standard practice of enteral feeding in patients with acute lung injury. The phase II study, however, has provided important feasibility and safety data and will provide important mechanistic data that will greatly complement the results of the proposed phase III trial.

1.5 Summary of Enteral Nutrition

A significant amount of time and resources are spent attempting to deliver enteral nutrition early in a patient's intensive care unit stay. Although there is general consensus that enteral nutrition is preferred over parenteral nutrition, the optimal timing, composition, and amount of enteral feeding is still unknown. Based on data from small surgical studies, some advocate that early enteral feeding improves outcomes in all critically ill patients, while others caution about interpreting the available data in mechanically ventilated, critically ill medical patients. The literature supports both improved and worsened outcomes when critically ill patients are fed as early as possible in their ICU stay, but no studies focus on patients with ALI/ARDS. There is biologic feasibility for both benefit and harm from early, more aggressive feeding, since more complete nutritional support may be accompanied by increased risk of hyperglycemia, uremia, or aspiration. Current practice is heterogeneous, and the reasons for this are uncertain. Further complicating the issue is the paucity of data on the optimal volume of enteral nutrition, especially early in the critical care course. In this trial, we will compare the clinical outcomes and systemic levels of inflammation of critically ill patients receiving initial trophic enteral feedings for 144 hours followed by advancement to full-calorie enteral feedings versus patients receiving initial full-calorie enteral feedings.

1.6 Fatty Acid Metabolism in ALI/ARDS

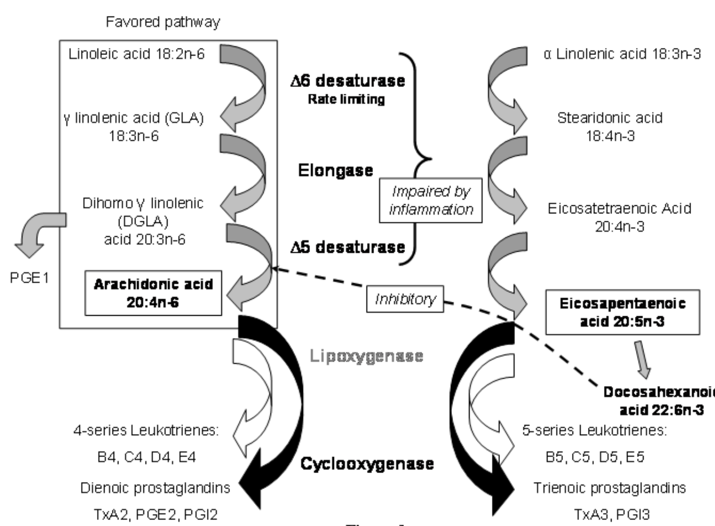


Figure 1

Linoleic and alpha linolenic acid are essential fatty acids classified by their double bond position as n-6 and n-3 fatty acids respectively. Chronic deficiency of these essential fatty acids is associated with a variety of clinical disorders including chronic degenerative neurological disease (Salem, 2001). In addition to their nutritional role, fatty acids profoundly influence inflammatory and immune events by changing lipid mediators and inflammatory protein and coagulation protein expression. After ingestion, n-6 and n-3 fats are metabolized by an alternating series of desaturase and elongase enzymes transforming them into the membrane associated lipids arachidonic acid (n-6), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids (n-3) respectively (figure 1) (Calder, 2004). All cells contain both n-6 and n-3 lipids but the central nervous system is particularly rich in DHA (Salem, 2001).

Because most humans consume a vast molar excess (>10:1) of n-6 fatty acids and n-6 lipids are the preferred substrate of the rate limiting Δ6 desaturase enzyme, arachidonate is the predominant membrane associated fatty acid and is the principal compound released when inflammation induced phospholipase activity liberates membrane associated fatty acids (Simopoulos, 1991). Normally, membrane composition is dynamic reflecting fatty acid intake. In healthy animals and humans the major dietary n-3 fatty acid α-linolenic acid, is rapidly metabolized and incorporated into plasma and platelets, leukocytes, and endothelial cells (De Caterina, 1994; Fischer, 1983). However, during inflammation, activity of the Δ5 and Δ6 desaturase enzymes is impaired, limiting the ability to transform membrane content merely by altering linolenic acid intake (Mandon, 1988). Thus, augmenting n-3 composition during inflammation requires direct supply of the end products EPA and DHA to circumvent what amounts to an effective enzymatic block (Tate, 1989).

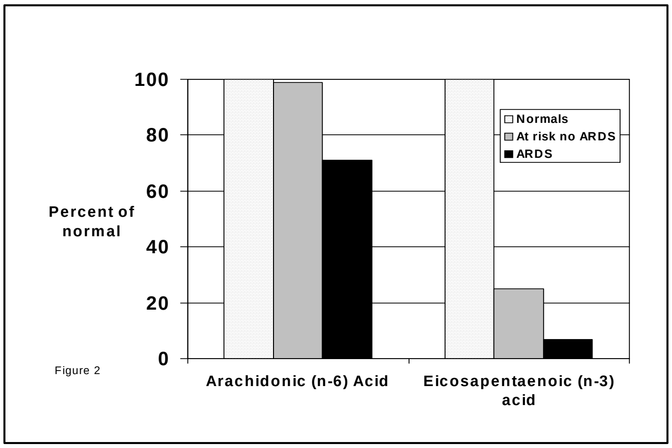


Figure 2

During inflammation, fatty acid profiles are abnormal with dramatic decreases in n-3 content. For example, in contrast to the largely preserved levels of n-6 fatty acids, patients at risk to develop ALI/ARDS have been reported to have n-3 levels approaching 25% of those of normal and those with established ARDS have levels near 6% of normal suggesting a potential role for n-3 replacement (Figure 2) (Kumar, 2000).

1.7 Cyclooxygenase and Lipoxygenase Metabolites

Once liberated, cyclooxygenase and lipoxygenase enzymes convert fatty acids to metabolically active lipids. The type and activity of the products depends upon the substrate: arachidonate (n-6) yields highly reactive dienoic prostaglandins and series 4 leukotrienes; n-3 fatty acids yield the far less active trienoic prostaglandins and series 5 leukotrienes (Calder, 2004; Prescott, 2005). Because arachidonic (AA) acid (n-6) usually predominates in plasma and membranes, dramatic elevations in dienoic products, e.g. thromboxane A2 (TxA_2), prostaglandin D2 (PGD_2) and E2 (PGE_2), occur in human plasma, urine, and bronchoalveolar lavage (BAL) fluid during ALI/ARDS (Lelcuk, 1984). Arguably TxA2 is most consequential in ALI/ARDS by causing platelet aggregation, vasoconstriction and bronchoconstriction (Lelcuk, 1984). Evidence supporting the role of these mediators comes from experiments showing that administration of n-6 precursors increases TxA2 production and worsens physiology (Palombo, 1999) and cyclooxygenase inhibitors (e.g. ibuprofen) and TxA2 inhibitors (e.g. ketoconazole) or receptor blockers improve gas exchange, oxygen consumption, and airway and vascular resistance (Bernard, 1997; The Acute Respiratory Distress Syndrome Network, 2000).

Similarly, increases in 5-lipoxygenase metabolites of AA (e.g. leukotriene B_4, C_4, D_4) have been demonstrated in ALI/ARDS (Bernard, 1991). Among these compounds LTB4 is most significant because of its potent neutrophil and macrophage chemoattractant properties and ability to increase vascular permeability and respiratory burst. Experimentally LTB_4 infusion has been associated with lung dysfunction, and antagonists of leukotriene production or receptor binding improve physiology in animal models of ALI/ARDS (Miller, R.F., 1992). Human clinical trials of leukotriene antagonists in ALI/ARDS have not been conducted.

Within hours of treating leukocytes *ex vivo* with EPA or DHA fatty acid composition and cell responses to stimuli are modified. *In vivo*, modest n-3 doses alter plasma and cellular content within 1-3 days (Chilton, F.H., 1993). Because of conformational differences, n-3 substrates have a 5-10 fold lower efficiency of conversion by desaturase enzymes than n-6 compounds (Simopoulos, 2000). Thus, when supplied in excess, n-3 fatty acids are competitive substrates decreasing total lipid mediator production. Moreover, the mediators formed from EPA (e.g. TxA_3 and LTB_5) are less active than those derived from AA. For example, TxA_3 is at least 10 fold less potent than TxA_2 and LTB_5 is significantly less active than LTB_4 (Palombo, 1996). Likewise, administration of n-3 fatty acids in animals and humans is proven to decrease and alter the leukotrienes produced (Kumar, 2000).

EPA has another potential benefit by inhibiting the conversion of dihomogammalinolenic acid to arachidonic acid via the $\Delta5$ desaturase enzyme thus shunting eicosanoid production from TxA_2, PGD_2 and PGE_2 to PGE_1 a compound with anti-aggregatory and vasodilatory properties (Fieren, 1992). Changes in lipid mediator production and profiles following n-3 fatty acid therapy have be en associated with improved physiological measures in numerous animal and human studies suggesting therapeutic potential (Breil, 1996; Gadek, 1999; Lee, 1985).

1.8 Omega-3 Fatty Acids and Cytokine Mediators

Neutrophilic inflammation is a pathologic hallmark of ALI/ARDS and strong evidence supports the roles of specific cytokines in this complex process. Very simplistically, LTB_4 and IL-8 attract and TNF and IL-1 promote adhesion and activation of neutrophils. Simultaneously TNF and IL-1 activate macrophages. Aggregates of activated neutrophils and alveolar macrophages release oxidants that subsequently damage membrane polyunsaturated fatty acids, further increasing alveolar-capillary permeability and disrupting epithelial barrier function. Reciprocally oxygen free radicals enhance TNF and other cytokine production. In addition to oxidant generation, TNF and IL-1 initiate pro-coagulant tissue factor activity on cell surfaces (Taylor, F.B., Jr., 1996). TNF and interleukins 1, 6 and 8 have been found in high concentrations in lung lavage fluid in ALI/ARDS victims (Donnelly, 1993; Donnelly, 1996) and persistent elevations, especially of IL-8 predict a poor prognosis (Miller, E.J., 1992). Because it is evanescent in plasma, TNF is uncommonly detected, and when present is seen at low levels. Instead, plasma IL-6 serves as a useful long-lived TNF surrogate. Although present at much lower levels than in BAL, plasma IL-8 can be detected and correlates with a poor outcome (Miller, E.J., 1992). Additional evidence suggests IL-10, a potent anti-inflammatory cytokine, exerts beneficial effects by down regulating TNF, IL-1, IL-6 and IL-8 production and increasing release of IL-1 soluble receptor (Chollet-Martin, 1994). Additional evidence supporting the beneficial role of IL-10 comes from the observation that low levels of IL-10 in BAL are associated with worse outcomes (Arndt, 2001).

Although pro-inflammatory cytokines are present in significant levels and correlate inversely with outcome, so far attempts to individually neutralize the effects of TNF or IL-1 using specific antagonists have not dramatically altered the course of human ALI/ARDS (Arndt, 2001).

Whether the failures reflect inefficiency of the antagonists tested, are indicative of the redundancy of inflammatory mechanisms, or are explained by the failure to adequately address the coagulopathic disease component is uncertain. Nevertheless, a simply administered, inexpensive, non-toxic therapy to decrease IL-1, IL-6, and IL-8 production while preserving IL-10 production remains an attractive notion for ALI/ARDS therapy.

Treatment of normal humans and ALI/ARDS patients with n-3 fatty acids dramatically decreases *ex vivo* production of TNF and IL-1 from stimulated mononuclear cells (Endres, 1989). In animal models of sepsis and ALI/ARDS, n-3 fatty acids significantly decrease inflammatory cytokine levels in plasma and lavage fluid and are associated with improved physiological parameters (Murray, 1995). In contrast to the suppression of pro-inflammatory cytokines seen following treatment with n-3 fatty acids, IL-10 levels are not suppressed suggesting n-3 fatty acids do not non-specifically down-regulate all cytokines. The mechanism(s) by which n-3 fatty acids inhibit cytokine activity are unknown. Hypotheses include: preserved or augmented IL-10 production (Donnelly, 1996); diversion of dihomogammalinolenic acid from AA production to E1 series prostaglandins discussed above; and altered nuclear kappa factor B activity mediated through changes in PPAR alpha and gamma activity (Schwartz, 1996; Sethi, 2002). In addition to the benefits of n-3 fatty acids in decreasing production of chemokines and lipid mediators, in higher doses they also decrease production and release of toxic oxidants.

Despite the convincingly beneficial results of n-3 fatty acids in experimental animals and *ex vivo* simulation studies of human leukocytes, their ability to reduce plasma or BAL pro-inflammatory cytokine levels in human ALI/ARDS remains largely un-investigated.

1.9 Human Clinical Studies of Omega-3 Fatty Acids in Inflammatory Disease

Biochemical changes in lipid mediators, cytokines and coagulation proteins induced by n-3 fatty acid therapy outlined above are associated with physiological improvements in chronic neutrophilic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease (Cleland, 2003; MacLean, 2005; Romano, 2005; Simopoulos, 2002). Interestingly, chronic neurologic diseases with cognitive impairment have also been demonstrated to benefit from n-3 fatty acid therapy (Fenton, 2001; Terano, 1999). Benefits of n-3 fatty acid therapy have extended to *chronic* neutrophilic inflammatory lung diseases, such as chronic obstructive pulmonary disease (Matsuyama, 2005; Romieu, 2001) and cystic fibrosis (Freedman, 2004; Panchaud, 2005).

Two studies have demonstrated benefit of n-3 fatty acid supplementation in patients with severe sepsis or septic shock (Pontes-Aruda, 2006; Galban, 2000). Both studies demonstrated a significant reduction in mortality using omega-3 fatty acids and anti-oxidants compared to standard enteral nutrition, although concern has been raised about an abnormally high mortality rate in the control arm of the most recent study. Further, Galban and colleagues demonstrated fewer infectious complications with omega-3 fatty acid supplementation (Pontes-Aruda, 2006; Galban, 2000), while Pontes-Aruda et al found improvements in oxidation, more ICU-free and ventilator-free days, and the development of fewer organ dysfunctions (Pontes-Aruda, 2006).

1.10 Human Studies of Omega 3 Fatty Acids in ALI/ARDS

Similarly, two randomized, controlled phase II studies of the effects of fish oil have been conducted in ALI patients using Oxepa®, a commercial product containing among many components, n-3 fatty acids (Gadek, 1999; Singer, 2006). Both studies suggest that Oxepa® is beneficial, but their results are not entirely similar, neither has been regarded as definitive, and the first trial published in 1999 has not changed clinical practice on a large scale. Of the hospitals involved in ARDSnet, only two use Oxepa® in their patients with ARDS. The two studies are summarized here.

The first trial (Gadek, 1999) included 148 patients with ALI randomized to Oxepa® or Pulmocare®. Treatment with Oxepa® changed plasma fatty acid profiles and decreased the total number of cells and neutrophils found in lung lavage fluid as early as day 4 of therapy. In addition, subsequent analysis of BAL fluid demonstrated a reduction in IL-8 and LTB_4 levels in patients fed the study formula (Pacht, 2003). Patients treated with Oxepa® also demonstrated significantly improved oxygenation, shorter ICU length of stay and duration of mechanical ventilation, and development of fewer new organ failures. Hospital mortality was also reduced in patients treated with Oxepa®, although not statistically significant (p=0.165). Interestingly, however, mortality in this study was much lower than expected in an era before lung protective ventilation (25% in the "control" patients receiving Pulmocare® and 16% in the Oxepa® patients), suggesting that these results may not be generalizable to all ALI patients. Further, only two-thirds of randomized patients were deemed evaluable and included in the analysis, dramatically limiting interpretation of the results (Gadek, 1999).

The second trial recently published (Singer, 2006) includes 100 patients, again randomized to Oxepa® or Pulmocare®. Physiologic variables including lung compliance and oxygenation were improved in the Oxepa® group. However, there was no difference in ICU length of stay or overall duration of mechanical ventilation, although the point estimates are similar to the Gadek trial, suggesting it was underpowered. Additionally, hospital mortality was not different and was very high, 75% in each group, again suggesting that the results are not generalizable. A second limitation is that the test article in both studies not only contained n-3 fatty acids, but also large amounts of gamma-linolenic acid (GLA), and other putative anti-oxidant compounds making attribution of the effect to omega-3 fatty acids difficult.

These preliminary data suggesting an improvement in outcomes are stronger for this nutriceutical intervention than for any other proposed study in ALI. Although the phase II data are suggestive, these two studies have significant limitations. Therefore, a large phase III trial as we have designed powered for clinically important endpoints is necessary to reproduce and confirm the prior results in a generalizable ALI patient population; such a study has the potential to change practice substantially.

1.11 Gamma-Linolenic Acid and Omega Three Fatty Acids

Gamma-Linolenic acid (GLA) is an 18-carbon n-6 polyunsaturated fatty acid present in borage oil, flax seed oil, and evening primrose oil. The body normally produces GLA from the n-6 essential fatty acid, linoleic acid. However, certain conditions, including highly inflammatory states, inhibit the action of the Δ-6 desaturase enzyme, impairing the body's ability to convert linoleic acid to GLA. This renders GLA a conditionally essential fatty acid. As very little GLA is found in the diet, supplementation may be required. Several prior studies have shown that GLA supplementation reduced the signs and symptoms of chronic inflammatory diseases such as rheumatoid arthritis and atopic dermatitis (Leventhal, 1993; Morse, 1989; Tate, 1989). Until recently, these clinical effects seemed to be inconsistent with its biosynthesis, since GLA is a potential precursor of AA, a highly proinflammatory eicosanoid precursor. Recent studies, however, have found that human neutrophils contain the elongase that metabolizes GLA to dihomo-γ-linolenic acid (DGLA) but not the Δ-5-desaturase that converts DGLA to AA (Chilton, Lopez, 1996). Therefore, it appears that GLA supplementation to neutrophils leads to the accumulation of DGLA and not AA intracellularly. However, in vivo GLA supplementation can increase serum AA in humans, thus raising the potential for an increase in proinflammatory mediators (Johnson, 1997).

Moreover, EPA is an inhibitor of human Δ-5-desaturase. In vitro and in vivo studies of GLA and EPA supplementation have found that the combination reduced serum and neutrophil leukotriene concentrations but did not increase serum AA concentrations (Barham, 2000; Johnson, 1997). It is important to note, however, that none of these human studies were performed in critically ill patients.

1.12 Summary of Omega-3 Fatty Acids and ALI / ARDS

Ex vivo and *in vivo* data from animals and humans indicate n-3 fatty acids inhibit inflammatory effects of relevant cytokines, cyclooxygenase, and lipoxygenase products. Treatment with n-3 fatty acids maintains or increases levels of the anti-inflammatory cytokine IL-10. Human data confirm safe, well-tolerated doses of n-3 fatty acids alter fatty acid levels within days resulting in anti-inflammatory and anti-thrombotic effects. Substantial data from chronic inflammatory human conditions other than ALI / ARDS (e.g. rheumatoid arthritis, inflammatory bowel disease) indicate n-3 fatty acids favorably alter clinical outcomes. The Phase II human study of n-3 fatty acids in ALI / ARDS noted above offers strong support for the concept. We hypothesize that increasing the ratio of n-3 fatty acids in patients with ALI / ARDS will acutely alter the lipid composition of plasma and cell membranes, decrease the levels of pro-inflammatory cytokines, and decrease cyclooxygenase and lipoxygenase products. Altering these inflammatory mechanisms will favorably impact the important clinical endpoints of mortality, ventilator free days, and organ failure free days.

1.13 Oxidative Stress and ARDS

Oxidative stress is elevated with many disease states (Cracowski, 2000; Montuschi, 2000; Wood, 2000), and it is reasonable to postulate that levels of oxidative stress are even higher in illnesses representing more severe perturbations of the disease spectrum. In many critical illnesses, especially ones emanating from infection, macrophages are increased, recruited, and activated. The resultant increase in macrophage oxidative burst is vital in helping to overcome the inflammatory process. In addition, energy expenditure increases in critical illness. Studies have demonstrated that patients with sepsis and septic shock demonstrate elevated levels of oxidant stress (Goode, 1995; Gutteridge, 1999). Furthermore, the acute respiratory distress syndrome, a predominantly neutrophilic inflammatory process, also results in increased levels of oxidative stress (Carpenter, 1998; Gutteridge, 1999; Schmidt, 2004). Some studies have suggested that levels of oxidative stress, as demonstrated by lipid peroxidation, correlate with worse outcomes in critically ill patients (Cowley, 1996). Studies of anti-oxidant therapy independently in patients with ARDS, however, are limited to trials investigating N-acetylcysteine (NAC). Despite demonstrating improved pulmonary physiology, three moderate-sized clinical trials investigating intravenous NAC failed to demonstrate any benefit in clinical outcomes, including no difference in 30-day or 60-day mortality, ventilator-free days, or ICU-free days (Bernard, 1997; Jepsen, 1992; Suter, 1994). Unfortunately, none of the studies measured changes in markers of oxidative stress. One study combining omega-3 fatty acid and anti-oxidant treatment, however, found normalization of low anti-oxidant levels, but no alteration in measures of oxidative stress (Bernard, 1997; Jepsen, 1992; Nelson, 2003; Suter, 1994).

1.14 Caloric Restriction, Energy Expenditure, and Oxidative Stress

Trophic feedings, as utilized in this proposal, provide an enteral feeding regimen low in calories compared to full-calorie feedings. Caloric restriction (Koubova, 2003) delays the development of a wide spectrum of diseases, including kidney disease, neoplasias, diabetes, and autoimmune diseases, resulting in prolonged survival in multiple species (Fernandes, 1976; Jolly, 2005; Lane, 2001; Sohal, 1996). Although the mechanism of its action remains unknown it has been proposed that caloric restriction reduces oxidative damage generated by ROS produced during respiration (Afanas'ev, 2005; Heilbronn, 2006). Normally about 3% of oxygen consumed is converted to ROS by mitochondria; hence as energy expenditure increases, the ROS burden increases. Likewise, reducing energy expenditure decreases formation of radical generating molecules. Caloric restriction effectively decreases energy expenditure (Heilbronn, 2006), and has been shown to decrease the production of ROS, resulting in less oxidative stress in animal models (Yu, 2005).

1.15 Nutrition and Oxidative Stress

Whereas antioxidants, when given as nutritional supplements, reduce oxidative stress (Dietrich, 2002; Nathens, 2002), the effect of feeding on these processes remains unknown, especially in critically ill patients. Polyunsaturated fats (PUFA), which make up the bulk of fats in standard tube feed formulas, are excellent substrates for lipid peroxidation (Napolitano, 2004). With rare exception (Stier, 2001), in vitro studies suggest that oxidative susceptibility increases with polyunsaturated fats (Cosgrove, 1987) and studies in non-critically ill patients have demonstrated similar increases in lipid peroxidation with PUFA intake (Abbey, 1993; Van Gossum, 1988). In addition providing differing amounts of fatty acids demonstrates increased oxidative stress in patients given additional PUFAs (Abbey, 1993; Van Gossum, 1988). Omega-3 fatty acid supplementation alters the lipid composition of plasma membranes, resulting in less arachidonic acid available for peroxidation. However, the effect of omega-3 fatty acid supplementation on levels of *in vivo* oxidative stress is unknown. In addition, since inflammation increases oxidative stress, decreasing inflammation either through administration of omega-3 fatty acids or trophic feeds, may also result in lower levels of oxidative stress.

1.16 Summary of Anti-oxidants and ALI / ARDS

Critically ill patients have increased levels of oxidative stress. We hypothesize that anti-oxidant supplementation, in the form of Vitamin C and Vitamin E, will scavenge free radicals and reduce oxidative stress in patients with ALI / ARDS. In addition, we hypothesize that omega-3 fatty acid supplementation will complement the anti-oxidant effect by reducing the availability of arachidonic acid, a major substrate for lipid peroxidation and that caloric restriction in the form of trophic feeds will further reduce oxidative stress by decreasing energy expenditure. Finally, both omega-3 fatty acid supplementation and trophic enteral feedings have the potential to indirectly attenuate oxidative stress by reducing inflammation. Decreasing oxidative stress by any or all of these methods will favorably impact the important clinical endpoints of mortality, ventilator free days, and organ failure free days.

1.17 Measurement of Long Term Outcomes and Acute Lung Injury

Emerging data indicate that survivors of acute lung injury have substantial disability after recovery from acute lung injury. After hospital discharge, only about one-third return to home and more than one-half reside in skilled nursing facilities or rehabilitation facilities (Rubenfeld, 2005). Up to one year later, most patients have serious deficits in health-related quality of life, functional performance, cognition, and employment (Herridge, 2003; Hopkins, 2005). Mortality and ventilator-free days, which have been the primary outcomes in most clinical trials of treatments for acute lung injury, do not capture these important longer-term decrements (Brower, 2004; Schoenfeld, 2002; The Acute Respiratory Distress Syndrome Network, 2000). Moreover, it has recently become clear that acute lung injury, contrary to previous belief, becomes a chronic, disabling pulmonary condition in many cases (Herridge, 2003). To capture the full impact of any treatment for acute lung injury, longer term outcomes must be assessed.

The effects of treatment for acute lung injury on short term mortality may not capture the full impact of treatment over the longer term. A treatment may have early benefit that is maintained, amplified, or attenuated over a longer time period. For example, an invasive strategy for diagnosing ventilator-associated pneumonia reduced 14 day mortality, but the benefit decreased thereafter and the mortality benefit was lost (Fagon, 2000). In addition, a treatment may improve mortality but have additional deleterious effects that adversely affect long term outcomes such as health-related quality of life and functional performance. For example, parenteral corticosteroids, which may have some immediate benefit in late-phase ARDS, may have detrimental longer-term effects on muscle function and weakness that lead to impaired physical functioning (Herridge, 2003; Steinberg, 2006). To fully evaluate new therapies for acute lung injury, a broad spectrum of long term outcomes must be ascertained. Moreover, measurement of long-term outcomes is necessary to compare the cost-effectiveness of different strategies for ARDS (Angus, 2001).

2 Objectives

2.1 Primary Objectives

- Evaluate the efficacy and safety of initial trophic enteral feeds followed by advancement to full-calorie enteral feeding vs. initial advancement to full-calorie enteral feeding management strategies on mortality, ventilator-free days, ICU-free days, and organ failure in patients with Acute Lung Injury or Acute Respiratory Distress Syndrome

- Evaluate the safety and efficacy of omega-3 fatty acid, gamma-linolenic acid (GLA), and anti-oxidant supplementation on mortality, ventilator-free days, ICU-free days, and organ failure in patients with ALI or ARDS

2.2 Secondary Objectives

To develop and analyze a clinical database of patients enrolled in the clinical trial who are well characterized and followed for 12 months for the purpose of answering questions about the natural history of ARDS and evaluating the effect of different interventions a and patterns of supportive care.

2.3 Primary Hypotheses

- Initial trophic feeding followed by full-calorie enteral feeding will increase the number of ventilator-free days to study day 28 in patients with ALI or ARDS by reducing systemic inflammation and the number of feeding complications as compared to early, full-calorie enteral feeding.

- Omega-3 Fatty Acid, GLA, and anti-oxidant supplementation, as compared to placebo, will increase the number of ventilator-free days to study day 28 in patients with ALI or ARDS by attenuating systemic inflammation.

2.4 Secondary Hypotheses

- Initial trophic feeding will have anti-inflammatory effects demonstrated by decreased plasma IL-6 and IL-8 levels in patients with ALI or ARDS compared to early, full-calorie enteral feeding.

- Initial trophic feeding followed by full-calorie enteral feeding will decrease the incidence of gastrointestinal intolerances (vomiting, aspiration, regurgitation, diarrhea, elevated gastric residual volumes, and abdominal distention and cramping) compared to early, full-calorie enteral feeding.

- Initial trophic feeding followed by full-calorie enteral feeding will decrease the incidence of ventilator-associated pneumonia in patients with ALI or ARDS compared to early, full-calorie enteral feeding.

- Initial trophic feeding followed by full-calorie enteral feeding will decrease the incidence of developing bacteremia in patients with ALI or ARDS compared to early, full-calorie enteral feeding.

- Omega-3 fatty acid supplementation, as compared to placebo, will selectively increase plasma levels of EPA and DHA, resulting in an increased omega-3 to omega-6 fatty acid ratio in plasma.

- Omega-3 fatty acid, GLA, and anti-oxidant supplementation, as compared to placebo, will decrease the plasma levels of pro-inflammatory cytokines IL-6 and IL-8.

- Omega-3 fatty acid and anti-oxidant supplementation will reduce the urinary ratios of stable metabolites of leukotriene B_4 to B_5 indicating alterations in lipid utilization by lipoxygenase enzymes among ALI/ARDS patients compared to placebo.

- Anti-oxidant supplementation will reduce the systemic oxidative stress as measured by urinary F_2-isoprostane metabolites (Morrow, 1999) among ALI/ARDS patients compared to placebo.

3 End Points

Analysis of primary and all secondary endpoints will be conducted on an intention to treat basis. A secondary analysis will be performed looking at patients who achieved greater than 70% of full-calorie feeds for the initial 6 days.

3.1 Primary Endpoint

1. Ventilator-Free Days to study day 28

VFDs is a composite endpoint that is affected by mortality and duration of mechanical ventilation in survivors (Schoenfeld, 2002), which has been chosen as the primary endpoint for a number of reasons. Preliminary data suggests that omega-3 fatty acid and anti-oxidant supplementation decreases inflammation in the lungs in patients with ALI. VFDs provide a validated measure of improved lung function, even if overall mortality is only minimally altered. In addition to also possibly altering inflammation, full-calorie feedings may place patients at risk for aspiration, which may result in increased mortality, but will result in fewer ventilator-free days, even in non-fatal cases. Further, VFDs is a measure of a morbidity outcome and it is directly related to "days of assisted ventilation." However, a trend in one treatment group toward early patient death would likely decrease the number of days of assisted ventilation. This example of decreased days of assisted ventilation is misleading as the treatment group actually had a worse outcome. Measuring ventilator days in survivors would offset the problem of early mortality decreasing ventilator days. However, if a treatment group had a favorable trend towards improved survival, but required additional ventilator days for survival, "average number of ventilator days in survivors" could also be misleading. VFDs represent a measurable outcome that is favorably affected by both shorter duration of assisted ventilation in survivors and lower mortality.

VFD to day 28 is defined as the number of days from the time of initiating unassisted breathing to day 28 after randomization, assuming survival for at least two consecutive calendar days after initiating unassisted breathing and remains free of assisted breathing to day 28. If a patient returns to assisted breathing and subsequently achieves unassisted breathing to day 28, VFD will be counted from the end of the last period of assisted breathing to day 28 unless a period of assisted breathing was less than 24 hours and the purpose of assisted breathing was a surgical procedure. If a patient was receiving assisted breathing at day 27 or dies prior to day 28, VFD will be zero. Patients transferred to another hospital or other health care facility prior to day 28 while still receiving assisted breathing will be followed to assess this efficacy measure. Unassisted breathing is defined as breathing with facemask or nasal prong oxygen (or room air) following extubation, T-tube breathing, breathing with continuous positive airway pressure (CPAP $\leq$ 5 cm H_2O without PS or IMV assistance), or tracheotomy mask breathing.

3.2 Secondary Endpoints

1. The secondary efficacy variable for the trial is mortality prior to hospital discharge with unassisted breathing. Patients alive in hospital at day 60 will be considered to have survived.
2. Mortality before hospital discharge home, with unassisted breathing, to day 90. Patients alive in hospital to day 90 will considered to have survived
3. Number of ICU-free days at 28 days after randomization
4. Number of organ failure-free days at 28 days after randomization. Organ failure will be defined by previously validated definitions for renal, circulation, central nervous system, hematologic, and hepatic organ and system failures (Bernard, 1997).

Organ failure is defined as present on any date when the most abnormal vital signs or clinically available lab value meets the definition of clinically significant organ failure according to the Brussels Organ Failure Table. Patients will be followed for development of organ failures to death, hospital discharge or study day 28, whichever comes first. Each day a patient is alive and free of a given clinically significant organ failure will be scored as a failure-free day. Any day that a patient is alive and free of all 5 organ failures will represent days alive and free of all organ failure. Central nervous system dysfunction is evaluated using the Glasgow Coma Scale.

5. Number of days between the day of first meeting criteria for weaning-readiness (see Appendix G, section G.2.) and day 28 after randomization.
6. Mortality and VFDs in patients with pre-randomization $PaO_2/F_IO_2 \leq 200$.
7. Change in plasma levels of IL-6, IL-8, VWF, SPD, and total protein concentrations from baseline to study day 3.
8. Ventilator free days and mortality prior to hospital discharge with unassisted breathing to day 60 and number of ventilator-free days to day 28 in patients with shock (defined in 2.1.2) at the time of randomization.

3.3 Other Endpoints

Many of these proposed outcomes are not meant to definitively establish the underlying mechanisms, but instead will explore biochemical endpoints to provide additional support or generate other hypotheses of how the interventions may result in different clinical outcomes.

1. Reduction of PaO_2 / FiO_2 ratio on study days 1-7
2. Improvement in Lung Injury Score on study days 1-7
3. Number of gastrointestinal intolerances (aspiration, vomiting, regurgitation, diarrhea, elevated gastric residual volumes, abdominal distention and cramping) on study days 1-7
4. Level of systemic inflammation, as measured by plasma IL-6 and IL-8 levels.
5. Degree of lipid utilization by lipoxygenase, as measured by the ratio of stable urinary metabolites of leukotriene B_4 to B_5.
6. Measure of oxidative stress on days 3, 6 and 12 compared to baseline as measured by urinary levels of F_2-isoprostane metabolites

7. Incidence of bacteremia developing
8. Incidence of *Clostridium dificile* induced diarrhea.
9. Incidence of ventilator-associated pneumonia
10. Serum levels of markers of nutrition, including albumin and total protein levels between baseline and days 6 and 12.

Clostridium dificile diarrhea will be diagnosed by one or more daily stool specimen positive for cytotoxin assay or enzyme immunoassay. Patients with more than 3 liquid stools totaling more than an estimated 500 ml of stool per day, or those with systemic inflammatory response syndrome unexplained by other infection, may have up to three daily stool samples sent for *C. dificile* investigation (either cytotoxin assay or enzyme immunoassay).

Bacteremia will only be considered if it develops greater than 24 hours after the initiation of study procedures and is documented with a positive blood culture. The primary medical team, using clinical judgment, will determine when blood cultures are sent. Coagulase negative (or thermo nuclease negative) *Staphylococci* or *Corynebacterium* bacteremia require the isolation of these organisms from at least two blood cultures drawn within 24 hours of each other containing the same organism in order to be deemed significant.

Ventilator-associated pneumonia (VAP) is a difficult diagnosis to make with certainty, especially in patients with underlying ALI or ARDS. However, for the purposes of this trial, an objective definition of VAP will be used in order to standardize the reporting and reduce bias during the first 6 days of enteral feeding given the unblinded administration of enteral feeding volumes. As such, VAP will be defined using the same scoring system as the ARDS network used for the LaSRS study The scoring system incorporates temperature, leukocyte count, sputum or tracheal aspirate Gram stain and culture, and chest radiograph results. This score will be calculated as available as long as the patient remains ventilated. The certainty of VAP will be graded as either suspected or possible vs. probable using the criteria listed in Appendix A.

4 Study Population and Enrollment

4.1 Number/Source/Screening

The trial will accrue a maximum of 1000 patients into a 2 x 2 factorial study design (see diagram below) over a 3-4 year interval. Patients with ALI or ARDS will be recruited from intensive care units at NIH ARDS Network centers. Study coordinators will visit intensive care units daily to identify potential candidates for enrollment (see inclusion criteria, section 4.2, and exclusion criteria, section 4.3. Permission to approach patients and/or their families will be requested from the attending physicians. All patients meeting the inclusion criteria will be entered on a screening log. If the patient is not enrolled, the screening log will include information explaining why enrollment did not occur (exclusion criteria, attending physician denial, patient refusal, etc. see Appendix L).

2 X 2 Factorial Study Design

Unblinded / Blinded	Trophic (10 cc / hr X 144 hrs)	Full-calorie (within 24 hr)
Study Solution (21 days or UAB)	n = 250	n = 250
Placebo	n = 250	n = 250

4.2 Inclusion Criteria

Patients will be eligible for inclusion if they meet all of the below criteria. Criteria 1-3 must all be present within a 24-hour time period:

Acute onset (defined below) of:

1. $PaO_2 / FiO_2 \leq 300$ (intubated). If altitude > 1000m, then $PaO_2 / FiO_2 \leq 300$ x (PB/760)
2. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph. The infiltrates may be patchy, diffuse, homogeneous, or asymmetric
3. Requirement for positive pressure ventilation via endotracheal tube, and
4. No clinical evidence of left -sided cardiac failure to account for bilateral pulmonary infiltrates.
5. Intention of primary medical team to enterally feed the patient

   The 48-hour enrollment time window begins when criteria 1-3 are met. If a patient meets the first three inclusion criteria but has a PAOP (Pulmonary Arterial Wedge Pressure) greater than 18 mmHg, then the first four criteria must persist for more than 12 hours after the PAOP has declined to ≤ 18 mmHg, and still be within the 48-hour enrollment window.

   "Acute onset" is defined as follows: the duration of the hypoxemia criterion (#1) and the chest radiograph criterion (#2) must be ≤ 28 days at the time of randomization. Opacities considered "consistent with pulmonary edema" include any opacities not fully explained by mass, atelectasis, or effusion or opacities known to be chronic (greater than 28 days). Vascular redistribution, indistinct vessels, and indistinct heart borders alone are not considered "consistent with pulmonary edema" and thus would not count as qualifying opacities for this study.

4.3 Exclusion Criteria

1. Age younger than 13 years.
2. Greater than 48 hours all since inclusion criteria met

3. Neuromuscular disease that impairs ability to ventilate with out assistance, such as cervical spinal cord injury at level C5 or higher, amyotrophic lateral sclerosis, Guillain-Barré Syndrome, or myasthenia gravis (See Appendix B)
4. Pregnant or breast-feeding
5. Severe chronic respiratory disease (See Appendix B for detailed exclusion criteria).
6. Burns greater than 40% total body surface area
7. Malignancy or other irreversible disease or condition for which 6-month mortality is estimated to be greater than 50% (See Appendix B).
8. Allogeneic bone marrow transplant within the last 5 years
9. Patient, surrogate, or physician not committed to full support (Exception: a patient will not be excluded if he/she would receive all supportive care except for attempts at resuscitation from cardiac arrest).
10. Severe chronic liver disease (Child-Pugh Score of 11-15)
11. Diffuse alveolar hemorrhage from vasculitis.
12. Morbid obesity (> 1kg/cm body weight)
13. No consent/inability to obtain consent
14. Unwillingness or inability to utilize the ARDS network 6 ml / kg PBW ventilation protocol
15. Moribund patient not expected to survive 24 hours
16. No intent to obtain central venous access for monitoring intravascular pressures.
17. > 72 hours since mechanical ventilation initiated
18. Refractory shock (See Appendix B)
19. Unable to obtain enteral access
20. Presence of partial or complete mechanical bowel obstruction, or ischemia, or infarction
21. Current TPN use or intent to use TPN within 7 days
22. Severe malnutrition with BMI < 18.5 or loss of > 30% total body weight in the previous 6 months
23. Laparotomy expected within 7 days
24. Unable to raise head of bed 30-45 degrees
25. Short-bowel syndrome or absence of gastrointestinal tract
26. Presence of high-output (> 500 cc/day) enterocutaneous fistula
27. INR > 5.0 or platelet count < 30,000 / mm^3 or history of bleeding disorder
28. Intracranial hemorrhage within the previous month
29. Allergy to enteral formula, n-3 fatty acids, gamma-linolenic acid, vitamin E, vitamin C, beta-carotene, taurine, or L-carnitine
30. Requirement for, or physician insistence on, enteral formula supplemented with omega-3 fatty acids (ex: Oxepa®, Impact®) or providing omega-3 fatty acid, GLA, or anti-oxidant supplementation

4.4 Enrollment, Randomization, and Study Initiation Time Window

All patients must be randomized within 48 hours of meeting inclusion criteria for ALI (inclusion criteria 1-3) and within 72 hours of initiating mechanical ventilation. The window for randomization will begin at the time of meeting all inclusion criteria and/or the time of documentation of mechanical ventilation, regardless of hospital location. The first three inclusion criteria may be met at either the Network or referring hospital. Following randomization, the low tidal volume protocol for mechanical ventilation must be initiated within one hour (if not already being utilized). Enteral feeds and the enteral feeding protocol must be initiated within 6 hours of randomization. The first dose of study emulsion or placebo must be administered within 6 hours of randomization.

4.5 Informed Consent

Informed consent will be obtained from each patient or surrogate prior to enrollment in the trial. No study procedures will be done prior to obtaining informed consent.

4.6 Randomization

After obtaining a signed and dated informed consent, the coordinating center will be called and an assignment, in the form of a study ID number, will be made by computer-generated randomization to OMEGA or placebo and early verses late trophic feedings.

Randomization will be accomplished with a web based randomization system. Each research coordinator will have a unique Personal Identification Number (PIN). The randomization will provide and patient ID number to the pharmacy that will dispense either active treatment, or placebo based on a predetermined list in the research pharmacy. The pharmacist will be unblinded to the treatment assignments. He or she will be responsible for treatment assignments, formulations, and maintaining the list of codes revealing which treatment is being taken by each study participants. In addition the study personnel will be informed if the patient is to receive trophic or full feedings.

The randomization will be stratified by institution, and by shock at study entry to one of the four nutrition study combinations.

4.7 Minorities and Women and Children

Gender and racial patient subsets were considered by the NHLBI in selecting the Network Centers. The demographic profiles of the Centers selected for the Network show that the aggregate patient population contains representative proportions of minorities and women. Recruitment of minorities and women will be monitored by the Network Coordinating Center. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate patient sample contains appropriate gender and minority subsets.

Children will be enrolled who are 13 years and older. There is general agreement that children in this age range have pathophysiology and outcomes similar to adults with ALI. In addition the study procedures called for in the protocol can be readily carried out safely and effectively in this population.

It is less clear that this is so for children under the age of 13. A joint committee of the ARDSnet and Pediatric ALI investigators (PALISI) is actively debating this issue. Once their report has been reviewed and accepted by the ARDSnet Steering Committee, it will be forwarded to the DSMB with or without proposed changes in the trial depending on the recommendations presented.

5 Study Procedures

5.1 Enteral Feeding Procedures

5.1.1 Enteral Feeding Formula

Feedings in both groups will employ a sterile, commercially available standard enteral formula (not supplemented with n-3 fatty acids) used in the ICU. Any formula that does not contain supplemental n-3 fatty acids or anti-oxidants will be acceptable to use. Enteral formulas supplemented with n-3 fatty acids will not be allowed to be utilized due to the confounding effects of the n-3 fatty acids. Neither n-3 fatty acid, nor anti-oxidant supplementation will be permitted during the study. The list of formulas that are not allowed includes: Oxepa®, Impact®, Peptamen AF®, Crucial®, Optimental® and Pivot 1.5®.

5.1.2 Enteral Feeding Site

The location and type of enteral feeding tube (nasogastric, nasoenteric, PEG, orogastric, oroenteric, etc.) will not be randomized, but will instead be determined by the patient's primary medical team. The location of the feeding will be documented on the case report form. Consideration should be made for advancing the feeding tube to a post-pyloric position in patients receiving gastric feeds who experience multiple elevated gastric residual volumes (see section 5.1.4) or vomiting (see section 5.1.7.4).

5.1.3 Enteral Feeding Rates

All patients will have enteral feeds started within 6 hours of being enrolled and randomized. Upon admission to the ICU, a full-calorie feeding rate should be determined. The full-calorie feeding rate will be calculated to deliver 25-35 kcal/kg PBW each day (Cerra, 1997). If a formal dietary evaluation is done, the dietary recommendation can be used as an acceptable alternative full-calorie rate.

The following formulas will be utilized to calculate predicted body weight (PBW):

For males: PBW (kg) = 50 + 2.3 [height (*inches*) - 60] = 50 + .91 (height (*cm*) - 152.4)
For females: PBW (kg) = 45.5 + 2.3 [height (*inches*) - 60] = 45.5 + .91 (height (*cm*) - 152.4)

5.1.3.1 Trophic Enteral Feeding Treatment Group (Trophic Feeding Group)

All patients randomized to trophic enteral feedings will have enteral feeds started at 10 cc / hr and continued at this rate for 144 hours (see Trophic Feeding Protocol, Appendix C) provided gastric residuals remain at an acceptable level (see Gastric Residuals, section 5.1.4) and provided the patient remains on the ventilator. After 144 hours of trophic enteral feeds, the feeding rate will be advanced to full-calorie rates using the same protocol as for the full-calorie feeding treatment group (see section 5.1.3.2 and Appendix D) provided the patient remains on the ventilator.

5.1.3.2 Full-calorie Enteral Feeding Treatment Group (Full-calorie Feeding Group)

The full-calorie feeding group will have enteral feeds initiated at 25 cc / hr. If gastric residuals remain at an acceptable level (see Gastric Residuals, section 5.1.4), the feeding rate will be increased by 25 cc / hr every 6 hours until goal rate (as determined by the dietary evaluation if available) is achieved (see Full-calorie Feeding Protocol, Appendix D). This pattern of advancement is similar to advancement rates used in other feeding trials (Adam, 1997; Rice, 2005).

The preliminary data from the single-center phase II study demonstrates that patients randomized to the 10 cc/hr of trophic feeding actually receive 220 cc / day of enteral feeds, which represents 15% of calculated target feeding rates (1480 cc/day). However, in the study design, every patient will also receive the equivalent of 240 additional cc of enteral feeding each day (120 cc of n-3 fatty acid study solution or placebo twice each day). Thus, patients in the trophic feeding arm will receive 480 cc, or 32% of target enteral feedings, each day. This volume of delivery lies within the range of data from published studies in animals and neonatal humans (Burrin, 2000; McClure, 2000; Ohta, 2003; Omura, 2000; Owens, 2002; Tyson, 2005).

5.1.4 Gastric Residuals

The gastric residual volume (GRV) will be the amount of gastric contents able to be withdrawn from the gastric tube using a 60 cc syringe. If gastric residuals exceed 400 cc (McClave, 2002), the feeding rate will be adjusted according to the full-calorie feeding protocol (see Appendix D). If the patient has a post-pyloric feeding tube, gastric residuals will be measured only if a separate gastric port on the feeding tube or a separate gastric tube is in place. GRV in patients receiving post-pyloric feeding will only be considered significant if they exceed 400 cc and contain tube feeding formula. The aspiration of gastric juice in patients fed through post-pyloric tubes will not be considered gastric residual for the purpose of adjusting tube feeding rates unless it contains enteral formula.

Since a single, isolated, elevated gastric residual has been shown to be a poor predictor of enteral feeding tolerance (Mentec, 2001; Spain, 1999), enteral feeding rates will not be adjusted after a single elevated gastric residual. However, enteral feeding rates will be decreased or held if two or more GRVs are elevated (see Appendix D) as this likely represents impaired gastrointestinal tolerance of enteral feeding (Mentec, 2001; Spain, 1999).

The use of pro-kinetic agents and/or advancing the distal location of the feeding tube to a post-pyloric position should be considered in patients experiencing more than one episode of elevated gastric residual volume.

5.1.4.1 Gastric Residuals -Trophic Feeding Group

GRVs will be checked every 12 hours while patients are receiving trophic feeding rates. If GRV remains less than or equal to 400 cc, GRV will be replaced and tube feeds will continue at 10 cc / hr (see Appendix C). If GRV exceeds 400 cc, 400 cc of the residual volume will be replaced, tube feeds will continue at 10 cc / hr and GRV will be rechecked in 2 hours. If GRV remains greater than 400 cc, tube feeds will be held. GRV will be replaced (up to 400 cc) and gastric residual rechecked every 2 hours. Tube feeds will continue to be held until residual volume is less than or equal to 400 cc. Once residual volume is less than or equal to 400 cc, trophic tube feeds will be restarted at a rate of 10 cc / hr.

When the trophic feeding group is advanced to full-calorie enteral feeds (after 144 hours of trophic feeds), GRVs will be checked according to the full-calorie feeding protocol (see section 5.1.4.2 and Appendix D).

5.1.4.2 Gastric Residuals – Full-calorie Feeding Group

Gastric residuals in the full-calorie feeding group will be checked every 6 to 12 hours according to the full-calorie feeding protocol (see Appendix D). If GRV remains less than or equal to 400 cc, GRV will be replaced tube feeds will be advanced or maintained if already at full-calorie rate. After the first episode of GRV greater than 400 cc, 400 cc of the GRV will be replaced, the feeding rate will be maintained at the current rate and residuals will be rechecked in 2 hours. After the second episode of GRV greater than 400 cc, tube feeds will be held. 400 cc of the GRV will be replaced and rechecked every 2 hours. Tube feeds will continue to be held until gastric residuals are less than 400 cc. Once GRV is less than or equal to 400 cc, GRV will be replaced and tube feeds will be restarted at 25 cc / hr less than the previous rate.

5.1.5 Patient Position

To decrease the risk of aspiration and nosocomial pneumonia, patients will be maintained in the semi-recumbent position (head of bed raised 30-45 degrees) at all times possible (Drakulovic, 1999; Mentec, 2001; Spain, 1999). Exceptions to the semi-recumbent position will include times when tube feeds are turned off because a patient is: having a bedside procedure performed, bathing, or hypotensive requiring flat or reverse Trendelenburg positioning.

5.1.6 Holding or Interrupting Enteral Feeds

Enteral feeds held for less than 30 consecutive minutes will not be considered interrupted, but when held for 30 minutes or more should be reported as interrupted on the case report form.

Enteral feeds should be held for no more than 4 hours prior to procedures, including surgical procedures in the operating room, and in anticipation of extubation. Alternatively, feedings can be continued up to the time of the procedure or extubation and the gastric volume, including enteral feeds, can be removed using manual suction through a 60 cc syringe. Post-procedure, or if patient deemed not ready for extubation, feedings should be restarted at the prior rate.

If enteral feeding is stopped for any reason other than gastrointestinal intolerance (see Gastrointestinal Intolerances, Section 5.1.7), then tube feeds are to be restarted at prior rate.

5.1.7 Gastrointestinal Intolerances

The action taken if a patient has one of the following gastrointestinal intolerances will be standardized. However, the patient's primary medical team will determine whether or not the patient fulfills the criteria for meeting the definition of the specific gastrointestinal intolerance.

5.1.7.1 Abdominal Distention/Cramping

Abdominal distention or cramping is defined as the presence of a tense abdomen, rigidity, guarding or rebound on exam.

For patients receiving trophic feeds, enteral feeds will be held for abdominal cramping or distention. Enteral feeding will be resumed at 10cc/ hr in this group after 6 hours or improvement of distention or cramping, whichever occurs first.

When abdominal distention or cramping occurs in patients randomized to full-calorie feeding rates, the enteral feeding rate will be decreased by 25 cc / hr to a minimum rate of 10 cc / hr. The abdomen will be re-evaluated 6 hours later. If the distention or cramping is improved, the enteral feeds will be advanced to full-calorie rates per the full-calorie feeding protocol as long as GRVs are acceptable (see Appendix D). If, after 6 hours, the abdominal distention or cramping is not improved, then enteral feeding will be held and resumed at the 25 cc less/ hr rate after 6 additional hours or improvement of the abdominal distention or cramping, which ever occurs first.

5.1.7.2 Aspiration

Aspiration is defined as the presence of food in the lungs. This will be determined by the primary medical team, but will include visualization of enteral feeds in the endotracheal tube or enteral formula suctioned from the endotracheal tube.

When aspiration occurs, the tube will be checked to confirm that it terminates in the correct location (gastric or post-pyloric). The manner in which the tube position is confirmed will be determined by the primary medical team, but will include either auscultation of air forced through the tube or obtaining a radiographic study. If the feeding tube terminates in the esophagus, it should be repositioned and tube feeds restarted at 25 cc / hr less than the previous rate (minimum of 10 cc / hr) for the full-calorie feeding group and 10 cc / hr for the trophic feeding group. If the feeding tube terminates distal to the esophagus, enteral feeds will be held for 6 hours. After 6 hours, enteral feeds will be restarted at 25 cc / hr less than the previous rate (minimum of 10 cc / hr) for the full-calorie feeding group and 10 cc / hr for the trophic feeding group.

5.1.7.3 Regurgitation

Regurgitation is defined as the presence of enteral feeds in the oropharynx or nasopharynx on routine oral care.

When regurgitation occurs, the tube will be checked to confirm that it terminates in the correct location (gastric or post-pyloric). The manner in which the tube position is confirmed will be determined by the primary medical team, but will include either auscultation of air forced through the tube or obtaining a radiographic study. If the feeding tube terminates proximal to the stomach, it will be repositioned and enteral feedings will continue at the previous rate. If the tube is in the correct position (i.e. gastric or post-pyloric), a GRV will be checked. If the GRV is greater than 400 cc, the enteral feeds will be held for 6 hours. After 6 hours, the enteral feeds will be restarted at 25 cc / hr less than the previous rate (minimum of 10 cc / hr) for the full-calorie feeding group and 10 cc / hr for the trophic feeding group. If the gastric residual is less than 400 cc, the residual will be replaced and the enteral feeds will continue at the current rate.

5.1.7.4 Vomiting

Vomiting is defined as the forceful expulsion of gastric contents from the oropharynx or nasopharynx.

When vomiting occurs, enteral feeds will be held for 6 hours. Six hours after the last episode of vomiting, enteral feeds will be restarted at 25 cc / hr less than the previous rate (minimum of 10 cc / hr) for the full-calorie feeding group, and 10 cc / hr for the trophic feeding group.

The use of pro-kinetic agents and/or advancing the distal location of the feeding tube to a post-pyloric position should be considered in patients who experience vomiting.

5.1.7.5 Diarrhea

Diarrhea is defined as more than 3 liquid bowel movements totaling more than an estimated 500 cc in a 24-hour period.

Since diarrhea is rarely caused solely by enteral feeds (Kandil, 1993), the treatment of diarrhea may include discontinuation of laxatives and/or pro-kinetics, initiation of anti-diarrheals, treatment for C. *dificile* infection, or addition of fiber to the diet. The treatment will be determined by the patient's primary medical team, but will not include decreasing the enteral feeding rate unless the primary medical team feels that the patient's health is at risk because of the severity or nature of the diarrhea.

5.1.7.6 Constipation

Constipation is defined as the absence of a bowel movement requiring a specific intervention (i.e. enema, laxative, disimpaction, etc.) at the discretion of the primary medical team.

The treatment will be determined by the patient's primary medical team, but will not include decreasing the enteral feeding rate unless the primary medical team feels that the patient's health is at risk because of the severity or nature of the constipation.

5.1.7.7 Use of Prokinetic Agents

The use of prokinetic agents, including erythromycin and metoclopramide is not protocolized. Use of these agents will vary between centers and investigators. It is reasonable to administer these agents in a patient who experiences elevated GRV, aspiration, or vomiting, but the final decision is left to the discretion of the primary medical team. The use of prokinetic agents will be prospectively collected on the case report form.

5.1.8 Completion of Enteral Feeding Procedures

Patients will be considered to have completed the study enteral procedures if any of the following conditions occur:

1. Death

2. Hospital discharge
3. Alive 28 days after randomization
4. Extubation or 48 hours of UAB, whichever occurs first (see Definition of Unassisted Breathing, Appendix G)

5.1.9 Premature Withdrawal from Treatment

The feeding protocol will be discontinued in any patient who develops an abdominal process requiring emergent surgical exploration or repair, or an allergy to the enteral feeding formula. Data will continue to be collected prospectively in these patients until day 28 after randomization or hospital discharge, whichever occurs first.

5.2 Composition of the N-3 Fatty Acid / GLA / Anti-Oxidant Solution (Study Emulsion)

The study emulsion will consist of a combination of omega-3 fatty acids (eicosapentaenoic and docosahexanoic acids), gamma-linolenic acid (in the form of borage oil), and antioxidants (vitamins C and E, selenium, beta-carotene, taurine, and L-carnitine). All ingredients in the study emulsion will be pharmaceutical grade.

The study emulsion will be commercially made and will contain all of the omega-3 fatty acids, GLA, and anti-oxidants in an emulsified form. The volume of the emulsion will be approximately 240 cc each day, given as two administrations of approximately 120 cc each about 12 hours apart. Each day's worth of study emulsion will contain 6 grams of EPA and 3.5 g of DHA, in two divided doses. This will come from 20 grams of refined fish oil which is 30% EPA and 20% DHA by concentration. The dose selected is based on published clinical trials documenting the safety, tolerance, and biochemical effect in humans and accounts for the possibility of moderately impaired absorption in the critically ill patient. Doses as low as 3 grams per day of EPA have been shown to alter fatty acid profiles in ambulatory humans and doses as high as 21 grams per day have been shown to be safe and well tolerated even in patients with gastrointestinal cancer. The chosen EPA/DHA doses approximate that used in the Phase II trials published by Gadek, Singer, and Pontes-Arruda (Gadek, 1999; Pontes-Arruda, 2006; Singer, 2006). We elected not to use the maximum tolerated dose to avoid the possibility of worsening diarrhea in a critically ill population. Even if only 50% of the dose is absorbed delivery will be sufficient and there is ample room to safely move the daily dose up to 3 fold. Interim analyses will allow the DSMB and Advisory groups to recommend lower doses if more diarrhea occurs in treated patients compared to other groups or higher doses if changes in n-3 fatty acid levels are suboptimal.

The study emulsion will also contain gamma-linolenic acid (GLA certified to be free of pyrrolizidine alkaloids (PA-free). Each day randomized patients will receive 5.5 grams of GLA in the study emulsion in two divided doses. This will be derived from 26 grams of borage oil containing 22% GLA by concentration. This dosage is similar to that patients received in the phase II trial published by Gadek, Singer, and Pontes-Arruda and has demonstrated safety (Gadek, 1999; Pontes-Arruda, 2006; Singer, 2006).

The anti-oxidants utilized will consist of vitamins C and E, selenium, and beta-carotene. A total of 294 mg of liquid soluble Vitamin E and 1000 mg of liquid vitamin C will be administered each day divided equally between two doses. Both of these dosages are within the FDA approved daily dosages for these vitamins. The study emulsion will also contain 85 mcg/day of Selenium, divided into two doses of 42.5mcg each. This dose is also within the FDA approved daily dosage for selenium. A total of 4.2 mg of beta-carotene will be administered each day, divided equally between two doses.

The study emulsion will also contain the amino acids taurine (420 mg/day) and L-carnitine (203 mg/day). These are also doses that approximate the average doses delivered in the three phase II studies (Gadek, 1999; Pontes-Arruda, 2006; Singer, 2006).

The study components will be emulsified with a casein protein and will be de-odorized of fish smell. Each dose of the study emulsion will consist of 120 cc of total volume. The two administrations daily will thus provide a total of 240 cc of volume and 414 kcal each day.

5.3 Administration of the Study Solution (or Placebo)

The study emulsion will be administered into the patient's gastric tube every 12 hours (plus or minus 2 hours) as a 120 cc (4 oz) bolus using a syringe. Dosing will continue for 21 days or until 48 hours of UAB or extubation, whichever occurs first.

If a study dosing time is missed, the emulsion should be administered as soon as possible after the missed dosing time. The dose should be skipped if more than 6 hours late. The next scheduled dose can be given at the originally scheduled time. All subsequent doses should continue on the original every 12-hour schedule.

In the event of regurgitation, aspiration, or vomiting no attempt will be made to replace expelled drug but the episode will be recorded on the case report form as an intolerance event. The gastric contents should not be removed for at least 2 hours after study emulsion administration.

Study emulsion or matching placebo will be delivered in 2 oz (60 cc) sterile, plastic bottles to the investigational pharmacy at each hospital. The investigational pharmacy will deliver two plastic bottles (2 x 60 cc) of emulsion every 12 hours to the patient's location for administration, beginning at the time of randomization. Two 60 cc bottles will be utilized for each administration (4 bottles or 240 cc each day). The bedside nurse should give the entire 120 cc of emulsion at one time through the enteral feeding tube. The feeding tube should be flushed with 60 cc of sterile water after the emulsion is given to ensure delivery of the entire volume of the study emulsion.

Study emulsion or placebo should be administered even when enteral feedings are being held. The exception to this is in cases when enteral feedings are being held because of vomiting. In these situations, the study emulsion/placebo should be held until 6 hours after the last episode of vomiting. All subsequent doses should continue on the original every 12-hour schedule.

Due to a theoretical risk of bleeding from omega-3 fatty acids, study emulsion/placebo should not be given in patients whose INR exceeds 5.0, platelets are less than 30,000 or in patients with intracranial hemorrhage. If any of these situations should arise, the study emulsion should be held until the INR is less than 5.0 or discontinued entirely in patients who develop intracranial hemorrhage.

5.4 Blinding of Study Emulsion or Placebo Treatment

An equal amount (120 cc per administration) of commercially available Two-Cal® enteral feedings will serve as the placebo. This placebo is essentially isocaloric. This study emulsion will be composed to be visually indistinguishable from the Two-Cal® placebo, removing the need for the study drug formulation to be delivered in an opaque syringe. The study emulsion will also be de-odorized to ensure the placebo and study emulsions have indistinguishable odors.

5.5 Glucose Control

As levels of hyperglycemia are likely to vary with different volumes of enteral feeds and can confound the results of this study, glucose control protocols will be utilized to maintain tight control of hyperglycemia in all study patients. Each participating institution will utilize their own standard management, including institution-specific insulin drip protocols, to maintain blood sugars within at least a target range of 80 –150 mg / dL. The use of protocols with tighter ranges of blood sugar control (i.e. 80 – 110 mg / dL) will be allowed at institutions where this is standard practice for the care of critically ill patients. After both the first 100 and 250 patients, the DSMB will evaluate the blood glucose levels for the trophic versus full-calorie feeding groups for the first 6 days of the study. If the blood glucose levels between the two groups are not adequately similar, the tight glucose control requirements will be adjusted in an attempt to make these values similar over the remaining course of the study.

5.6 Ventilator Procedures

Ventilator management, including weaning, will be a simplified version of the 6 ml / kg PBW lung protective ventilation protocol from ARDSNet Study 01 – ARMA (See Appendix G) (The Acute Respiratory Distress Syndrome Network, 2000). If not already being utilized, this low tidal volume protocol for mechanical ventilation must be initiated within one hour of randomization.

Since the time a patient achieves unassisted ventilation affects some secondary endpoints, and because recent evidence-based consensus recommendations have identified a best practice for weaning, a weaning strategy will also be controlled by protocol rules in accordance with these evidence-based recommendations. This will assure similar weaning methods and provide potential benefit to both study groups. This newer weaning strategy is a simplified version of the protocolized weaning strategy used in prior ARDS Network studies (see Appendix G, section G.2.).

5.7 On-Study Fluid Management

Fluid management during shock will be unrestricted. However, in patients not in shock, a conservative fluid approach will be required for all patients enrolled in the study. This conservative fluid management approach will represent a simplification of the algorithm utilized in the ARDS Network FACTT study (see Appendix H). If not already being utilized, this conservative fluid management approach must be initiated within four hours of randomization, and continued until UAB or study day seven, whichever occurs first.

5.8 Procedures After Re-Intubation

In the event patients are extubated but re-intubated within the 21-day treatment period, study emulsion procedures will be resumed and continued through day 21. Treatment is discontinued at 21 days because most patients have either succumbed to ALI/ARDS or have been liberated from the ventilator by that point in their illness. Ventilator and feeding procedures should be resumed and continued through day 28.

6 Data Collection

6.1 Background Assessments

1. Demographic and Admission Data
2. Pertinent Medical History and Physical Examination
3. Height; measured Body Weight (mBW); calculated predicted body weight (PBW); body mass index (BMI)
4. Time on ventilator prior to enrollment
5. Type and location of ICU Admission
    a. Medical
    b. Surgical scheduled
    c. Surgical unscheduled
    d. Trauma
6. Risk factors for ALI/ARDS (sepsis, aspiration, trauma, pneumonia, drug overdose, other)
7. Presence of following chronic diseases:
    a. Metastatic cancer (proven by surgery, computed tomographic scan, biopsy or other documented method)
    b. Hematologic malignancy (ex: lymphoma, acute leukemia, or multiple myeloma)
    c. AIDS with complications (ex: PCP pneumonia, Kaposi's sarcoma, lymphoma, tuberculosis, or toxoplasmosis).
8. Weight loss in the last 6 months

6.2 Baseline Assessments

The following information will be recorded during the 24-hour interval encompassing the 12 hours prior to randomization and the 12 hours after randomization. If more than one value is available for this 24-hour period, the value closest to the time of enrollment will be recorded. If no values are available from the 12 hours prior to randomization, then values will be measured during the 12 hours post randomization and prior to initiation of enteral feeds.

1. Vital Signs: heart rate (beats / min), systemic systolic and diastolic BP (mmHg), body temperature (^{0}C)

2. Ventilator mode, rate, minute ventilation, tidal volume, FiO_2, PEEP, plateau, peak, and mean airway pressures
3. Arterial pO_2, pCO_2, pH and SpO_2
4. Serum electrolytes, magnesium, phosphorous, BUN, creatinine, bilirubin, and glucose
5. Blood hemoglobin, hematocrit, WBC, and platelets, Prothrombin time (PT) International normalized ration (INR)
6. Serum total protein, albumin
7. Glasgow Coma Score
8. Frontal Chest Radiograph – radiographic lung injury score (# of quadrants)
9. Administration of the following medications (Y / N):
   (a) Sedatives
   (b) Vasopressors
   (c) Pro-kinetic agents (cisapride, metoclopramide, lactulose, sorbitol, or erythromycin)
10. Location of feeding tube (orogastric, nasogastric, oro-enteral, naso-enteral, PEG or jejunostomy tube)
11. Presumed site of infection, if sepsis is the etiology of ALI / ARDS
12. APACHE III score
13. Blood for DNA banking (appendix K)
14. Blood for cytokines, mediators, and markers of inflammation. Plasma obtained from two, 10 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 4 equal 2 ml aliquots in specified tubes and frozen at -70^0C.
15. Blood for EPA, DHA and AA levels and ratio of n-3 to n-6 fatty acids. Plasma obtained from one, 5 ml blood sample collected in an EDTA vacutainer will be placed immediately after centrifugation into one 2 ml aliquot in specified tube and frozen at -70^0C
16. Urine for leukotriene B series and F_2- isoprostane metabolites. Urine obtained from the patients will be collected in an 8 ml sample tube and divided into 4 equal aliquots in specified tubes and frozen at -70^0C.

6.3 Assessments During Study

The following data will provide the basis for assessing protocol compliance and safety as well as between-group differences in several efficacy variables. Data for each of the variables will be recorded on the days shown in the Time-Events schedule (Appendix E) or until death, discharge from the intensive care unit, or unassisted ventilation for 48 hours.

Reference Measurements

The following parameters will be measured and recorded between 4:00 and 10:00 A.M. using the values closest to 8:00 A.M. on the days specified in the Time-Events schedule (Appendix E). The following conditions will be ensured prior to measurements: no endobronchial suctioning for 10 minutes; no invasive procedures or ventilator changes for 30 minutes. All vascular pressures will be zero-referenced to the mid-axillary line.

1. If receiving positive pressure ventilation:
   (a) Ventilator mode
   (b) PEEP level
   (c) Total minute ventilation
   (d) Tidal Volume
   (e) Plateau airway pressures
2. FiO_2
3. PaO_2, $PaCO_2$, pH, and SpO_2
4. Hemodynamic values
   (a) Systemic arterial systolic, diastolic and mean blood pressures
   (b) Heart Rate (beats/min)

Values for the following variables will be recorded for the dates shown in the Time-Events Schedule (Appendix E). If the measurements are not obtained during the 6-hour reference interval (4:00 to 10:00 A.M.), then the value obtained closest in time to the reference interval on the respective date will be recorded. If more than one value is obtained during the reference interval, then the value obtained closest to 8:00 A.M. will be recorded.

5. Blood hemoglobin concentration, white blood cell count, prothrombin time (PT), International normalized ratio (INR) and platelet count
6. Serum electrolytes, creatinine, and glucose
7. Units of insulin at time of daily glucose value (infusion) or total insulin in the 6 hours prior to the glucose value (subcutaneous)
8. Serum total protein, albumin, magnesium, phosphorus
9. Requirements for the following medications (Y / N):
   (a) Sedatives and narcotics
   (b) Neuromuscular blocking agents
   (c) Vasopressors
   (d) Pro-kinetic agents (e.g. metoclopramide, erythromycin, lactulose, or sorbitol)
   (e) Laxatives and fiber products
   (f) Anti-diarrheal agents
   (g) Methylprednisolone equivalents greater than 20 mg
10. Frontal Chest Radiograph – Lung Injury Score
11. Brussels Score data (Bernard, 1997)
   (a) Worst PaO_2 / FiO_2 ratio for the date.
   (b) Worst systolic blood pressure for that date
   (c) Worst creatinine, bilirubin, and platelet count for the date
   (d) Use of vasopressors
   (e) Glasgow Coma Score

12. Blood for cytokines, mediators, and markers of inflammation. Plasma obtained from two, 10 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 4 equal 2 ml aliquots in specified tubes and frozen at -70^0C. Blood will be collected on days 0, 3, 6, and 12.
13. Blood for EPA, DHA and arachidonic acid levels and ratio of n-3 to n-6 fatty acids. Plasma obtained from one, 5 ml EDTA anti-coagulated blood sample will be placed immediately after centrifugation into one 2 ml aliquot in specified tubes and frozen at -70^0C. Specimens will be collected on days 0, 3,6 and 12. This will be done in the first 60patients to ensure absorption of the n-3 fatty acids.
14. Urine for leukotriene and isoprostane metabolites. Urine obtained from the patients will be collected in an 8 ml sample tubes and divided into 4 aliquots of 2 ml each in specified tubes and frozen at -70^0C. Urine will be collected on days 0, 3 and 6
15. Enteral feeding volume and number of calories received for the previous 24 hours.
16. Number and type of gastrointestinal intolerances for the previous 24 hours.
17. Episodes of VAP
18. Episodes of bacteremia
19. Episodes of *Clostridium dificile*-induced diarrhea
20. Cardiac arrhythmias occurring from the time of enrollment to ICU discharge (or study day 21, whichever comes first).

Samples will be sent to a central repository to be stored (as described below). Samples will be identified by random number during shipment and storage in the central repository. In the future, when approved studies are received at the CCC the CCC will instruct the repository to prepare the appropriate samples for shipment. The key relating the ARDSNet study number to the new specimen number will be kept at the CCC in a locked file. The CCC does not record or store unique patient identifiers (such as initials, date of birth, hospital record numbers, addresses, phone numbers, etc.) in the database. All data released by the CCC for studies will be linked to the specimen but will be de-identified. The link (key) between the de-identified database and the patient will be removed two years after the primary publication.

Note: Urine and plasma collected for this trial will be frozen and stored at a Bio repository for future research.

6.4. Assessments after Hospitalization

As explained in the Background and Significance section of this proposal, it is very important to obtain long term outcomes data on the patients enrolled in this ALTA trial and the Nutrition trial as either or both interventions may have a significant effect on long term morbidity, and it would be very unfortunate to carry out a large trial of this kind and have no mechanism to determine longer term outcomes.

The following data, as well as vital status, will be collected at 6 and 12 months after ICU discharge. We will collect this data through telephone interviews with patients. In addition, we will verify duration of survival for patients lost to follow-up or noted to have died using the Centers for Disease Control and Prevention's National Death Index (National Death Index, 2000). We will use each patient's social security number (SSN) for an exact NDI match. We will collect contact information for the patient and alternative contact information on up to 3 individuals. This information and the SSN will be collected on paper at the time of consent, and forward via secure fax to the CCC. Contact information and SSN will be maintained on paper and will not appear in the CCC database.

The following instruments will be used in data collection. This battery of instruments will be pilot tested to guarantee feasibility. The text explains the alternative tests available pending the results of the pilot testing.

1. Health-related Quality of Life:
    a. SF-36 (consider the SF-12 if the length is too long in pilot testing). *Estimated administration time: 6 minutes.*
    b. Euro-QOL (EQ-5d)*: Estimated administration time 2 minutes.*
    c. Functional Assessment of Chronic Illness Therapy (FACIT; 13 questions) (if length is too long in pilot testing, this instrument will be deleted due to over-lap with SF-36); *Estimate administration time: 3 minutes*

2. Psychological Outcomes:
    a. Depression and Anxiety: Hospital Anxiety and Depression Scale (14 questions) *Estimated administration time: 5 minutes*
    b. Post-Traumatic Stress Disorder (PTSD): Impact of Events Scale—Revised (22 questions); *Estimated administration time: 3 minutes.*

3. Neurocognitive Outcomes:

Telephone version of the Mini-Mental State Examination (TMMSE) (16 items); *Estimated administration time: 5 minutes*

4. Physical Activity Outcomes:
    a. Overall: Functional Performance Inventory-Short Form (32 questions) (alternative: deleting this instrument (due to overlap with the Physical Function Domain of SF-36) or use the Katz ADL (6 questions) & the Lawton IADL, (8 questions), if length is too long in pilot testing) *Estimated administration time: 5 minutes*
    b. Work disability: Return to Work Custom-made Questionnaire (12 questions—will reduce number of questions if length is too long in pilot testing); *Estimated administration time: 2 minutes.*

5. Health care utilization: Custom-made instrument developed based on University of Toronto ARDS Outcome Study instrument provided by Margaret Herridge (27 questions), will reduce number of questions if this instrument is too lengthy in pilot testing; *Estimate administration time: 8 minutes*

6.5 Other Data Collected

Pre-morbid condition

a. APACHE III Demographics plus history of: hypertension, prior myocardial infarction, congestive heart failure, peripheral vascular disease, prior stroke with sequelae, dementia, chronic pulmonary disease, arthritis, peptic ulcer disease
b. Survey of smoking history including:
    - Ever smoker (>100 cigarettes in lifetime)?
    - If Yes, current smoker?
    - If ever smoker, estimate pack years:
    - Pack years = (# packs per day) x (number of years smoked)
    - If former smoker, when quit?
c. Survey of alcohol history (see Appendix O)

6.6 Endpoint Determinations

1. Vital status at 28, 60, and 90 days until discharged home on UAB.
2. Time of initiation of unassisted breathing (assuming patient achieves 48 consecutive hours of unassisted breathing)
3. Need for re-instituting assisted or mechanical ventilation after achieving 48 consecutive hours of unassisted breathing
4. Status 48 hours after initiation of unassisted breathing
5. Date of ICU discharge
6. Date of Hospital discharge

7 Statistical Considerations

Primary Endpoint

The primary endpoint will be ventilator free days. All analyses will be intent to treat. A three-way analysis of variance will be used with factors shown in Table 1. The primary model will be a main effects model. A secondary analysis will test for the significance of two-way interactions between Omega and time of feeding. The primary comparisons will be the main effects for feeding and nutriceuticals. That is, we separately test whether Omega-3 Fatty Acid, Gamma-linolenic acid (GLA), and anti-oxidant supplementation leads to an improvement in ventilator free days as compared to placebo and whether initial trophic feeding followed by full-calorie enteral feeding is different than initial advancement to full-calorie enteral feeding.

Table 1: Factorial Design

Factor	Level
Nutriceutical (Medical Food)	1. Omega-3 Fatty Acid, Gamma-linolenic acid (GLA), and anti-oxidant supplementation
	2. Placebo
Time of Feeding	1. Initial trophic feeding followed by full-calorie enteral feeding
	2. Initial advancement to full-calorie enteral feeding
Shock at baseline	1. Yes

	2. No

The maximum sample size will be 1000 patients. The study will be monitored using a flexible group sequential design that stops for both efficacy and futility. Since patients will be co-enrolled on two studies, the DSMB will meet at the same time for both studies
Each of the primary comparisons will be monitored independently. For instance, if the medical food comparison crosses a boundary, then the accrual to the study will continue until the time of feeding comparison crosses a boundary. The reported confidence intervals on the treatment difference will be adjusted for the group sequential design using the method of Jennison & Turnbull.

In order to allow flexibility we will use alpha and beta spending boundaries as described by DeMets and Ware (zl=2.277,delta=1.663,zu=2.025,m=4, mu=3.3837) (DeMets, 1982). Each of the treatment factors in the study will be monitored separately and could be stopped before the other stratification has stopped. Factors will be considered either as one or two sided. The OMEGA factor is one sided while the trophic verses full feeding is two sided. One-sided factors will have an upper efficacy boundary and a lower futility boundary. Two sided factors will have a two-sided efficacy boundary and an inner wedge futility boundary that will be formed by reflecting the lower futility boundary about the abscissa. There would be no chance of futility stopping of two sided factors at the first look.

In this method of interim monitoring we specify a function a(t) and b(t) called the alpha and beta spending functions. The function a(t) gives the amount of the p-value that will be "spent" by a given time "t" in the study, where time runs from 0 at study start to 1 when all patients have been entered. It is the probability under the null hypothesis that the trial will stop for efficacy at or before time t. The function b(t) is the type II error that will be "spent" by the interim monitoring plan to allow futility stopping. It is the probability under the alternative hypothesis that the study will stop for futility at or before time t or that, at the last look, the efficacy boundary will not be exceeded. The reason that we use alpha and beta "spending" functions rather than p-values to stop the trial is that with two co-enrolled trials we may not be monitoring the data of both trials at 250 patient intervals.

Table 2 shows the alpha-spending boundary a(t) where t is the proportion of patients accrued at that DSMB meeting. In the table we have assumed 5 meetings at t= .10, .25, .50, .75 and 1.0 This function a(t) will be extended to a smooth function of t using a cubic spline as suggested by (Pampallona, 1994) and at each DSMB meeting the actual stopping boundary will be calculated so that the probability of stopping at or before that meeting is a(t). Similarly the futility boundary is defined by the beta-spending function b(t). The number b(t) is the cumulative probability that the results would be below the futility stopping boundary given the alternative hypothesis of a 2.25 day increase in VFD with a standard deviation of 10.5. At each DSMB meeting a futility stopping boundary will be calculated so that the probability of futility stopping at or before that meeting is b(t) at this alternative hypothesis.

The overall one-sided significance level of the study will be 0.025 which is equivalent to a two sided p=0.05 significance level. Five analyses are planned after 100, 250, 500, 750, and 1000 patients. Under the assumption that there are five equally spaced interim analyses the power of the study will be 90.7%. Changes in the number or spacing of the interim analyses will have a minor effect on the power. With this design, assuming that the pattern of deaths and extubations is similar to the FACTT fluid study, there is a 82% chance that the study will show both a significant effect of VFD and a nominally positive benefit in mortality.

The DSMB will be advised to consider mortality differences in deciding whether to stop the trial. For example, they might decline to stop the trial for efficacy if the mortality difference would make the positive benefit in ventilator free days difficult to interpret and they might decline to stop the trial for futility if there is a positive mortality benefit. For example, if there was no difference in vent free days but a trend towards a survival benefit the DSMB might continue past a futility boundary. The stopping rules have been set up so that this would not invalidate the trial if such judgments were made. The efficacy boundary has been developed without regard to the futility boundary. Thus if the futility boundary is crossed but the trial is not stopped the trial can still achieve a 0.025 one-sided significance level.

Table 2 shows the characteristics of this boundary if we had the interim reports described above. The second column is the nominal p-value to stop for efficacy; the third and fourth columns are the difference in VFD to stop for efficacy and futility. The next columns are the error spending functions. The type I error spending function is the probability that the upper boundary will be exceeded under the null hypothesis. The type II error spending function is the probability that the statistic will be below the lower boundary at an interim analysis or under the upper boundary at the final analysis under the alternative hypothesis. The probability of stopping for futility is given in the seventh column and the probability of stopping for efficacy in the eighth column. The final column shows the confidence interval for the difference in VFD if the trial stopped for efficacy at that look and the treatment effect just exceeded the stopping boundary.

Table 2: Stopping Boundaries

Number of patients	P-value Efficacy 2-sided	Difference Efficacy	Difference Futility	Type I Error Spending 1-sided	Type II Error Spending	Prob stop futility	Prob Stop efficacy	Confidence interval when no difference
100	1.5 E-6	9.5		7.6E-11	0	0	5E-8	9.3-17.6
250	5 E-5	3.8	-0.50	2.56 E-5	0.0128	0.30	0.009	2.8-8.0
500	0.0042	1.9	0.14	0.0021	0.0232	0.31	0.31	.8-4.5
750	0.0194	1.3	0.35	0.0104	0.0287	0.17	0.41	.3-3.2
1000	0.0429	0.95	0.46	0.0250	0.0923	0.09	0.18	0.0-2.6

Secondary Endpoints

Mortality

Mortality will be compared at interim data analyses using Kaplan Meier estimates at 60 days and their associated standard errors. This analysis will be stratified as above and a test for interaction of treatment with strata will be presented. At the end of the study sixty-day mortality will be compared using a Mantel-Haenzel test as long as all patients can be followed. If not the method used for the interim analyses will be used.

Other Endpoints

The number of ICU-free days, Organ-Failure Free days, and days from first weaning readiness will be analyzed in the same manner as is described above for the primary endpoint. Subset analysis defined in the secondary analyses section will use an analysis of variance. First we will check for interactions and then, if significant, analysis will be performed for each of the specified subsets. In addition we will test for interactions between treatment and gender and race as per NIH guidelines (National Institutes of Health, 2001).

Changes in plasma levels of IL-6, IL-8, and protein will be compared in two analyses. An analysis of covariance will test for a treatment effect on the day 3 value of these variables using the day 0 value as a covariate. In addition, a multivariate analysis of variance will test for a baseline difference between day 3 and day 0.

Table 3 illustrates the detectable differences for endpoints, assuming 1000 patients enrolled, 90% power, and a two-sided alpha-level of 0.05.

Table 3: Detectable Differences for Secondary Endpoints

Variable	Incidence or Mean	Standard Deviation	Detectable Difference
PaO_2 / FiO_2	155	73	15
ICU free days	13.4 days	12.6 days	2.6 days
Shock free days	19.1 days	4.93 days	2.23 days
Plasma IL-6 (pg/ml)	1252	862	177
Plasma IL-8 (pg/ml)	149	93	19
28 day hospital mortality	22%		8.2%
90 day hospital mortality	25.4%		8.6%

Changes in physiologic lung indices on days 1-7 will be compared using a multivariate analysis of variance.

Phase 1 Pharmacokinetics

Plasma will be collected for measuring DHA, EPA and AA levels at days 0, 3, 6 and 12 from the first 30 patients receiving the placebo and the first 30 patients receiving the omega-3 fatty acid study emulsion. When the DSMB meets after approximately 100 patients have been enrolled, the DSMB will look at plasma EPA, DHA, and arachidonic acid and the ratio of omega-3 to omega-6 fatty acids in the blood on day 3 in these 60 patients to ensure that the omega-3 fatty acids are being absorbed. An estimate of the detectable difference is given in table 4 below, assuming 80% power and two-sided alpha level of 0.05. These estimates are conservative because they don't take into account the reduction of variance due to the use of the day zero value as a covariate.

Table 4: Detectable Difference for Plasma Omega-3 Fatty Acids

Variable	Incidence or Mean	Standard Deviation	Detectable Difference
Plasma EPA (% total	0.645	0.2	0.15

lipid)			
Plasma DHA (% total lipid)	2.54	0.13	0.10
Plasma arachidonic acid (% total lipid)	9.8	0.38	0.28
EPA+DHA / AA ratio	0.32	0.1	0.07

Twice during the early part of the study, the DSMB will evaluate the glucose control between the trophic and full-calorie arms to ensure that the levels of blood glucose are not clinically different between the groups over the first 6 days of the study. These evaluations will occur after approximately 100 and 250 patients are enrolled in the study. Should the blood glucose values differ between the groups at these evaluations, the guidelines for controlling blood glucose levels for the remainder of the study will be adjusted in an attempt to equalize the blood glucose levels for the study.

8 Data Collection and Site Monitoring

8.1 Data Collection

The research coordinators will collect data and record it either on paper data sheets or in a custom-designed computer database. Data will be transferred to the Clinical Coordinating Center on a prescribed basis through a web-based data collection program.

8.2 Site Monitoring

Site visits will be performed on a regular basis by the Data Coordinating Center, to ensure that all regulatory requirements are being met and to monitor the quality of the data collected. Records of Institutional Review Board approvals and patients' charts will be examined on a spot check basis to evaluate the accuracy of the data entered into the database.

9 Risk Assessment

This study involves randomization of two separate (but potentially interacting) interventions: 1) Initial trophic enteral feeds followed by advancement to full-calorie enteral feeds vs. initial full-calorie enteral feeds, and 2) omega-3 fatty acid supplementation vs. placebo. Each of the two randomizations carries with it potential risks (and potential offsetting benefits), and the possible interactions between the two trials may also have risk or benefit.

9.1 Risks of Enteral Feedings

Potential risks of enteral feedings exist in both feeding groups. Common risks of enteral feeding are abdominal distention, cramping, nausea, and diarrhea. Uncommon risks of enteral feeding include vomiting, aspiration, and intestinal ischemia.

9.2 Risks of Full-calorie Enteral Feedings

Potential common risks to patients in the early full-calorie feeding group are more episodes of gastrointestinal intolerance of the tube feedings. GI intolerance includes abdominal distention, abdominal cramping, nausea, vomiting and diarrhea. GI intolerance could lead to more vomiting and aspiration. The early full-calorie feeding group may also experience more diarrhea. Additionally, patients receiving early full-calorie enteral feeds may be at an increased risk for intestinal ischemia or infarction. Because they will receive more calories, patients in this group may be at risk of having higher blood glucoses. The treatment of the higher blood glucoses will be standardized to help control for this confounder, which will likely precipitate the full-calorie feeding group receiving more insulin. The clinical significance of additional insulin is uncertain. Early full-calorie feedings could theoretically reduce the risk of infection by improving nutritional status, but could also be associated with increased infection risk from hyperglycemia or aspiration pneumonia.

9.3 Risks of Trophic Enteral Feedings

Patients in the initial trophic feeding group will receive less calories and protein for the first six days. The clinical importance of this is uncertain, but could lead to more protein catabolism and weight loss. In addition, trophic feedings could reduce immune function and impair control of infections. The trophic feeding group may have a decreased incidence of abdominal distention, abdominal cramping, vomiting, aspiration, and diarrhea.

9.4 Risks of Omega-3 Fatty Acids

The second trial consists of randomization to either omega-3 fatty acid supplementation or placebo, in the form of isocaloric, commercially available, Two-Cal® enteral nutrition. The risks of additional enteral nutrition to that given in the first trial are probably minimal, but may include some of the risks listed for the full-calorie feeding group. Omega-3 fatty acids are available over the counter as dietary supplements. As such, they have demonstrated an excellent safety profile. As the amount of EPA and DHA in this study is above the Food and Drug Administration recommended daily supplement, unknown or unforeseen side effects may occur. Common side effects of omega-3 fatty acids include fishy aftertaste (Kris-Etherton, 2002) and mild gastrointestinal disturbances such as bloating and belching. Omega-3 fatty acids also may increase plasma low-density lipoprotein cholesterol levels, but the clinical significance of this is unknown (Harris, 1997). Uncommonly, omega-3 fatty acids result in diarrhea, nausea, and worsening hyperglycemia in diabetics (Kris-Etherton, 2002). Rarely, patients taking omega-3 fatty acid supplements have experienced increased bleeding times resulting in easy bruisability or nosebleeds (Sanders, 1992) and/or allergic reactions.

9.5 Risks of GLA

Gamma-linolenic acid will also be administered as part of the study emulsion. The dose of GLA in this protocol is higher than that used for daily supplementation, but similar to the amount patients received in a previously published phase II study (Gadek, 1999). No serious adverse events were reported in that study or other clinical studies of people taking GLA. Most of the side effects of GLA and borage oil are gastrointestinal in nature, including nausea, diarrhea, intestinal gas, burping and stomach bloating (Leventhal, 1993). Borage oil may increase the risk of bleeding and bruising in selected patient populations (Chaintreuil, 1984). Some borage seed oil preparations contain pyrrolizidine alkaloids (found in the leaves and roots of borage plants) which can harm the liver or worsen liver disease. To minimize this possibility, the borage oil utilized in this study will be derived from borage seeds and certified to be pyrrolizidine alkaloid free (PA-free). As GLA may decrease the metabolism of cyclosporine causing levels to increase, patients taking cyclosporine should have close monitoring of their levels while taking GLA (Morphake, 1994).

9.6 Risks of Anti-Oxidants

Vitamins C and E in this trial are administered at dosages routinely used in daily supplementation. Both are generally tolerated with few side effects. Vitamin E may uncommonly cause gastrointestinal discomfort, including stomach pain, nausea and diarrhea. Headaches and fatigue are also uncommon side effects of vitamin E. Rarely, patients will develop a rash from vitamin E. Similarly, vitamin C also may cause nausea, vomiting, or diarrhea. Mouth sores, headaches, skin flushing, and increased urination have also been reported with vitamin C supplementation. Rarely, patients taking vitamin C for long periods of time have reported kidney stones, side, flank or back pain, or painful urination. Both vitamins C and E are excreted in breast milk. The adverse effects of beta-carotene commonly include skin discoloration (yellowing of the palms, soles, and/or face) and diarrhea. Rarely, beta-carotene may cause bruising, dizziness, or joint pain. Smokers who took 20-30 mg of beta-carotene for years were found to have an increased risk of both lung and prostate cancer. It is not known if this occurs with lower doses and/or short term use. L-carnitine, especially at the low doses utilized in this study, is well-tolerated with only a few uncommon side effects, including high blood pressure, tachycardia, fever, rash, body odor, and in high dosages, diarrhea. Similarly, taurine also has few reported side effects. Rare side effects of taurine include low blood pressure, rash, and diarrhea, but these were all seen in studies using ten times the dosage proposed for this study.

9.7 Risks of Blood Draws

All patients will have blood drawn for research purposes. The risks of drawing blood are uncommon and include bleeding and bruising. Commonly, drawing blood is painful, and rarely, drawing blood can lead to infections at the site of the blood draw.

9.8 Risk of Death

It is possible that one treatment arm may lead to more deaths and mortality is a secondary outcome and will be monitored during the course of the study.

9.9 Minimization of Risks

Federal regulations at 45 CFR 46.111(a)(1) requires that risks to subjects are minimized by using procedures which are consistent with sound research design. There are several elements of study design inherent in the present protocol that meet this human subject protection requirement. First, several of the exclusion criteria prohibit participation of patients who might be at increased risk of enteral nutrition (e.g. bowel obstruction, bowel ischemia, bowel infarction, severe malnutrition). Safeguards with regard to intolerance of tube feeds have been incorporated into the protocol, most notably frequent evaluation of residuals and gastrointestinal intolerances (nausea, vomiting, diarrhea, abdominal distention, constipation). Actions to be taken for either elevated residual volumes or gastrointestinal intolerances have been systematically included in the protocol. These actions include adjustments to or temporary discontinuation of the enteral feedings in cases of elevated residual volumes, nausea, diarrhea, and abdominal distention. For diarrhea and constipation, the choice of action is left to the discretion of the treating physician. The DSMB will be reviewing data as outlined above and will examine not only efficacy but safety (inclusive or mortality) and reserves the right to halt the study at any time.

9.10 Potential Benefits

Study subjects may or may not receive any direct benefits from their participation in this study. Potential benefits from immunonutrition include reductions in inflammation, more rapid resolution of ALI/ARDS with increased survival and ventilator free days. Full calorie feeds may be result in improved nutritional status and facilitate disease resolution. Trophic feeds may lead to reduction in the volume of recurrent aspiration and avoid prolongation of ALI/ARDS.

9.11 Risks in Relation to Anticipated Benefits

Federal regulations at 45 CFR 46. 111 (a)(2) require that "the risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result." Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits.
Procedures – blood draws. The risks associated with these common clinical practices are small, however the knowledge gained in furthering our understanding of the pathophysiology and potentially leading to better and targeted therapy may be substantial.
Treatments – The nutrition regimens chosen are consistent with clinical practice. There is potential for benefit to society and individual patients should one treatment arm prove to be of benefit. Should one treatment arm, again consistent with clinical practices, prove to be harmful, the benefit will be in avoiding such therapies for future patients with ALI/ARDS.

In summary, investigators have reviewed enteral nutrition literature through May 2006 in regard to clinical practices, expert opinions and consensus recommendations and conclude the following:

1. Equipoise is present with regard to the nutritional issues to be addressed in this trial (inclusive of but not limited to caloric intake, time to implement full feeding, and immunonutrition).
2. Treatment arms in the Omega and EDEN protocol are within the spectrum of clinical practice and the potential risks and benefits have been weighed and equipoise between the nutrition strategies remains.
3. Evidence does not support supplementing enteric nutrition with parenteral nutrition, and may actually suggest harm in so doing.

10 Human Subjects

Each study participant or a legally authorized representative must sign and date an informed consent form. Institutional review board approval will be required before any subject is entered into the study.

10.1 Selection of Subjects

10.1.1 Equitable Selection of Subjects

Federal regulations at 45 CFR 46(a)(3) require the equitable selection of subjects. The ICUs will be screened to determine if any patient meets the inclusion and exclusion criteria. Data that have been collected as part of the routine management of the subject will be reviewed to determine eligibility. No protocol-specific tests nor procedures will be performed as part of the screening process. If any subjects meet criteria for study enrollment, then the attending physician will be asked for permission to approach the patient or his/her surrogate for informed consent. Justifications of exclusion criteria are given in Section 4.3. These exclusion criteria neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals from participation in the research. Hence, the recruitment of subjects conforms to the principle of distributive justice.

10.1.2 Justification of Including Vulnerable Subjects

The present research aims to investigate the safety and efficacy of a type of treatment for patients with acute lung injury and acute respiratory distress syndrome. Due to the nature of these illnesses, the vast majority of these patients will have impaired decision-making capabilities. This study cannot be conducted if limited to enroll only those subjects with retained decision-making capacity. Hence, subjects recruited for this trial are not being unfairly burdened with involvement in this research simply because they are easily available.

10.2 Informed Consent

Federal regulations 45 CFR 46.111(a)(5) require that informed consent will be sought from each prospective subject or the subject's legally authorized representative. The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, and answering any questions the patient may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's willingness to continue his or her participation in the trial. The consenter will make every effort to minimize coercion. All study participants or their surrogates will be informed of the objectives of the study and the potential risks. The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study agent.

10.3 Continuing Consent

For subjects for whom consent was initially obtained from a surrogate, but who subsequently regains decision-making capacity while in hospital, all sites will obtain formal consent for continuing participation, inclusive of continuance of data acquisition. The initial consent form signed by the surrogate should reflect that such consent will be obtained.

10.4 Identification of Surrogates

Many of the patients approached for participation in this research protocol will invariably have limitations of decision-making abilities due to their critical illness. Hence, most patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's legally authorized representative.

Regarding proxy consent, the existing federal research regulations ('the Common Rule') states at 45 CFR 46.116 that "no investigator may involve a human being as a subject in research…unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative"; and defines at 45 CFR 46 102 (c) a legally authorized representative (LAR) as "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedures(s) involved in the research." OHRP defined examples of "applicable law" as being state statutes, regulations, case law, or formal opinion of a State Attorney General that addresses the issue of surrogate consent to medical procedures. Such "applicable law" could then be considered as empowering the surrogate to provide consent for subject participation in the research. Interpretation of "applicable law" is therefore state specific and hence, will be left to the discretion of the individual IRBs of the respective clinical centers involved in the ARDSNet.

According to a previous President's Bioethics Committee (National Bioethics Advisory Committee), an investigator should accept as an LAR…a relative or friend of the potential subject who is recognized as an LAR for purposes of clinical decision making under the law of the state where the research takes place (National Bioethics Advisory Commitee (NBAC), 1998). Finally, OHRP has opined in their determination letters that a surrogate could serve as a LAR for research decision making if such an individual is authorized under applicable state law to provide consent for the "procedures" involved in the research study (Office of Human Research Protections (OHRP), 2002).

10.5 Justification of Surrogate Consent

According to the Belmont Report, respect for persons incorporates at least two ethical convictions; first, that individuals should be treated as autonomous agents, and second, that person with diminished autonomy are entitled to protection. One method that serves to protect subjects is restrictions on the participation of subjects in research that presents greater than minimal risks. Commentators and Research Ethics Commission have held the view that it is permissible to include incapable subjects in greater than minimal risk research as long as there is the potential for beneficial effects and that the research presents a balance of risks and expected direct benefits ***similar*** to that available in the clinical setting (Dresser, 1999). Several U.S. task forces have deemed it is permissible to include incapable subjects in research. For example, the American College of Physicians' document allows surrogates to consent to research involving incapable subjects only "if the net additional risks of participation are not substantially greater than the risks of standard treatment." (American College of Physicians, 1989). Finally, the National Bioethics Advisory Committee (NBAC) stated that an IRB may approve a protocol that presents greater than minimal risk but offers the prospect of direct medical benefits to the subject, provided that…the potential subject's LAR gives permission…" (National Bioethics Advisory Commitee (NBAC), 1998)

Consistent with the above ethical sensibilities regarding the participation of decisionally incapable subjects in research and the previous assessment of risks and benefits in the previous section, the present trial presents a balance of risks and potential direct benefits that is ***similar*** to that available in the clinical setting, with the exception of the additional blood draws.

10.6 Additional Safeguards for Vulnerable Subjects

The present research will involve subjects who might be vulnerable to coercion or undue influence. As required in 45CFR46.111(b), we recommend that additional safeguards be included to protect the rights and welfare of these subjects. Such safeguards might include, but are not limited to: a) assessment of the potential subject's capacity to provide informed consent, b) requirement for subject's assent, c) the availability of the LAR to monitor the subject's subsequent participation and withdrawal from the study; d) augmented consent processes. The specific nature of the additional safeguards will be left to the discretion of the individual IRBs.

Minors (13-18 years old)
This study will enroll minors between the ages of 13-18. As this is a vulnerable population the consent form will include a section for obtaining assent for the minor coupled with permission from a parent for both study enrollment and continuation. The assent for continuation will be invoked when surrogate consent is obtained initially (subject not able to self enroll due to illness). In accordance to the decision matrix provided in 45 CFR 46, Children as subjects of research (http://www.hhs.gov/ohrp/panels/407-01pnl/riskcat.htm) we have designed the IRB approved project assent form to require one parental permission signature, as the study is greater than minimal risk with the potential for direct benefit to the subject (46.405).

10.7 Confidentiality

Federal regulations at 45 CFR 46 111 (a) (7) requires that when appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data. To maintain confidentiality, all laboratory specimens, evaluation forms, and reports will be identified only by a coded number. The coded number will be generated at random by a computer, and only the study investigators will have access to the codes. All records will be kept in a locked, password protected computer. All computer entry and networking programs will be done with coded numbers only. All paper case report forms will be maintained in a locked cabinet inside a locked office. Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the National Heart, Lung, and Blood Institute, and the ARDS Clinical Coordinating Center.

11 Adverse Event Reporting

Investigators will determine daily if any clinical adverse experiences occur during the period from enrollment through study day 23 or ICU discharge, whichever occurs first. The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of treatment of patients with ALI or ARDS.

For this trial, a *reportable adverse event* is defined as:

1. Any clinically important untoward medical occurrence in a patient receiving study solution or undergoing study procedures which is different from what is expected in the clinical course of a patient with ALI, or:

2. Any clinically important, untoward medical occurrence that is thought to be associated with any component of the study solution (i.e. omega-3 fatty acids, gamma-linolenic acid, or anti-oxidants), or nutritional procedures, regardless of the "expectedness" of the event for the course of a patient with ALI.

3. The following protocol specified adverse events should always be reported as adverse events:
   a. Hypersensitivity to enteral feeds
   b. Hypersensitivity to omega-3 fatty acids
   c. Intestinal ischemia or infarction
   d. Severe bleeding (defined as any central nervous system bleeding or any bleeding event that leads to the administration of three or more units of packed red blood cells per day for two consecutive days).

The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the clinical course patients with ALI. *Expected events for ALI* are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of patients with ALI treated in an intensive care unit with mechanical ventilation. Examples of adverse events that are expected in the course of ALI include transient hypoxemia, agitation, delirium, nosocomial infections, intolerance of gastric feeding, skin breakdown, and gastrointestinal bleeding. Such events, which are often the focus of prevention efforts as part of usual ICU care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study drug or procedures, or unexpectedly severe or frequent for an individual patient with ALI. Examples of unexpectedly frequent adverse events would be repeated episodes of unexplained hypoxemia, in contrast to an isolated episode of transient hypoxemia (eg. Sp02 ~85%), particularly if related to positioning of suctioning. This latter event would not be considered unexpected by nature, severity or frequency.

11.1 Clinical Outcomes

Events leading to death and organ failure are being systematically captured in the case report forms and will be systematically analyzed per protocol as part of the safety and efficacy analysis.

The following clinical outcomes will not be considered to be adverse events *if the investigator determines the outcomes were not study solution or procedure-related:*

1. Death
2. Respiratory: worsening hypoxia, prolonged need for ventilation, hypoxemia, hypercarbia, respiratory acidosis, high airway pressures.
3. Circulatory: circulatory shock requiring vasopressors.
4. Hepatic: hepatic injury that leads to a rising bilirubin.
5. Renal: rising creatinine.
6. Coagulopathy: falling platelet count.

In addition, patients with acute lung injury who receive enteral nutrition often experience gastrointestinal intolerances (see Section 5.1.7). These gastrointestinal intolerances, including diarrhea, vomiting, constipation, nausea, and abdominal distention will be systematically collected and analyzed as part of the protocol. As such, they will not be considered to be adverse events. Similarly, patients with acute lung injury also often have elevated gastric residual volumes, which is also being systematically collected and analyzed as part of the protocol and will not be considered an adverse event.

An event will be considered to be study-related if the event follows a reasonable temporal sequence from the study drug/procedure and could readily have been produced by the study drug/procedure. An event will be considered to be *unexpected for study drug* if it is not identified in the study protocol.

11.2 Adverse Event Reporting Timeline

Investigators will report all serious, and unexpected, and study-related adverse events, as defined in Appendix F, to the Clinical Coordinating Center within 24 hours. The local Institutional Review Board must also be notified in a timely manner. The investigator will then submit a detailed written report to the Clinical Coordinating Center and the Institutional Review Board no later than 5 days after the investigator discovers the event.

The Clinical Coordinating Center will report all serious, unexpected, and study-related adverse events to the NHLBI within 24 hours. A written report will be sent to the DSMB within 15 calendar days and these reports will be sent to investigators for submission to their respective Institutional Review Boards. The DSMB will also review all adverse events during scheduled interim analyses. The Clinical Coordinating Center will distribute the written summary of the DSMB's periodic review of adverse events to investigators for submission to their respective Institutional Review Boards in accordance with NIH guidelines.

APPENDICES

A Identification of Ventilator-Associated Pneumonia

Suspected or Possible Pneumonia: patient must meet at least one criterion from two categories below (i, ii or iii).

Probable Pneumonia: patient must meet at least one criterion from all three categories below (i and ii and iii).

i. Chest radiograph shows new infiltrate corresponding in size (although not necessarily to segmental anatomical boundaries) to at least one segment or cavitation with an air-fluid level within an area of infiltrate (*i.e.*, not a simple subpleural air cyst). The qualifying radiographic abnormality must persist over at least 48 hours with no decrease in its size.

ii. New onset of or increase in fever ($T \geq 38.3^{\circ}C$ or increase $\geq 1^{\circ}C$ over the previous 24 hour T_{max} if T already $\geq 38.3^{\circ}C$) or new hypothermia ($T \leq 36.0^{\circ}C$) or increase in WBC (WBC > 10,000 and a 25% increase or an increase in band forms to > 10% of total WBC) or new decrease in WBC to < 4,000.

iii. Bacteriological confirmation of pulmonary infection (can be any of the following):

- quantitative culture of tracheal secretions with $> 10^6$ cfu/mm^3
- quantitative culture of bronchoalveolar lavage with $> 10^4$ cfu/mm^3
- quantitative culture of protected specimen brush with $> 10^3$ cfu/mm^3
- positive Gram stain with ≥ 3+ of at least one type of bacteria.
- positive semi-quantitative sputum culture with ≥ 3+ growth of at least one type of potentially pathogenic bacteria
- positive blood culture for bacterial pathogen also identified in sputum or other respiratory specimens
- positive Gram stain or culture of pleural fluid for bacterial pathogen

Only one episode will be considered to be present during the 28-day period for the following due to difficulty in defining successful therapy during this time period.

B Exclusion Definitions

1. Malignant and Irreversible Conditions

a. Poorly controlled neoplasms (proven by surgery, computed tomographic scan, biopsy or other documented method)
b. Known HIV positive with known end stage processes (e.g., progressive multifocal leukoencephalopathy, systemic mycobacterium avium infection) with known CD4 count < 50.
c. Prior cardiac arrest requiring CPR without fully demonstrated neurologic recovery
d. New York Heart Association Class IV subjects (defined as subjects who have cardiac disease resulting in inability to carry out physical activity without discomfort. Symptoms of cardiac insufficiency or of anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased).
e. Chronic respiratory condition making patient respirator dependent.

2. Refractory Shock

Refractory shock is defined as the requirement of any of the following to obtain a blood pressure adequate for perfusion of tissues

a. Dopamine infusion at rate ≥ 15 mcg / kg / min
b. Dobutamine infusion at rate ≥ 15 mcg / kg / min
c. Epinephrine or Norepinephrine infusion at rate ≥ 30 mcg / min
d. Phenylephrine infusion at rate ≥ 50 mcg / min
e. Milrinone infusion at rate ≥ 0.5 mcg / kg / min
f. Vasopressin infusion at rate > 0.04 U / min
g. Intra-aortic Balloon Pump

3. Child-Pugh Score (Pugh, 1973)

Points	Class
5-6	A
7-9	B
≥ 10	C

	Numerical Score for Increasing Abnormality		
Measurement	1	2	3
Ascites	None	Present	Tense
Encephalopathy	None	Grade I or II	Grade III or IV
Bilirubin (mg/dl)	< 2	2-3	> 3
Albumin (g/L)	> 35	28-35	< 28
Prothrombin time (sec. prolonged)	1-4	4-10	> 10

4. Neuromuscular Disease Impairing the Ability to Ventilate Spontaneously

a. Amyotrophic lateral sclerosis
b. Guillain-Barré Syndrome
c. Myasthenia gravis
d. Upper spinal cord injury at level C5 or above
e. Kyphoscoliosis or chest wall deformity resulting in severe exercise restriction (unable to climb stairs or perform household duties), secondary polycythemia, or respirator dependence

5. Severe Chronic Respiratory Disease

Any of the following is considered severe chronic respiratory disease and excludes a patient from being eligible for enrollment:

1. FEV_1 less than 20 ml/kg PBW (e.g. 1.4 L for a 70 kg person), or

2. FEV_1/VC less than 50% predicted, or

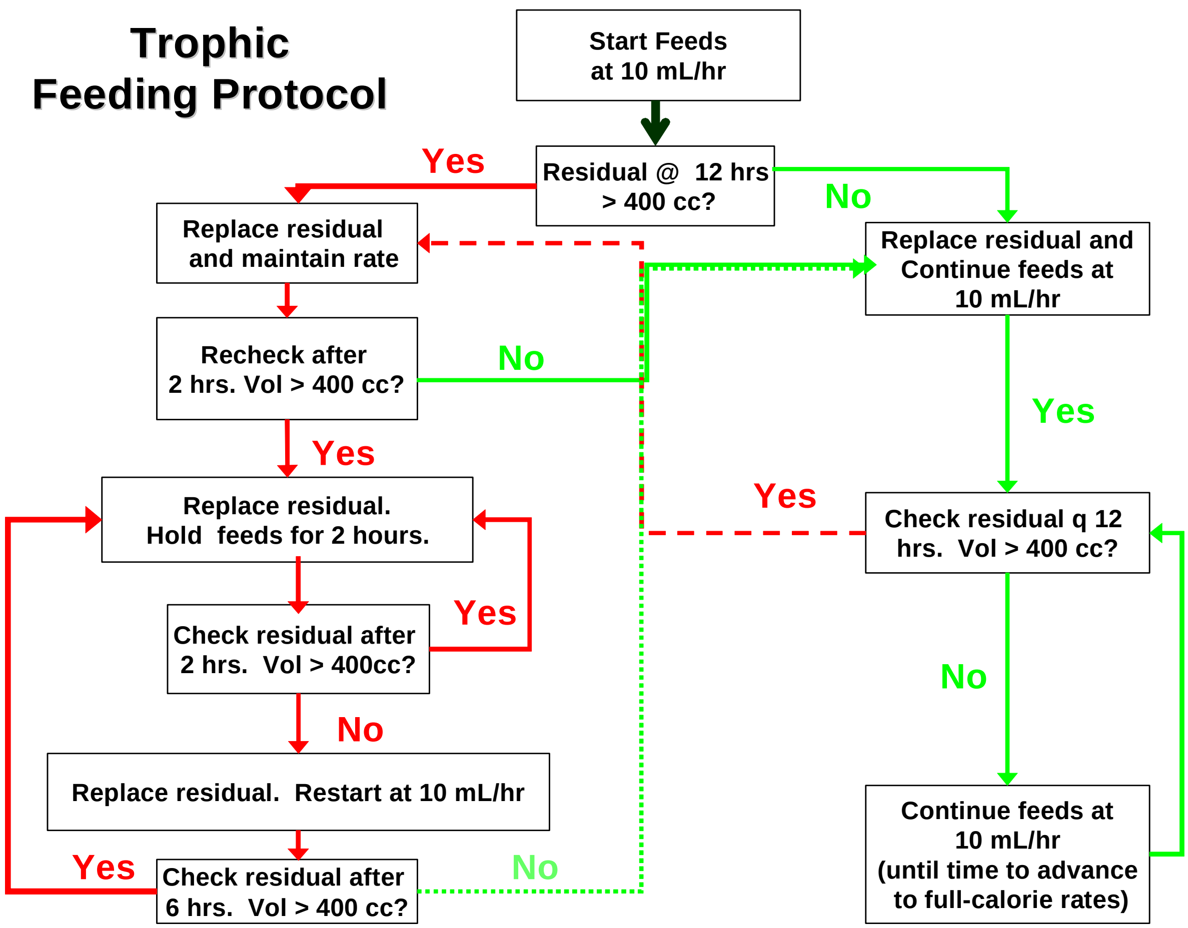
3. Chronic hypercapnia ($PaCO_2$ greater than 45 mmHg) and/or chronic hypoxemia (PaO_2 less than 55 mmHg) on $F_IO_2 = 0.21$, or

4. Radiographic x-ray evidence of any chronic over-inflation or chronic interstitial infiltration, or

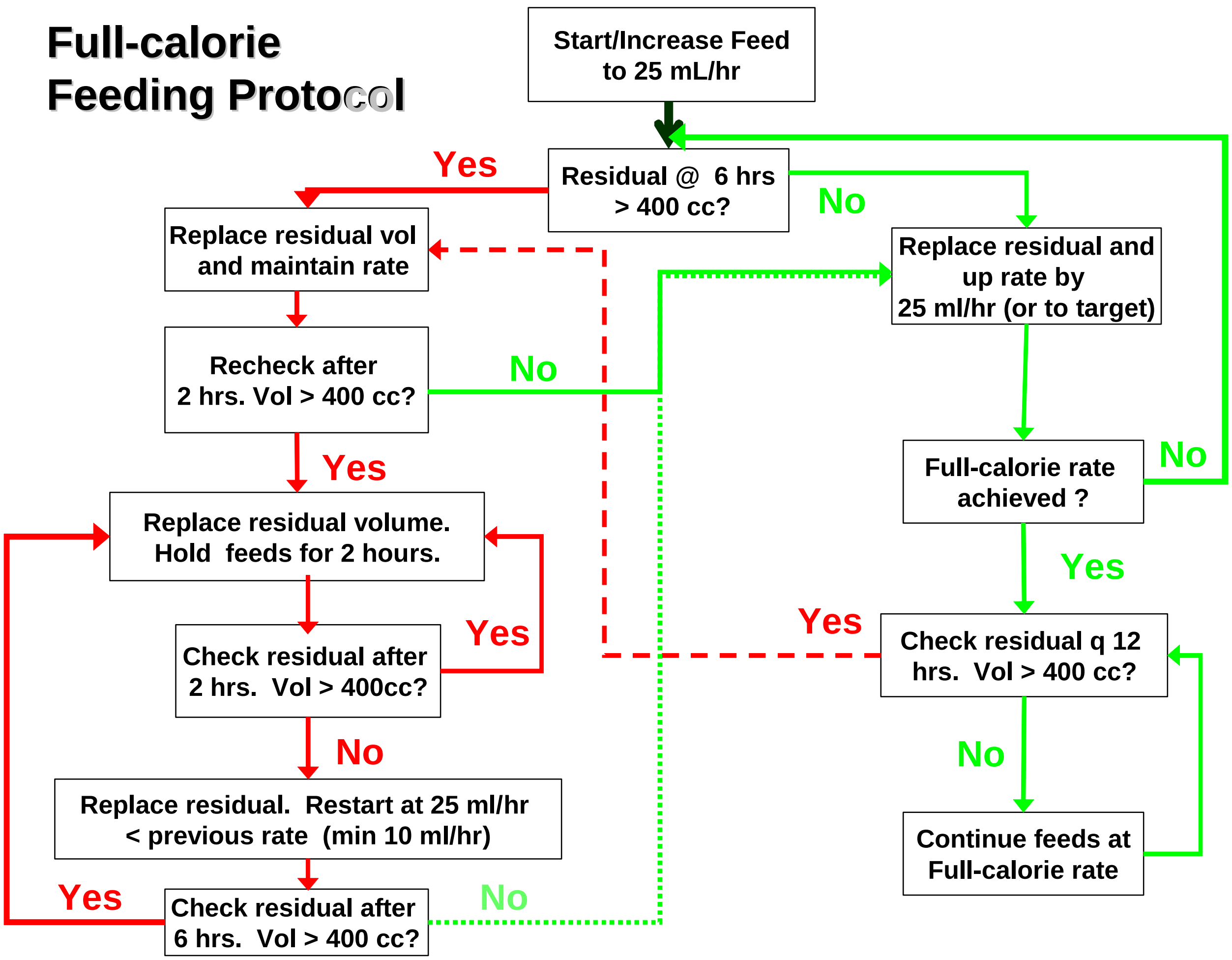
5. Hospitalization within the past six months for respiratory failure in patients with chronic respiratory disease. ($PaCO_2$ greater than 50 mmHg or PaO_2 less than 55 mmHg or O_2-Sat < 88% on $FiO_2 = .21$).

6. Chronic restrictive, obstructive, neuromuscular, chest wall or pulmonary vascular disease resulting in severe exercise restriction, e.g., unable to climb stairs or perform household duties, secondary polycythemia, severe pulmonary hypertension (mean PAP greater than 40 mmHg), or respirator dependency.

C Trophic Feeding Protocol



D Full-calorie Feeding Protocol



E Time-Events Schedule

Measurement/Event	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	14	21	28	60
Demographics, History & Physical, Height, Weight	X																
Etiology of ARDS, site of sepsis if septic etiology	X																
APACHE III Score [C]	X																
HCG (in females)	X																
Vital Signs (HR, SBP, DBP, Temp ^oC) *	X	X	X	X	X	X	X	X	X	X	X						
Central Venous Pressure *	A	A	A	A	A	A	A	A	A	A	A						
Fluids (In and Out) *	X	X	X	X	X	X	X	X									
Brussels Score[B] ~	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Glasgow Coma Scale	X							X								X**	
Ventilator Parameters (including FiO_2) *#	X	X	X	X	X		X						X		X	X	
Arterial Blood Gases (PaO_2, $PaCO_2$, pH) and SpO_2	X	A	A	A	A		A						A		A	A	
Serum Glucose, Na+, K+, HCO3-, Hgb	X	A	A	A	A	A	A	A	A	A	A	A	A				
Creatinine, Platelets, Bilirubin, BUN Hct,	X																
Chest X-ray (# quadrants for lung injury score)	A	A	A	A	A	A	A	A	A	A	A						
Record Sedatives, narcotics, pressors * (Y/N)	X	X	X	X	X	X	X	X	X	X	X	X	X				
Serum magnesium and phosphorus	X	X	A	X	A	A	A	A	X	A	A	A	A				
Total Protein and Albumin	X	X	A	A	A	A	A	X	A	A	A	A	X				
Ventilator–Associated Pneumonia assessment[V]	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
Insulin dose at time of glucose level	A	A	A	A	A	A	A	A	A	A	A	A	A				
Location of Feeding tube (e.g., gastric, post-pyloric) *	X	A	A	A	A	A	A	A	A	A	A	A	A				
Volume of tube feeds / Calories delivered *		X	X	X	X	X	X	X	X	X	X	X	X				
Number and type of Gastrointestinal Intolerances *		X	X	X	X	X	X	X	X	X	X	X	X				
Pro-kinetic agents, anti-diarrheals, anti-emetics * (Y/N)	X	X	X	X	X	X	X	X	X	X	X	X	X				
Serum fatty acids *** (no longer required)	X			X			X						X				
Plasma for Cytokines IL-6 and IL-8	X			X			X						X				
Urine leukotriene B and isoprostane metabolites	X			X			X										
Whole blood for DNA	X																
Episode of bacteremia (record positive blood cultures) *		A	A	A	A	A	A	A	A	A	A	A	A	A			
Clostridium dificile diarrhea tests		A	A	A	A	A	A	A	A	A	A	A	A	A			
Study Drug Administration Record *	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Vital Status §																X	X

X=Required
A=When available
C=Labs not available in the 24 hours before randomization must be obtained
V = VAP assessment from available CXR, sputum culture, gram stain and WBC until extubated or day 28, whichever occurs first
*= Data gathered at times indicated or until 48 hours UAB, whichever occurs first
**=On day 28 or hospital discharge date
***=Collect in the first 30 patients of the placebo and study emulsion groups.
B=Records clinically available creatinine, platelets, bilirubin, SBP and vasopressor use
~=Data gathered on days 0-28 or until d/c from study hospital
#=Measure during reference period (0600-1000); other values may be obtained closest to 0800 on the specified calendar date
§=Measure at 90 days and 12 months as part of Long Term outcome.

F Adverse Events

1. Procedures for Reporting Adverse Events

   Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The Principal Investigator will evaluate all adverse events. The Study Coordinator must view patient records for possible adverse events throughout the study period. All adverse events occurring within the study period must be reported in the participants' case report forms.

   Investigators will report all *serious, unexpected, and study-related* adverse events to the Clinical Coordinating Center within 24 hours. The local Institutional Review Board must also be notified in a timely manner. The investigator will then submit a detailed written report to the Clinical Coordinating Center and the local Institutional Review Board no later than 5 days after the investigator discovers the event.

2. Definitions of Adverse Events

   A *serious* adverse event is any event that is fatal or immediately life threatening, is permanently disabling, or severely incapacitating, or requires or prolongs inpatient hospitalization. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

   Life-threatening means that the patient was, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This definition does not include a reaction that, had it occurred in a more serious form, might have caused death. Assessment of the cause of the event has no bearing on the assessment of the event's severity.

   An *unexpected* event is any experience not identified by the type, severity, or frequency in the current study protocol or an event that is unexpected in the course of treatment for ALI or ARDS.

   Adverse events will be considered to be study-related if the event follows a reasonable temporal sequence from a study procedure and could readily have been produced by the study procedure.

   Organ failures related to ALI or ARDS or the patient's underlying condition should not be reported as adverse events *if the investigator determines the outcomes were not study solution or procedure-related* since they are systematically captured by the protocol data collection.

The following protocol-specified events should always be reported as adverse events:

a. Hypersensitivity to enteral feeds
b. Hypersensitivity to omega-3 fatty acids
c. Intestinal ischemia or infarction
d. Severe bleeding (defined as any central nervous system bleeding or any bleeding event that leads to the administration of three or more units of packed red blood cells per day for two consecutive days).

G Ventilator Procedures

G.1 Ventilator Management

A modified, simplified version of the ARDS Network lung protective lower tidal volume strategy will be used in this trial. This strategy, which was associated with unprecedented low mortality rates in three previous ARDS Network trials (ARMA, ALVEOLI, and FACTT), will ensure that study subjects receive the beneficial effects of lung protection as part of their participation in this trial (Brower, 2004; The Acute Respiratory Distress Syndrome Network, 2000). ARDS Network personnel have substantial experience in the application of this protocol from the three completed trials noted above.

1. Any mode of ventilation capable of delivering the prescribed tidal volume (6ml/kg PBW, +/- 2ml/kg) may be used, provided the V_T target is monitored and adjusted appropriately. During APRV, tidal volume is defined as the sum of the volume that results from the ventilator pressure-release and an estimation of the average spontaneous $V_{T.}$

2. Tidal Volume (Vt) Goal: 6 ml / kg PBW

   Predicted body weight (PBW) is calculated from age, gender, and height (heel to crown) according to the following equations:

   Males: PBW (kg) = 50 + 2.3 [height (inches) – 60]
   Females: PBW (kg) = 45.5 + 2.3 [eight (inches) – 60]

3. Measure and record inspiratory plateau pressure (Pplat) according to ICU routine (at least every four hours and after changes in Vt and PEEP recommended)

4. If Pplat > 30 cm H_20, reduce Vt to 5 ml / kg and then to 4 ml / kg PBW if necessary to decrease Pplat to $\leq$ 30 cm H_20.

5. If Vt < 6 ml / kg PBW and Pplat < 25 cm H_20, raise Vt by 1 ml / kg PBW to a maximum of 6 ml / kg.

6. If "severe dyspnea" (more than 3 double breaths per minute or airway pressure remains at or below PEEP level during inspiration), then raise Vt to 7 or 8 ml / kg PBW if Pplat remains below 30 cm H_20. If Pplat exceeds 30 cm H_20 with Vt of 7 or 8 ml / kg PBW, then revert to lower Vt and consider more sedation.

7. If pH < 7.15, Vt may be raised and Pplat limit suspended (not required).

8. Oxygenation target: 55 mmHg < PaO2 < 80 mm Hg or 88% < SpO2 < 95%. When both PaO_2 and SpO_2 are available simultaneously, the PaO_2 criterion will take precedence.

9. Minimum PEEP = 5 cm H_20

10. Adjust FiO_2 or PEEP upward within 5 minutes of consistent measurements below the oxygenation target range

11. Adjust FiO_2 or PEEP downward within 30 minutes of consistent measurements above the oxygenation target range.

12. There are no requirements for maintaining a specific PEEP to FiO_2 ratio. The lower PEEP / higher FiO_2 table represents a consensus approach developed by ARDS Network investigators in 1995. The higher PEEP / lower FiO_2 table (ALVEOLI) yielded equivalent results in a randomized trial (Brower, 2004) and would be acceptable and perhaps preferable in patients who appear to respond with substantial increase in arterial oxygenation in the transition from lower to higher PEEP.

Lower ELV/Higher FiO_2 Treatment Group

FiO_2	.30	.40	.40	.50	.50	.60	.70	.70	.70	.80	.90	.90	.90	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18-24

Higher ELV/Lower FiO_2 Study Group

FiO_2	.30	.30	.30	.30	.30	.40	.40	.50	.50	.50 – .80	.80	.90	1.0	1.0
PEEP	5	8	10	12	14	14	16	16	18	20	22	22	22	24

(Levels of PEEP in these FiO_2 / PEEP scales represent levels set on the ventilator, not levels of total-PEEP, auto-PEEP, or intrinsic-PEEP.)

13. No specific rules for respiratory rate, but incremental increase in the RR to maximum set rate of 35 if pH < 7.30.

14. No specific rules about I:E. Recommend that duration of Inspiration be ≤ duration of Expiration.

15. Bicarbonate is allowed (neither encouraged nor discouraged) if pH < 7.30.

Changes in more than one ventilator setting driven by measurements of PaO_2, pH, and Pplat may be performed simultaneously, if necessary.

G.2 Weaning

G.2.1 Commencement of Weaning

Patients will be assessed for the following weaning readiness criteria each day between 0600 and 1000. If a patient procedure, test, or other extenuating circumstance prevents assessment for these criteria between 0600 and 1000, then the assessment and initiation of subsequent weaning procedures may be delayed for up to six hours.

(a) At least 12 hours since enrollment in the trial.

(b) $FiO_2 \leq 0.40$ and PEEP ≤ 8 cm H_2O or $FiO_2 \leq 0.50$ and PEEP = 5 cm H_2O

(c) Values of both PEEP and FiO_2 ≤ values from previous day (comparing Reference Measurement values, section 6.3).

(d) Not receiving neuromuscular blocking agents and without neuromuscular blockade

(e) Patient exhibiting inspiratory efforts. If no efforts are evident at baseline, ventilator set rate will be decreased to 50 % of baseline level for up to 5 minutes to detect inspiratory efforts.

(f) Systolic arterial pressure ≥ 90 mm Hg without vasopressor support (≤ 5 mcg / kg / min dopamine or dobutamine will not be considered a vasopressor).

G.2.2 Spontaneous Breathing Trial Procedure and Assessment for Unassisted Breathing

If criteria a-f above are met, then initiate a trial of up to 120 minutes of spontaneous breathing with $F_IO_2 \leq 0.5$ using any of the following approaches:

1. Pressure support ≤ 5cm H_2O, PEEP ≤ 5cm H_2O
2. CPAP ≤ 5 cm H_2O
3. T-piece
4. Tracheostomy mask

Monitor for tolerance using the following:

1. $SpO_2 \geq 90\%$ and / or $PaO_2 \geq 60$ mmHg
2. Mean spontaneous tidal volume ≥ 4 ml / kg PBW (if measured)
3. Respiratory Rate ≤ 35 / min
4. pH ≥ 7.30 (if measured)
5. No respiratory distress (defined as 2 or more of the following):
    a. Heart rate ≥ 120% of the 0600 rate (≤ 5 min at > 120% may be tolerated)
    b. Marked use of accessory muscles
    c. Abdominal paradox
    d. Diaphoresis
    e. Marked subjective dyspnea.

If any of the goals 1-5 are not met, revert to previous ventilator settings or to PS + 10 cm H_2O with Positive End-expiratory Pressure and FiO_2 = previous settings and reassess for weaning the next morning.

The clinical team may decide to change mode of support during spontaneous breathing (PS = 5, CPAP, tracheostomy mask, or T-piece) at any time.

G.2.3 Decision to remove ventilatory support

For intubated patients, if tolerance criteria for spontaneous breathing trial (1-5 above) are met for at least 30 minutes, the clinical team may decide to extubate. However, the spontaneous breathing trial can continue for up to120 minutes if tolerance remains in question. If any of criteria 1-5 are not met during unassisted breathing (or 120 minutes has passed without clear tolerance), then the ventilator settings that were in use before the attempt to wean will be restored and the patient will be reassessed for weaning (see section G.2.1) the following day.

G.3 Definition of Unassisted Breathing

(a) Extubated with face mask, nasal prong oxygen, or room air, OR

(b) T-tube breathing, OR

(c) Tracheostomy mask breathing, OR

(d) CPAP ≤ 5 without PS or IMV assistance

G.4 Completion of Ventilator Procedures

Patients will be considered to have completed the study ventilator procedures if any of the following conditions occur:

a. Death
b. Hospital discharge
c. Alive 28 days after enrollment

If a patient requires positive pressure ventilation after a period of unassisted breathing, the study ventilator procedures will resume unless the patient was discharged from the hospital or > 28 days elapsed since enrollment.

G.5 Removal from the Ventilator Management Protocol

Patients may be removed from the 6 ml / kg tidal volume ventilation requirement if they develop neurologic conditions where hypercapnia would be contraindicated (e.g., intracranial bleeding, GCS ≤ 8, cerebral edema, mass effect [midline shift on CT scan], papilledema, intracranial pressure monitoring, fixed pupils).

H Conservative Fluid Management Approach

This fluid protocol captures the primary positive outcome of the FACTT trial on increasing ventilator free days. This protocol should be initiated within four hours of randomization in enrolled patients, and continued until UAB or study day 7, whichever occurs first.

1. Discontinue maintenance fluids.
2. Continue medications and nutrition.
3. Manage electrolytes and blood products per usual practice.
4. For shock, use any combination of fluid boluses[#] and vasopressor(s) to achieve MAP ≥ 60 mmHg as fast as possible. Wean vasopressors as quickly as tolerated beginning four hours after blood pressure has stabilized.
5. Withhold diuretic therapy in renal failure [§] and until 12 hours after last fluid bolus or vasopressor given.

CVP (recommended)	PAOP (optional)	MAP ≥ 60 mm Hg AND off vasopressors for ≥ 12 hours	
		Average urine output < 0.5 ml/kg/hr	**Average urine output ≥ 0.5 ml/kg/hr**
>8	**> 12**	Furosemide* Reassess in 1 hour	Furosemide* Reassess in 4 hours
4-8	**8-12**	Give fluid bolus as fast as possible[#] Reassess in 1 hour	
< 4	**< 8**		No intervention Reassess in 4 hours

§ Renal failure is defined as dialysis dependence, oliguria with serum creatinine > 3mg/dl, or oliguria with serum creatinine 0-3 with urinary indices indicative of acute renal failure.

[#] Recommended fluid bolus= 15 mL / kg crystalloid (round to nearest 250 mL) or 1 Unit packed red cells or 25 grams albumin

* Recommended Furosemide dosing = begin with 20 mg bolus or 3 mg / hr infusion or last known effective dose. Double each subsequent dose until goal achieved (oliguria reversal or intravascular pressure target) or maximum infusion rate of 24 mg / hr or 160 mg bolus reached. Do not exceed 620 mg / day. Also, if patient has heart failure, consider treatment with dobutamine.

I Genetic Testing

Portions of the blood specimens as specified in this protocol will be used for genetic analyses either for beta-receptor polymorphisms as part of an ancillary study, or for future genetic studies of ARDS that are presently undefined. ALI is a complex inflammatory condition of the lungs, and many of the inflammatory pathways thought to be involved in lung injury are associated with genetic polymorphisms. It is likely that there are, as yet undetermined, important gene/environment interactions that impact on clinical outcome. Thus it is important to collect and store DNA from large, carefully described cohorts of patients with ALI to facilitate discovery in this field with the aim to better understand the pathogenesis of ARDS and how treatment may be tailored to individual patient needs.

Genetic analysis will involve, in part, the analysis of genomic DNA and will attempt to link genotypic information to the extensive phenotypic information measured as part of this study. A layered informed consent will be used to obtain the study subjects' consent for genetic testing as follows: 1) consent for genetic studies related to ARDS, or; 2) consent for future studies not necessarily related to ARDS. The level of consent for testing (e.g. none, for ARDS studies, for future studies, or all studies) will be recorded in the Case Report Forms and stored in the Clinical Coordinating Center Data Base. All patients who recover decision-making capacity will be approached for written re-consent for genetic testing.

Two 7.5 ml EDTA plastic monovette tubes will be used to collect up to 10 ml of blood on each patient with consent for genetic testing. Samples will be labeled with pre-printed label with the subjects ARDSNet study number. DNA extraction will be done centrally.

Following extraction, DNA will be sent to a central repository to be stored (as described below). DNA will first be stored the extraction laboratory for seven years and then shipped to the central repository. Random number will identify samples during shipment, extraction, and storage in the central repository. In the future, when approved studies for genetic testing are received at the CCC, the CCC will identify samples that have the necessary level of informed consent for genetic testing. The CCC will then instruct the repository to prepare the appropriate samples for shipment. The key relating the ARDSNet study number to the specimen number will be kept at the CCC in a locked file. The CCC does not record nor store unique patient identifiers (such as initials, date of birth, hospital record numbers, addresses, phone numbers, etc.) in the data base. All data released by the CCC for genetic studies will be linked to the specimen but will be de-identified. The link (key) between the de-identified database and the patient will be removed two years after the primary publication.

Should patients or surrogates revoke their consent for genetic testing, the clinical sites will notify the CCC. The CCC will then contact the repository and request that all samples collected for genetic analysis for that patient be destroyed. Confirmation of destruction of samples will be sent to the CCC and forwarded to the clinical site.

J De-identified Data Elements for Screened, Non-Enrolled Subjects

- Was onset of ALI acute?
- Did frontal CXR show bilateral infiltrates consistent with pulmonary edema?
- Number of quadrants with opacities?
- Is patient intubated?
- PaO2
- FiO2
- Was there evidence of left atrial hypertension?
- Month of the year that patient met screening criteria (1-12).
- Gender
- Ethnicity
- Age (if age >89, 89 will be entered for age)
- Patient location (e.g. MICU, SICU, etc.) and if regularly screened
- Reason(s) patient excluded from study.
- If not excluded, not enrolled, why?
- Lung injury category (e.g. sepsis, pneumonia)
- If lung injury category=sepsis, site of infection

K Long Term Outcomes

K.1 Phone Surveys for Survivors from All 12 ARDSNet Study Sites

Table 1 summarizes the proposed measurement instruments and their rationale for each of the outcome domains evaluated in the phone-based assessments of ALI survivors from all ARDSNet study sites. These domains and instruments were determined based on a comprehensive assessment performed by the ARDSNet Long-Term Outcomes Committee and by the investigators for this proposed study.

Table 1. Phone assessments of ALI survivors from all 12 ARDSNet study sites at 6 and 12 months

Outcome Domain	Instrument	Rationale	No. of items; Time Req'd; Scale
Mortality	Custom (date & cause of death)*	- Used in existing long-term ALI study (2)	3 item; <1 min.
Physical function	Functional Performance Inventory - Short Form (FPI-SF)	-Developed in chronic pulmonary patients -Comprehensive, reliable and valid (11;12)	32 items; 5 minutes; Continuous
Mental health			
a) Depression & General Anxiety	Hospital anxiety & depression (HAD) scale (13)	-Most widely used survey in medical patients(14) -Separate subscale for depression & anxiety -Reliable and validated in medical patients (14) -Highly correlated with psychiatric evaluation (13;15)	14 items; 5 minutes (2) Continuous
b) Post-traumatic stress disorder	Impact of Events Scale – Revised (IES-R) (16)	-IES is the most commonly used instrument for assessing PTSD in the ICU (15) -Revised version (IES-R) follows DSM-IV (17) criteria -Reliable and valid (16;18)	22 items; 3 minutes (2) Continuous
Cognitive status	Telephone Mini-Mental State Examination (TMMSE) (19;20)	-MMSE is the most widely used instrument -TMMSE is designed specifically for phone use -Reliable and valid (19;20)	16 items; 5 minutes; Continuous
Health-related quality of life			
a) Generic	1. SF-36 version 2 (21)	-Most widely used instrument, esp in ALI (1-3;6-7) -Reliable and validated in ICU patients (23) -US population norms available (21)	36 items; 6 minutes; Continuous
	2. EQ-5D (EuroQOL) (22)	-Feasible for patients with inattention& fatigue (6;22) -Recommended for use in ICU patients (5) -Provides utility estimate with US norms (24)	6 items; 2 minutes (2) Continuous
b) Fatigue	Functional Assessment of Chronic Illness Therapy (FACIT)	-Designed for patients with chronic illness -Assesses both functional & emotional impact (25;26) -Reliable and validated (27;28)	13 items; 3 minutes (25) Continuous
Return to work	Custom instrument	-Developed & used in large cohort of ALI survivors (2)	12 item; 2 min. Categorical
Health care utilization	University of Toronto ARDS Outcome study instrument (4)	-Developed and used in large longitudinal cohort of ALI survivors (4)	27 items; 8 minutes; Continuous

*** Also will be determined from a National Death Index via participant's Social Security Number.**

Administration of phone surveys will be centralized at 2 sites: Johns Hopkins and LDS Hospital, where the 2 Principal Investigators are affiliated. Being in different time zones, this 2-site approach will allow flexibility in accessing patients across the US while also concentrating our oversight activities. Manuals of Operations will be used for training, reference and quality assurance review.

NOTES:

(1) Estimated time for completion. This was based on pilot testing, published estimates and the experience of the ARDS Network investigators. The full telephone interview will be piloted prior to implementation.

(2) Return to Work assessment. There are no pre-existing comprehensive survey instruments for measuring return to work and work disability in patients with lung disease. We derived our custom-made instrument from an approach used by one member of the Long-term Outcomes Committee (Dr. Eisner and colleagues) to measure work disability in asthma and COPD.

K.2 Statistical Considerations for Long Term Outcomes

A number of dichotomous and continuous measures of long-term efficacy of the treatment will be analyzed.

Dichotomous measures:

1) Survival times will be compared for the treatment arms using log rank test.
2) Proportions of patients alive without major disabilities will be compared between the treatment arms using Cochran-Mantel-Haenszel test. Major disability is defined for surviving patients that are prevented from working due to a respiratory condition.
3) Proportions of patients alive without disability in activities of daily living (ADL) or instrumental activities of daily living (IADL) will be compared across treatment arms using Cochran-Mantel-Haenzsel test. Major disability ADL and IADL are defined based on functional performance inventory for a patient who has at least one activity in the “body care” and “maintaining household” subscales, respectively, that s/he cannot perform at all due to health reasons or does it with much difficulty.

Each of the comparisons will be done based on the data collected at 6 months, and 1-year follow up times.

Continuous measures:

1) Primary measure of disability defined by functional performance inventory.
2) Eight subscales and two summary measures of the SF-36 instrument
3) Depression measure defined by Beck Depression Inventory II
4) Cognitive measure

Continuous measures will be analyzed using analysis of variance stratified by the treatment arm.

Each of the comparisons we will be done based on the data collected at 6 months, and at 12 months follow up times. We will compare the raw continuous measures in the groups of patients available for the follow up. There is a concern that those patients that survive and are contactable to obtain information will potentially belong to different populations for different treatment arms. If true, this will make comparison between the treatment arms no longer randomized. To address this we will compare the treatment arms using survival average causal effect (SACE). This method (Hayden 2005) uses concepts of casual inference by adjusting the estimates of the population parameters based on the model covariates. First the expected probabilities of survival and ability to contact and obtain information from a patient are computed using logistic regression. Then estimates are weighted by these computed survival and contactability to correct for potential differences in the patient populations across treatment arms selected by survival and contactability of patients. The model depends on the assumption that conditional on the values of the covariates the probabilities of a patient surviving and being contactable are independent across treatment arms. The effects of this assumption will be evaluated via a sensitivity analysis.

K.3 Citations for M1 (Choice of survey instruments)

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L. Data and Safety Monitoring Board

The principal role of the DSMB is to regularly monitor data from this trial, review and assess the performance of its operations, and make recommendations, as appropriate, to the NHLBI with respect to:

- Review of adverse events
- Interim results of the study for evidence of efficacy or adverse events
- Possible early termination of the trial because of early attainment of study objectives, safety concerns, or inadequate performance
- Possible modifications in the clinical trial protocol
- The performance of individual centers

The NHLBI ARDS Network DSMB is appointed by the Director, NHLBI. The DSMB reviews all new protocols for safety following review by an independent NHLBI Protocol Review Committee. The DSMB will consist of members with expertise in acute lung injury, biostatistics, ethics, and clinical trials. Ad hoc members have been appointed with particular expertise where necessary. Appointment of all members is contingent upon the absence of any conflicts of interest. All the members of the DSMB are voting members. The DSMB will review data prepared by the CCC. Decisions regarding issues such as stopping guidelines or whether the DSMB may at times remain blinded to study group identity will be made jointly by the DSMB members and the NHLBI representatives. The Principal Investigator and the Medical Monitor of the CCC will be responsible for the preparation of DSMB and adverse event reports and may review unblinded data. DSMB meetings will be scheduled by the NHLBI at intervals as described in section 7, and the DSMB will review the protocol during its first meeting. When appropriate, conference calls may be held in place of face-to-face meetings. Recommendations to end, modify, or continue a trial will be prepared by the DSMB executive secretary for review by Director, NHLBI, no more than two working days after a DSMB meeting. When appropriate, conference calls may be held in place of face-to-face meetings. Recommendations for major changes, such as stopping, will be communicated immediately, and followed by a written summary. The NHLBI will act on recommendations expeditiously; the NHLBI Project Officer or Program Scientist will communicate the recommendations promptly to the ARDS Network Steering Committee and the CCC with instructions for reporting to local IRBs when appropriate. The executive secretary of the DSMB will be responsible for preparing the minutes for each meeting or conference call. Details of the NHLBI policies regarding DSMBs can be found at the following URL: http://www.nhlbi.nih.gov/funding/policies/dsmb_inst.htm

The ARDS Network Steering Committee is comprised of the Principal Investigators and Co-investigators of all the Clinical sites, the CCC, and the NHLBI Project Officer who represents the NHLBI. All sites and the CCC have one vote, which is advisory to the NHLBI.

M. AUDIT Questionnaire

The Alcohol Use Disorders Identification Test (Babor, 1992)

The Alcohol Consumption Questionnaire is important to administer because there is a common association between alcohol abuse and Acute Lung Injury (ALI) (Moss, 1996). It will be important to have this information for a subgroup analysis. Knowledge of alcohol abuse will also help the primary team better care for the patient and improve patient outcome, as there are alcohol specific disorders in critically ill patients that often are not diagnosed and therefore not treated effectively. This survey will not be completed on subjects less than 18 years of age.

1. How often do you have a drink containing alcohol?
   (0) Never [Skip to Qs 9-10]
   (1) Monthly or less
   (2) 2 to 4 times a month
   (3) 2 to 3 times a week
   (4) 4 or more times a week

2. How many drinks containing alcohol do you have on a typical day when you are drinking?
   (0) 1 or 2
   (1) 3 or 4
   (2) 5 or 6
   (3) 7, 8, or 9
   (4) 10 or more

3. How often do you have six or more drinks on one occasion?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily
   Skip to Questions 9 and 10 if Total Score for Questions 2 and 3 = 0

4. How often during the last year have you found that you were not able to stop drinking once you had started?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

5. How often during the last year have you failed to do what was normally expected from you because of drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remorse after drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year

10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year

Record total of specific items here

If total is greater than recommended cut-off, consult User's Manual.

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