# Enteral nutrition with eicosapentaenoic acid, γ-linolenic acid, and antioxidants reduces alveolar inflammatory mediators and protein influx in patients with acute respiratory distress syndrome

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*Objective:* **Previously, we showed that acute respiratory distress syndrome patients fed an enteral diet containing eicosapentaenoic acid and γ-linolenic acid and elevated antioxidants (EPA+GLA; Oxepa) had significantly reduced pulmonary inflammation, increased oxygenation, and improved clinical outcomes. In a subset of acute respiratory distress syndrome patients from this trial, we performed a preliminary examination of the potential mechanisms underlying these clinical improvements by retrospectively testing the hypothesis that enteral feeding with EPA+GLA could reduce alveolar-capillary membrane protein permeability and the production of interleukin (IL)-8, IL-6, tumor necrosis factor-α, and leukotriene $B_4$ that are responsible, in part, for pulmonary inflammation.**

*Design:* **Prospective, randomized, double-blind, controlled clinical trial.**

*Setting:* **Intensive Care Unit of the Ohio State University Medical Center.**

*Patients:* **A total of 67 patients were enrolled who met defined criteria for acute lung injury/acute respiratory distress syndrome.**

*Interventions:* **A total of 43 of 67 evaluable patients randomly received either EPA+GLA or an isonitrogenous, isocaloric standard diet that was tube fed at a minimum caloric delivery of 75% of basal energy expenditure times 1.33 for at least 4 to 7 days.**

*Measurements and Main Results:* **Bronchoalveolar lavage (BAL) was performed at baseline and study days 4 and 7 to obtain BAL fluid (BALF) for measurement of total protein, ceruloplasmin, and transferrin, total neutrophil count, IL-8, IL-6, tumor necrosis factor-α, and leukotriene $B_4$. Oxygenation, measured as $Pao_2/Fio_2$, was assessed before BAL. Patients fed EPA+GLA had a significant reduction in BALF ceruloplasmin and IL-8 during the study as compared with patients fed the control diet. BALF levels of total protein, neutrophils, and leukotriene $B_4$ tended to decrease in EPA+GLA patients over the course of the study as compared with control patients. BALF levels of IL-6 declined similarly during the study in both groups. A trend toward a reduction in BALF tumor necrosis factor-α was observed on study day 7 in the EPA+GLA group as compared with control patients. Significant improvements in oxygenation ($Pao_2/Fio_2$) occurred in EPA+GLA patients on study day 4 as compared with controls. Correlation analysis revealed significant relationships between BALF neutrophil counts and indices of alveolar-capillary membrane protein permeability, IL-8, and leukotriene $B_4$.**

*Conclusions:* **This preliminary investigation showing a decrease in BALF levels of IL-8 and leukotriene $B_4$ and the associated reduction of BALF neutrophils and alveolar membrane protein permeability in acute respiratory distress syndrome patients fed EPA+GLA support, in part, the potential mechanisms underlying the previously described clinical improvements with this diet. Additional controlled studies are needed to confirm these findings. (Crit Care Med 2003; 31:491–500)**

KEY WORDS: **acute respiratory distress syndrome; γ-linolenic acid; eicosapentaenoic acid; protein permeability; pulmonary inflammation; acute lung injury; enteral nutrition; fish oil; borage oil**

Recent studies have shown that the use of specialized enteral nutrition formulas are becoming a useful adjuvant therapy in the clinical management of critically ill patients. The use of key ingredients, such as novel oil blends containing fatty acids with anti-inflammatory potential and elevated antioxidants (1–5), can aid in the modulation of the systemic inflammatory response. Although animal (6–10) and clinical studies (2, 3) have shown that nutritional intervention with dietary fish oil containing eicosapentaenoic acid (EPA) can favorably modulate proinflammatory eicosanoid production from arachidonic acid, a recent advancement has been the inclusion of γ-linolenic acid (GLA; borage oil) to fish oil–containing formulas (11) to promote the production of prostaglandin $E_1$, an eicosanoid with known anti-inflammatory and immunoregulatory properties.

Supplementation of nutritional formulas with a combination of EPA and GLA can favorably reduce an elevated inflammatory response while promoting vasodilation and oxygen delivery (7). These physiologic benefits have been shown using animal models of sepsis-induced acute respiratory distress syn-

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drome (ARDS). These studies showed that enteral nutrition with a low carbohydrate, high fat diet containing EPA, GLA, and elevated antioxidants (EPA+GLA): 1) rapidly decreased the level of arachidonic acid in key immune cell (alveolar macrophage, Kupffer cell) phospholipids (12–14), 2) attenuated endotoxin-induced increases in pulmonary neutrophil recruitment (8) and microvascular protein permeability (9), 3) reduced the production of proinflammatory eicosanoids associated with lung injury (7–10), 4) improved cardiopulmonary hemodynamics and respiratory gas exchange (7), and 5) did not impair alveolar macrophage functions, including respiratory burst, bactericidal, and phagocytois (10). These observed physiologic benefits could play an important role in governing physiologic responses within the lung, especially with the pathophysiology associated with ARDS.

Results from this research led to the initiation of a prospective, double-blinded, randomized, controlled, multicenter trial assessing the effects of EPA+GLA on clinical physiology and outcomes in patients with ARDS (1). Patients fed EPA+GLA for at least 4 to 7 days had significantly reduced pulmonary inflammation, increased oxygenation, and improved clinical outcomes (reduced time on the ventilator and intensive care unit [ICU] stay) and significantly reduced the number of patients who developed a new organ failure during the study as compared with patients fed an isocaloric, isonitrogenous standard control diet.

There exists a growing body of literature that states that one of the early pathologic changes in acute lung injury/ARDS is the recruitment of neutrophils into the alveolar-capillary barrier with the release of proinflammatory eicosanoids and cytokines (15–18). This increase in pulmonary inflammation leads to capillary permeability with severe acute hypoxemia due to pulmonary edema. With this in mind, we chose to do a preliminary examination of the potential mechanisms underlying the clinical improvements noted above in ARDS patients from the primary center of this multicenter trial. We tested the hypothesis that enteral feeding with EPA+GLA could reduce alveolar-capillary membrane protein permeability and the production of interleukin (IL)-8, IL-6, tumor necrosis factor-α (TNF-α), and leukotriene (LT)$B_4$ that are responsible, in part, for pulmonary inflammation. The relationship between oxygenation and indices of lung permeability and inflammation was also evaluated.

## MATERIALS AND METHODS

*Study Design and Patients.* The study design, patient selection, experimental groups, and study methods were the same as previously reported for the prospective, double-blinded, randomized, multicenter trial (1). The study was approved by the institutional review board before patient enrollment. A total of 67 patients with illnesses known to be associated with acute lung injury, pulmonary inflammation, and ARDS that satisfied all study entry criteria were prospectively recruited from the ICU of the Ohio State University Medical Center and randomized. These patients represent a subset of ARDS patients from the original multicenter trial of 146 patients (1) whose data were retrospectively analyzed for this study. Informed consent was obtained from all patients or their legal guardian before any study inclusion procedures being performed. The inclusion criteria (Table 1) represents a modification of the criteria for acute lung injury and ARDS from the American-European Consensus Conference definition (19). Patients meeting entry criteria and without exclusions (Table 1) were prospectively randomized to the study groups in a blinded fashion with the use of a permuted-block randomization design as previously described (1). Clinical investigators, patient caregivers, and patients were blinded to diet identity.

Patients received either a control diet or EPA+GLA (Oxepa, Ross Products Division, Abbott Laboratories, Columbus, OH). The control diet was a ready-to-feed high fat, low carbohydrate enteral nutrition formula designed to reduce $CO_2$ production in patients with respiratory insufficiency while providing complete and balanced nutrition. This diet has been shown to reduce minute ventilation and

**Table 1.** Inclusion/exclusion Criteria for Patient Selection

Inclusion criteria: Patient must have met all entry criteria
1) The patient must have been between 18 and 80 yrs of age, inclusive
2) Diagnosis of predisposing condition resulting in acute lung injury, which included the following
   a) Acute bacterial, viral, or fungal pneumonia
   b) Sepsis syndrome
   c) Aspiration injury
3) Bronchoalveolar lavage evidence of pulmonary inflammation as indicated by polymorphonuclear neutrophil count of >10%
4) One of the following clinical criteria of acute lung injury/acute respiratory distress syndrome
   a) $P(A-a)O_2$>100 torr
   b) $PaO_2/FiO_2$<250 torr but >100 torr
   c) Continuous positive airway pressure≥10 cm $H_2O$
5) Mechanism for enteral feeding—gastric, duodenal, or jejunal tube feeding
6) Chest radiography demonstrating diffuse pulmonary infiltrates
7) The patient, legal guardian, or authorized patient representative must have voluntarily signed an informed consent statement approved by the appropriate institutional review board(s). If the patient signed the consent form(s), the person obtaining consent must have ensured that the patient was in adequate condition to provide informed consent.

Exclusion criteria: Patients with any of the following diagnoses were excluded from the study
1) Clinical diagnosis of left ventricular failure without bronchoalveolar lavage evidence of acute lung injury
2) Lung cancer—primary or metastatic
3) Hematologic malignancy
4) Acute gastrointestinal bleeding precluding enteral feeding
5) Head trauma (Glasgow coma score, ≤5), stroke, or subarachnoid hemorrhage
6) Severe immunosuppression, defined as cytotoxic therapy within 15 days or White blood cell count of <5000
7) Use of steroids: >0.25 mg/kg/day of prednisone (or an equivalent dose of corticosteroids)
8) Use of nonsteroidal anti-inflammatory (including aspirin) drugs within the last 24 hrs
9) Known to be HIV positive
10) Moribond at entry
11) Pregnancy or positive pregnancy test

ventilatory demand by lowering the respiratory quotient and $CO_2$ production (20). The experimental diet (EPA+GLA) was isocaloric and isonitrogenous to the control diet, differing only in terms of its lipid composition and level of antioxidant vitamins. The lipid blend provides a mixture of EPA and docosahexaenoic acid (DHA) from fish oil and GLA from borage oil. A complete analysis of the two diets is outlined in Table 2.

Before the initiation of enteral feeding, a baseline medical history, physical examination, and other general clinical information (primary diagnosis and associated conditions) were recorded to determine the etiology of the pulmonary inflammation thought to be responsible for the development of acute lung injury/ARDS. Patients received enteral formula within 24 hrs of meeting entry criteria via a gastric, duodenal, or jejunal feeding tube at the discretion of the physician investigator. The day of entrance into the study was defined as baseline. If a patient had been in the ICU and was receiving enteral nutrition, the feeding was discontinued for 24 hrs before the start of the study. The study diets were delivered at a constant rate to achieve a minimum of 50% of basal energy expenditure (BEE; calculated) $\times$ 1.33 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE $\times$ 1.33 within 72 hrs of initiation of enteral feeding. The first day that a patient received enteral nutrition at a minimum of 75% of BEE $\times$ 1.33 was considered study day 1. Enteral nutrition was delivered continuously for a minimum of 4 $\pm$ 1 study days at a rate not to exceed BEE $\times$ 1.33. Daily enteral intake was recorded for total volume and calories delivered to each patient.

*Assessment of Gas Exchange.* Gas exchange was assessed by measurement of arterial blood gases at baseline, study day 4 ($\pm$1 day), and study day 7 ($\pm$1 day). Simultaneous recording of ventilator settings were also performed that enabled calculation of $Pao_2/Fio_2$. Patients were mechanically ventilated with conventional modes of ventilation (assist/control volume-limited, pressure support, and pressure controlled) and positive end-expiratory pressure using normal inspiratory-expiratory ratios. The overall goal of mechanical ventilation was to maintain acceptable gas exchange with minimal complications by utilizing patient-specific goals of ventilation, rather than a standardized strategy, to guide ventilator settings.

*Bronchoalveolar Lavage.* To assess the effects of the study diets on pulmonary neutrophil recruitment, alveolar-capillary membrane protein permeability, and inflammatory mediators, bronchoalveolar lavage (BAL) was performed at baseline and repeated at study day 4 ($\pm$1 day) and study day 7 ($\pm$1 day) to obtain BAL fluid (BALF) for analysis as previously discussed (1). Briefly, bronchoscopy was performed using a fiberoptic bronchoscope inserted through the endotracheal tube in intubated patients. The bronchoscope was wedged in a single segment of either the right middle lobe or lingula. Five aliquots of 30 mL each of sterile saline (0.9% NaCl) were infused through the bronchoscope and gently withdrawn after each infusion into the injection syringe. The lavage fluid was immediately pooled and placed on ice. Any lavage fluid foam created during the procedure was included in the pooled sample. Subsequent lavages were performed in a segment other than the one utilized the first time. Aliquots of the fluid were removed for total neutrophil counts, and the remaining lavage fluid, including foam, was centrifuged at 250 $\times$ *g* for 20 mins at 4°C to remove the cell pellet. The supernatant was removed aseptically, and aliquots were stored at −70°C. To minimize the potential for the BAL procedure to cause a deterioration in oxygenation or a change in ventilator variables (minute ventilation and positive end-expiratory pressure) (21), blood gases and ventilator settings were taken before the scheduled BAL for that day.

To assess the effects of the study diets on alveolar-capillary membrane protein permeability, BALF concentrations of total protein, ceruloplasmin, and transferrin were determined. The total protein content of the BALF was measured on an aliquot of the supernatant using a modification of a commercially available protein microassay procedure (Bio-Rad Laboratories, Hercules, CA) (22). BALF concentrations of ceruloplasmin and transferrin were determined by an enzyme-linked immunoassay technique (ELISA) as reported by Lykens et al (23).

*Cytokine and Eicosanoid Measurements.* Cytokine and eicosanoid concentrations in the BALF samples were measured in duplicate by enzyme-linked immunoassay in dilutions that allowed interpolation from simultaneously run standard curves. IL-6 was measured with a commercially available enzyme-linked immunoassay (Quantikine, R&D Systems, Minneapolis, MN). The minimum detectable concentration for IL-6 was 0.70 pg/mL with an intra-assay precision of <4.5% and an inter-assay precision of <6.5%. The IL-6 assay was highly specific, without significant cross reactivity or interference with other cytokines or soluble receptors. The concentration of IL-8 was determined using methods as previously described (24). BALF levels of TNF-α were measured using antihuman TNF antibody and

**Table 2.** Analyzed composition of enteral diets[a,b]

| Nutrient | Control | EPA+GLA |
|---|---|---|
| Protein | | |
| % of total calories | 16.7 | 16.7 |
| g/L | 62.6 | 62.5 |
| Source | 87% sodium caseinate, 13% calcium caseinate | 87% sodium caseinate, 13% calcium caseinate |
| Carbohydrate | | |
| % of total calories | 28.1 | 28.1 |
| g/L | 105.7 | 105.5 |
| Source | 46% maltodextrin, 54% sucrose | 45% maltodextrin, 55% sucrose |
| Lipids | | |
| % of total calories | 55.2 | 55.2 |
| g/L | 92.1 | 93.7 |
| Source | 96.8% corn oil, 3.2% soy lecithin | 31.8% canola oil, 25% MCT, 20% borage oil, 20% fish oil, 3.2% soy lecithin |
| Vitamins | | |
| Vitamin E, IU/L | 47.6 | 317 |
| Vitamin C, mg/L | 317 | 844 |
| β-carotene, mg/L | – | 5.0 |
| Taurine, mg/L | – | 316 |
| L-carnitine, mg/L | – | 181 |
| Caloric density, kcal/mL | 1.5 | 1.5 |
| Osmolality, mOsm/kg/$H_2O$ | 465 | 493 |

[a]Each liter of both formulas contained the following vitamins: 5360 IU, vitamin A, 425 IU of vitamin D, 101 μg of vitamin $K_1$, 850 μg, of folic acid, 3.2 mg of thiamine, 3.6 mg of riboflavin, 4.3 mg of vitamin $B_6$, 13 μg of vitamin $B_{12}$, 43 mg of niacin, 635 mg of choline, 635 μg of biotin, 22 mg of pantothenic acid; [b]Each liter of both formulas contained the following trace minerals: 1310 mg of Na; 1960 mg of K; 1690 mg of Cl, 1060 mg of Ca, 1060 mg of P; 425 mg of Mg, 160 μg of I; 5.3 mg of Mn, 2.2 mg of Cu, 24 mg of Zn, 20 mg of Fe, 77 μg of Se, 130 μg Cr, 160 μg of Mo.

EPA + GLA, eicosapentaenoic acid, γ-linolenic acid, and antioxidants, MCT, medium chain triglycerides.

previously described methodology (25). $LTB_4$ was measured with a commercially available enzyme-linked immunoassay (Oxford Biomedical Research, Oxford, MI). The minimum detectable concentration for $LTB_4$ was 100 pg/mL with an inter-assay precision of <5%. The $LTB_4$ assay was highly specific, without significant cross reactivity or interference with other leukotrienes.

*Statistical Analysis.* Study randomization plan, data management, and statistical analyses were performed at SCIREX, Bloomingdale, IL. Patient demographic data and baseline values were compared across the two dietary groups using a one-way analysis of variance for all continuous measurements. Data were analyzed using one-way analysis of variance, repeated measures analysis of variance, chi-square, Fisher's exact test, and Wilcoxon's rank-sum test. The relationship between oxygenation and indices of lung permeability and inflammation was tested using Pearson's correlation coefficient analyses. Data are expressed as mean ± SEM or as indicated. A probability value of $p \leq .05$ was considered statistically significant; $p$ values of .05 to .1 were considered important trends toward an increase or decrease for a specific variable.

## RESULTS

*Baseline Demographics and Comparison of Study Groups.* On review of the eligibility criteria, a total of 43 patients were deemed evaluable. Of the evaluable patients, 22 were randomized to receive the control diet and 21 patients were randomized to receive the EPA+GLA diet. An evaluable patient was defined as one having baseline measures of oxygenation and pulmonary inflammation, a feeding period of 4 ± 1 days from study day 1, and all measures of oxygenation or pulmonary inflammation that were available. On review of the data, 82% (35 of 43) of the evaluable patients started the study within 7 days of hospital admittance; four additional patients (39 of 43, 92%) began the study within 14 days of admittance, and the remaining four patients began the study after 2 wks of hospitalization. The percentage of patients within these categories were comparable between the two groups. Before unblinding of the study, data from the 24 nonevaluable patients were excluded from the final analysis due to protocol violations. The reasons for exclusion were inability to meet caloric goal (4 of 15 in the control group, 3 of 9 in the EPA+GLA group), inadvertent feeding tube removal (1 of 15 in the control group, 0 of 9 in the EPA+GLA group), withdrawal by attending physician (3 of 15 in the control group, 2 of 9 in the EPA+GLA group), administration of steroids or nonsteroidal anti-inflammatory drugs during study protocol (1 of 15 in the control group, 2 of 9 in the EPA+GLA group), aspiration (0 of 15 in the control group, 1 of 9 in the EPA+GLA group), extubation from ventilator (4 of 15 in the control group, 1 of 9 in the EPA+GLA group), and death (2 of 15 in the control group, 0 of 9 in the EPA+GLA group).

Table 3. Reasons for patients not completing through study day 7

| Reasons for Study Termination | Control (n = 7) | EPA+GLA (n = 10) |
|---|---|---|
| Unable to meet caloric goal (gastrointestinal complications[a] or NPO) | 3 | 1 |
| Patient pulled feeding tube | 1 | 0 |
| Withdrawal by attending physician | 0 | 1 |
| Administration of steroids or NSAIDS | 2 | 2 |
| Extubation from ventilator | 1 (14%) | 6 (60%) |

EPA+GLA, eicosapentaenoic acid, γ-linolenic acid, and antioxidants; NPO, nothing orally, NSAIDS, nonsteroidal anti-inflammatory drugs.

[a]Gastrointestinal complications: abdominal distension, high residuals, diarrhea

A total of 17 patients did not complete from study day 4 through study day 7 (seven control patients and ten EPA+GLA patients). When compiling the reasons for dropping out, there was a substantial difference in the number of patients removed from the study due to weaning from the ventilator. Sixty percent (6 of 10) of the patients fed EPA+GLA were extubated, whereas only 14% (1 of 7) of the control patients were weaned from the ventilator (Table 3).

Conclusions from data presented are based on patients who met all eligibility criteria and received enteral nutrition for a minimum of 4 ± 1 days from study day 1 at a rate not to exceed BEE × 1.33 as previously described (1). An intent-to-treat analysis of the study data was not performed. Analyses of the baseline demographic and characteristics of the two study groups showed that the EPA+GLA and control group were balanced with respect to a variety of clinical measures that are associated with morbidity and mortality in patients with acute lung injury/ARDS (Table 4).

*Nutritional Intake Variables.* The majority of the patients were gastrically or jejunally fed either formula within 24 hrs of meeting entry criteria (Table 5). No patients were dropped due to safety concerns related to either study formula. The time in hours from study entry to 75% of BEE × 1.3 (study day 1) was approximately 37–38 hrs for patients randomized to either the control or EPA+GLA diet. Analyses of the nutritional intake variables indicated that no significant differences existed between the groups with respect to daily intake of total calories, lipid, and protein ($p > .1$) (Table 5). Patients given EPA+GLA received substantial amounts of EPA, DHA, and GLA, whereas patients fed the control diet did not receive these fatty acids. Patients fed EPA+GLA as compared with the control diet also received significantly higher daily intakes of the antioxidant vitamins: vitamin C, all-natural vitamin E, and β-carotene.

*Alveolar-Capillary Membrane Protein Permeability.* Alveolar-capillary membrane protein permeability was assessed by measuring the amounts of total protein, ceruloplasmin, and transferrin in recovered BALF from patients at baseline, study day 4 (±1 day) and study day 7 (±1 day). There were no significant differences in the percentage of BALF recovered between the groups at baseline, study day 4, and study day 7 ($p > .1$). The mean percentage of recovered BALF for patients given the control diet and for patients fed EPA+GLA was 46% ± 2% and 37% ±2%, respectively. The change from baseline data for total protein, ceruloplasmin, and transferrin are shown in Figure 1. At baseline, there was no significant difference in the levels of total protein (EPA+GLA: 855 ± 255 μg/mL, n = 17, vs. control: 530 ± 109 μg/mL, n = 21; $p = .22$) and transferrin (EPA+GLA, 13.6 ± 4.6 μg/mL, n = 16; vs. control, 7.6 ± 1.8 μg/mL, n = 21; $p = .19$) in BALF for both groups. BALF levels of ceruloplasmin (19.0 ± 6.1 μg/mL, n = 16; vs. 4.5 ± 1.1 μg/mL, n = 21; $p = .01$) were higher at baseline for EPA+GLA vs. control patients, respectively. Patients fed EPA+GLA tended to have a greater reduction in BALF levels of total protein during the study as compared with the

control group ($p = .08$ by repeated measures analysis of variance). BALF levels of ceruloplasmin significantly decreased 63% from baseline to study day 4 in EPA+GLA patients vs. no change in patients given the control diet ($p = .03$). The decrease in ceruloplasmin from baseline to study day 7 tended to continue in the EPA+GLA group, with a 89% vs. a 38% decline in controls. Due to the small sample size remaining on study day 7, this difference did not reach statistical significance ($p = .2$). Both groups of patients showed comparable decreases in BALF transferrin from baseline to study day 4 and baseline to study day 7 ($p = .2$).

*BAL Inflammatory Mediators.* To assess the potential relationship underlying the observed reduction in alveolar-capillary membrane protein permeability in patients fed EPA+GLA, measurements of IL-8, $LTB_4$, IL-6, and TNF-α were chosen as a representative sample of inflammatory mediators previously shown to be elevated in the BALF of ARDS patients (26–33). Baseline BALF levels of the neutrophil chemoattractant, IL-8 (EPA+GLA, 2849 ± 1458 pg/mL, n = 16; vs. control, 1224 ± 347 pg/mL, n = 21), and $LTB_4$ (EPA+GLA, 596 ± 495 pg/mL, n = 17; vs. control, 145 ± 36 pg/mL, n = 21) were not statistically different between the EPA+GLA and control groups ($p > .1$). There was a significant decrease in BALF IL-8 during the study in patients fed EPA+GLA ($p = .05$ by repeated measures analysis of variance) vs. no reduction in control patients (Fig. 2). There was a trend toward a decrease in BALF $LTB_4$ during the study in EPA+GLA patients, whereas paradoxically, there was an increase in patients fed the control formula ($p = .1$ by repeated measures analysis of variance) (Fig. 2).

Baseline levels of BALF IL-6 (EPA+GLA, 282 ± 145 pg/mL, n = 16;

**Table 4.** Baseline demographic and clinical characteristics of the treatment groups

| Parameter | Control, n = 22 | EPA+GLA, n = 21 |
|---|---|---|
| Age, yrs | 55 ± 4 | 53 ± 3 |
| Sex, n (%) | | |
| Male | 6 (27) | 10 (48) |
| Female | 16 (73) | 11 (52) |
| Admission weight, kg | 76 ± 3 | 74 ± 6 |
| Classification of patient, n | | |
| Sepsis/pneumonia | 15 | 15 |
| Aspiration | 7 | 5 |
| Inhalation injury | 0 | 1 |
| Laboratory and physiologic data | | |
| Serum albumin, g/dL | 2.3 ± 0.1 | 2.4 ± 0.1 |
| Serum creatinine, mg/dL | 1.5 ± 0.2 | 1.3 ± 0.3 |
| Total bilirubin, mg/dL | 1.2 ± 0.3 | 1.3 ± 0.3 |
| WBC, $10^9$ cells/L | 14.6 ± 2.4 | 16.3 ± 1.8 |
| Organ system failure at baseline, n (%) | | |
| Respiratory | 22 (100) | 21 (100) |
| Cardiovascular | 5 (23) | 5 (24) |
| Renal | 3 (14) | 3 (14) |
| Hematologic | 1 (5) | 4 (19) |
| Hepatic | 1 (5) | 3 (14) |
| Neurologic | 1 (5) | 2 (10) |
| Gastrointestinal | 2 (9) | 1 (5) |
| No. of patients with | | |
| 1 organ system failure | 14 | 13 |
| 2 organ system failures | 5 | 1 |
| 3 organ system failures | 2 | 5 |
| 4 organ system failures | 0 | 1 |
| 5 organ system failures | 1 | 1 |

EPA+GLA, eicosapentaenoic acid, γ-linolenic acid, and antioxidants; WBC, white blood cell count. Values are mean ± SE.

**Table 5.** Nutritional intake parameters

| | Control, n = 22 | EPA+GLA, n = 21 |
|---|---|---|
| Type of feeding, % | | |
| Nasogastric tube | 73 | 76 |
| Nasojejunal tube | 14 | 24 |
| Jejunostomy | 5 | 0 |
| Gastric tube | 4 | 0 |
| Permanent gastrostomy tube | 4 | 0 |
| Enteral formula[a] calories, kcal/day | 1350 ± 58 | 1317 ± 59 |
| Lipid, g/day | 86 ± 4 | 86 ± 4 |
| EPA, (g/day) | 0 | 5.3 ± 0.2 |
| DHA, (g/day) | 0 | 2.2 ± 0.1 |
| GLA, (g/day) | 0 | 4.4 ± 0.2 |
| Protein, g/day | 58 ± 3 | 56 ± 3 |
| Vitamin C, mg/day | 485 ± 19 | 867 ± 39[b] |
| Vitamin E, IU/day | 57 ± 2 | 318 ± 14[b] |
| β-carotene, μg/day | 0 | 5076 ± 230[b] |

1 EPA, eicosapentaenoic acid; 2 GLA, γ-linolenic acid; 3 DHA, docosahexaenoic acid.

[a]Represents mean of intake from study day 1 to study day 4; [b]$p < .01$, one-way analysis of variance. Values are mean ± SE.

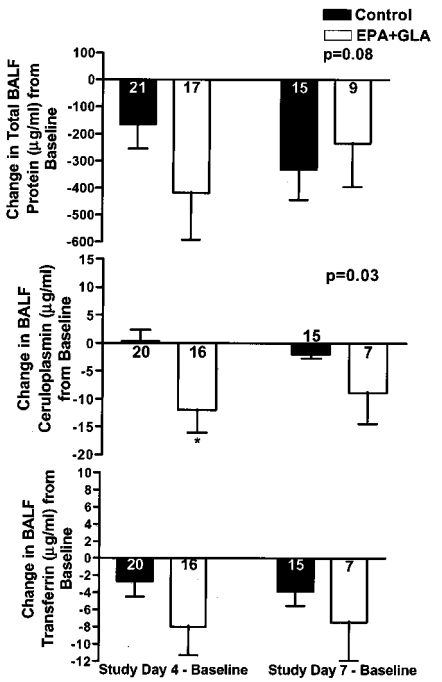


Figure 1. Changes on study days 4 and 7 from baseline in the absolute amounts of bronchoalveolar lavage fluid (*BALF*) total protein, ceruloplasmin, and transferrin. Numbers within *bars* indicate number of patients per group. Data are shown as mean ± SE. The *p* values are for the comparison between groups using repeated measures analysis of variance (total protein) and one-way analysis of variance (ceruloplasmin).

vs. controls, 386 ± 146 pg/mL, n = 21) and TNF-α (EPA+GLA, 75 ± 41 pg/mL, n = 17; vs. controls, 86 ± 41 pg/mL, n = 21) were similar for both groups ($p > .1$). Patients fed EPA+GLA tended to have a greater reduction in BALF IL-6 on study day 4 (54 ± 30 pg/mL, n = 16) as compared with control patients (196 ± 94 pg/mL, n = 20; $p = .1$). BALF IL-6 levels on study day 7 were similar for both groups (EPA+GLA, 117 ± 59 pg/mL, n = 9; vs. controls, 111 ± 48 pg/mL, n = 15; $p > .1$). BALF levels of TNF-α were similar in both groups on study day 4 (EPA+GLA, 81 ± 33 pg/mL, n = 17; vs. controls, 43 ± 23 pg/mL, n = 20; $p > .1$). There was a trend toward a greater reduction in BALF TNF-α observed on study day 7 in the EPA+GLA group (6 ± 6 pg/mL, n = 9) as compared with control patients (88 ± 36 pg/mL, n = 15; $p = .09$).

*Lung Neutrophil Recruitment and Gas Exchange.* The effects of EPA+GLA on pulmonary inflammation in this subset of ARDS patients was assessed by measuring total neutrophil counts in recovered BALF from patients at baseline, study day 4, and study day 7. Changes on study days 4 and 7 from baseline in BALF total neutrophil counts are shown in Figure 3. There were no significant baseline differences ($p > .1$) in total neutrophil counts × $10^3$/mL of recovered BALF for the EPA+GLA (64 ± 28, n = 17) and control (22 ± 4, n = 21) groups, respectively. Patients fed EPA+GLA tended to have a lower neutrophil count adjusted for volume of recovered BALF during the study period as compared with control patients ($p = .09$ by repeated measures analysis of variance).

At baseline, both groups of patients had similar compromised oxygenation status ($Pao_2/Fio_2$; $p > .1$), placing them in the ARDS category as previously defined (19) (Table 6). Patients fed EPA+GLA showed a significant increase from baseline in oxygenation status by study day 4 ($p = .03$). In contrast, patients fed the control diet had no improvement in $Pao_2/Fio_2$ on both study day 4 and day 7.

No significant differences were observed in ventilation and oxygenation variables at baseline between the groups ($p > .1$) (Table 6). Patients receiving EPA+GLA tended to have a higher $Pao_2$ on study days 4 and 7 as compared with controls. Patients given the control diet did not show an improvement in $Pao_2$, despite a maintenance in ventilator variables.

*Relationship Between Oxygenation and Indices of Lung Permeability and Inflammation.* Estimated Pearson's correlation coefficients between $Pao_2/Fio_2$, BALF neutrophil counts, total protein, ceruloplasmin, transferrin, IL-8, IL-6, $LTB_4$, and TNF-α are given in Table 7. There was a significant negative correlation between $Pao_2/Fio_2$ and BALF neutrophil count, suggesting that when pulmonary inflammation (BALF neutrophil count) was lowered, oxygenation ($Pao_2/Fio_2$) was increased. Markers of alveolar-capillary membrane protein permeability (BALF total protein, ceruloplasmin, and transferrin) were found to be significantly correlated to each other, indicating that when one of these proteins decreased in BALF, the others were also found to decrease. BALF neutrophil count was significantly correlated with BALF total protein, transferrin, and ceruloplasmin, suggesting that when lung inflammation decreased, alveolar-capillary membrane protein permeability was also decreased. Significant positive correlations were observed between BALF neutrophil counts and IL-8 and $LTB_4$, suggesting that when a reduction in lung inflammation was observed, BALF levels of IL-8 and $LTB_4$ were shown to decrease as well. No significant correlations were observed for BALF IL-6 and TNF-α.

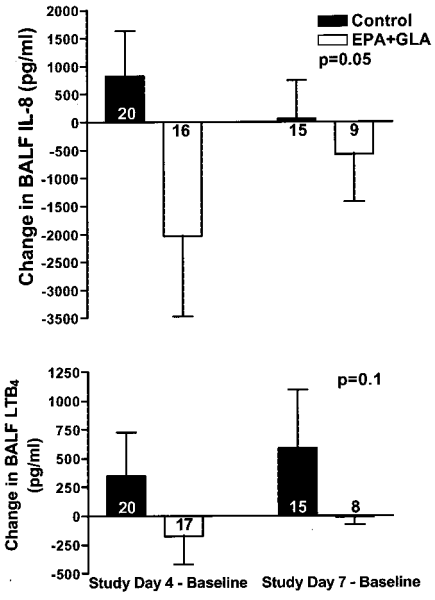


Figure 2. Changes on study days 4 and 7 from baseline in the absolute amounts of bronchoalveolar lavage fluid (*BALF*) interleukin-8 (*IL-8*) and leukotriene $B_4$ ($LTB_4$). Numbers within *bars* indicate number of patients per group. Data are shown as mean ± SE. The *p* values are for the comparison between groups using a repeated measures analysis of variance.

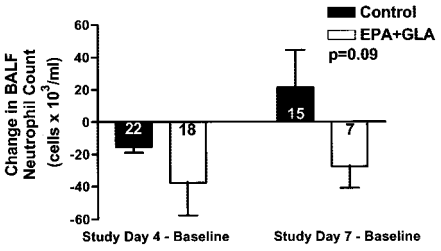


Figure 3. Effects of eicosapentaenoic acid, γ-linolenic acid, and elevated antioxidants (*EPA+GLA*) on pulmonary inflammation in this subset of patients was assessed by measuring total neutrophil counts in recovered bronchoalveolar lavage fluid (*BALF*). Numbers within *bars* indicate number of patients per group. Data are shown as mean ± SE. The *p* values are for the comparison between groups using a repeated measures analysis of variance.

## DISCUSSION

The goals of this preliminary study were to explore the potential mechanisms underlying the previously described clinical improvements in ARDS patients fed EPA+GLA (1). Results from this multicenter trial showed that patients fed EPA+GLA for at least 4 to 7 days had significantly reduced pulmonary inflammation, increased oxygenation, and improved clinical outcomes. ARDS is characterized by a massive inflammatory response associated with an accumulation of alveolar neutrophils that participate in the initiation and propagation of lung injury (34). We tested the hypotheses that enteral feeding with EPA+GLA could reduce alveolar-capillary membrane protein permeability and the production of selected BALF inflammatory mediators (IL-8, IL-6, $LTB_4$, and TNF-α). We also evaluated whether a relationship existed between measurements of pulmonary inflammation and indices of alveolar-capillary membrane protein permeability and oxygenation.

This study demonstrated that enteral nutrition in ARDS patients for at least 4 to 7 days with EPA+GLA as compared with a control diet: 1) significantly reduced BALF ceruloplasmin and IL-8, 2) tended to reduce BALF levels of total protein, neutrophils, and $LTB_4$, and 3) significantly improved oxygenation on study day 4 as measured by $Pao_2/Fio_2$. Correlation analyses revealed that BALF neutrophil count was significantly correlated with indices of alveolar-capillary membrane permeability, suggesting that when

**Table 6.** Mean ventilatory and oxygenation variables at baseline, and study days 4 and 7

| Variable | Control | | | EPA+GLA | | |
|---|---|---|---|---|---|---|
| | Baseline, n = 22 | Study Day 4, n = 21 | Study Day 7, n = 14 | Baseline, n = 21 | Study Day 4, n = 20 | Study Day 7, n = 10 |
| $Pao_2$, mmHg | 74 ± 3 | 75 ± 2 | 68 ± 3 | 75 ± 3 | 85 ± 6[a] | 76 ± 5[b] |
| $Fio_2$ | 0.47 ± 0.03 | 0.47 ± 0.03 | 0.42 ± 0.03 | 0.49 ± 0.02 | 0.43 ± 0.03 | 0.47 ± 0.03 |
| $Pao_2/Fio_2$ | 169 ± 13 | 170 ± 10 | 170 ± 12 | 160 ± 10 | 210 ± 19[c] | 165 ± 22 |
| $Paco_2$ mmHg | 37 ± 2 | 38 ± 1 | 39 ± 2 | 38 ± 1 | 41 ± 2 | 42 ± 4 |
| PEEP, cm $H_2O$ | 6.0 ± 0.9 | 4.6 ± 1.4 | 4.4 ± 1.5 | 5.3 ± 0.9 | 4.6 ± 1.2 | 3.9 ± 1.3 |
| PIP, cm $H_2O$ | 31.8 ± 1.7 | 32.5 ± 2.0 | 28.6 ± 1.7 | 30.8 ± 1.6 | 28.8 ± 2.0 | 30.4 ± 2.6 |
| Minute ventilation, L/min[d] | 10.5 ± 0.8 | 10.9 ± 1.1 | 11.0 ± 1.1 | 11.5 ± 1.0 | 10.5 ± 1.2 | 9.9 ± 1.3 |
| Tidal volume, mL | 657 ± 23 | 602 ± 18 | 567 ± 25 | 637 ± 17 | 589 ± 33 | 589 ± 53 |
| Tidal volume, mL/kg | 8.9 ± 0.5 | 8.1 ± 0.5 | 8.0 ± 0.7 | 9.1 ± 0.5 | 8.9 ± 0.8 | 9.1 ± 1.7 |

EPA+GLA, escosapentaenoic acid, γ-linolenic acid and antioxidants; PEEP, positive end-expiratory pressure; PIP, peak inspiratory pressure.

[a] $p = 0.08$ vs. control study day 4; [b] $p = 0.10$ vs. control study day 7; [c] $p < .05$ vs. control study day 4; [d] minute ventilation (L/min) = (tidal volume [liters]) × (total ventilation rate [breaths/min]). Values are mean ± SE.

**Table 7.** Pearson correlation coefficients examining the relationship between oxygenation and indices of lung permeability and inflammation

| | | Bronchoalveolar Lavage Fluid (BALF) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $Pao_2/Fio_2$ | Ceruloplasmin | Transferrin | Total Protein | IL-8 | IL-6 | $LTB_4$ | TNFα |
| $Pao_2/Fio_2$ | | | | | | | | |
| *r* | — | .07 | −.11 | — | −.18 | −.10 | −.02 | −.09 |
| *p* | — | .52 | .31 | — | .09 | .36 | .83 | .42 |
| n | — | 90 | 90 | — | 91 | 91 | 92 | 93 |
| BALF neutrophil count | | | | | | | | |
| *r* | −.27 | .23 | .34 | .33 | .60 | .08 | .23 | .07 |
| *p* | .007 | .02 | .001 | .001 | .001 | .42 | .02 | .51 |
| n | 103 | 96 | 96 | 99 | 96 | 96 | 97 | 98 |
| BALF total protein | | | | | | | | |
| *r* | −.03 | .77 | .87 | — | .12 | .20 | .03 | .05 |
| *p* | .81 | .001 | .001 | — | .25 | .05 | .75 | .61 |
| n | 94 | 96 | 96 | — | 97 | 97 | 98 | 99 |

IL, interleukin; $LTB_4$, leukotriene $B_4$; TNF, tumor necrosis factor.

lung inflammation decreased, alveolar protein influx was also decreased. IL-8 and $LTB_4$ were also significantly correlated with the observed reduction in pulmonary neutrophil recruitment.

Early pathologic changes in acute lung injury/ARDS include pulmonary neutrophil sequestration with subsequent injury to the alveolar-capillary barrier leading to increased pulmonary vascular permeability, progressive lung inflammation, and edema (35, 36). Normally, small amounts of plasma proteins reach the alveolar epithelial surface by a size-selective process that restricts the passage of very large molecules. However, size selectivity of plasma proteins have been found to be compromised in the lungs of ARDS patients (22). The increases in BALF protein most likely is due to a loss of integrity of the lung epithelial lining that results in a bulk flow of plasma proteins into the alveoli (22, 37). In this study, we found that ARDS patients had elevated baseline levels of BALF total protein, ceruloplasmin, and transferrin, confirming previous studies (22, 38, 39). Patients fed EPA+GLA had either a significant reduction or a trend toward reduction in these BALF proteins as compared with control patients, suggesting an improvement in the integrity of the endothelial-capillary barrier function. Although both ceruloplasmin and transferrin have antioxidant properties (38), it may be important in the ARDS patient to decrease lung edema and increase oxygenation at the expense of lowering endogenous antioxidants.

We tested the hypothesis that the decrease in alveolar-capillary membrane protein permeability in ARDS patients fed EPA+GLA may have resulted from modulation of the inflammatory response within the lung. Because evidence from experimental studies indicates that neutrophils are associated with altered alveolar permeability, we assessed changes in pulmonary neutrophil recruitment by measuring BALF neutrophil counts during the study. Patients who received EPA+GLA tended to have a lower number of neutrophils in recovered lavage fluid during the study as compared with control patients. These results are in agreement with previous animal (8) and clinical (1) reports showing a reduction in the level of pulmonary neutrophil recruitment and inflammation after enteral feeding with EPA+GLA.

Although neutrophils have received much attention as a key part of the common pathway underlying ARDS, proinflammatory eicosanoids and cytokines play a role in the inflammatory response through neutrophil influx and activation. In this study, we evaluated for the first time the effects of EPA+GLA on reducing BALF inflammatory mediators by assess-

ing the levels of IL-8, IL-6, TNF-α, and $LTB_4$ in BALF. We chose to measure these proinflammatory mediators because they have been found by several laboratories to be elevated in BALF of patients with ARDS. IL-8, IL-6, and $LTB_4$ serve to recruit and activate neutrophils and have been implicated with alterations in microvascular permeability. We chose to sample alveolar fluid because BAL is a relatively safe procedure (21) and the recovered alveolar fluid is a good reflection of the inflammatory process within the lung. Experimental studies have suggested that cytokine responses are compartmentalized among the alveoli, interstitium, and circulation and that analysis of plasma samples provides an incomplete reflection of the inflammatory events in the lungs (30, 32). During the severe inflammatory response with ARDS, this compartmentalization can be lost, thus making measurements of cytokines and eicosanoids in the lung more valuable than those performed in plasma or serum.

The most significant findings of our analyses showed that BALF from ARDS patients had elevated baseline levels of IL-8, IL-6, TNF-α, and $LTB_4$ that is consistent with other previously reported findings (26–33). However, patients fed EPA+GLA tended to have a greater reduction in BALF IL-8 and $LTB_4$ from baseline during the study as compared with the patients fed the control formula. This is an important finding given the fact that both inflammatory mediators are involved in the pathogenesis of ARDS by having potent neutrophil chemoattractant properties. $LTB_4$ also can promote the adherence of leukocytes to microvascular endothelial cells (40–42), thus increasing permeability of pulmonary microvessels (40, 43, 44). Previous studies have suggested that TNF-α and IL-6 play a role in initiating and mediating many of the host's nonspecific responses to inflammation. Patients fed EPA+GLA tended to have a greater decrease on study day 4 and study day 7 in BALF IL-6 and TNF-α, respectively. However the pattern of TNF-α and IL-6 levels in the lungs of patients with ARDS needs to be better understood before any definitive conclusions can be made as to the specific effects of EPA+GLA on their production.

In ARDS, a primary therapeutic goal is to increase oxygenation by decreasing pulmonary inflammation and permeability. There is convincing evidence that the severity of gas exchange abnormalities, as measured by $Pa_{O_2}/F_{IO_2}$ ratio, is correlated with the extent of neutrophil influx and the accumulation of inflammatory neutrophil products in the alveolar spaces (16, 45). In this study, we found a significant negative relationship between BALF neutrophil counts and $Pa_{O_2}/F_{IO_2}$, suggesting that when pulmonary inflammation (BALF neutrophil counts) was lowered, oxygenation ($Pa_{O_2}/F_{IO_2}$) was increased. Because the inflammatory process in ARDS patients has been associated with hypoxemia and pulmonary edema, we evaluated whether correlations existed between lung inflammation (BALF neutrophil counts) and indices of alveolar-capillary membrane permeability and BALF levels of IL-8, $LTB_4$, IL-6, and TNF-α. BALF neutrophil count was significantly correlated with total protein, ceruloplasmin, transferrin, IL-8, and $LTB_4$, suggesting that when there was a reduction in lung inflammation, an associated decrease in alveolar-capillary membrane permeability and inflammatory mediators was present. These findings demonstrate a central role for neutrophils in the pathogenesis of ARDS and suggest that interventions should be aimed at decreasing the inflammatory response in the lung.

Supportive care along with the increasing use of nutritional support in patients with acute lung injury/ARDS during the past 20 yrs has improved the rate of survival (46). Cytokines and eicosanoids possess potent inflammatory properties, and their regulation must be modulated to decrease an exaggerated inflammation in ARDS or bring the balance of anti-inflammatory and proinflammatory forces together. Thus far, clinical trials investigating the safety and efficacy of new pharmaceuticals aimed at blocking a single inflammatory mediator or cyclooxygenase products in ARDS have not proved successful (47). A variety of these agents include ibuprofen (48), prostaglandin $E_1$ (49, 50), prostacyclin (51, 52), antiendotoxin antibodies (53), IL-1 receptor antagonists (54, 55), anti-TNF antibodies (56), intravenous N-acetylcysteine (57, 58), nitric oxide (59), and Ketoconazole, a thromboxane $A_2$ synthetase inhibitor (60). Although this preliminary study's findings do not provide direct evidence for a cause and effect relationship between feeding EPA+GLA and improvements in alveolar-capillary membrane permeability and pulmonary inflammation, they do support the original assumptions of our hypothesis. Those assumptions are that the beneficial effects of EPA+GLA aids in restoring the balance between proinflammatory and anti-inflammatory forces during severe acute lung injury by modulating arachidonic acid metabolism while increasing the synthesis of more anti-inflammatory eicosanoids. In addition, we postulate that although it is necessary to modulate an exaggerated and persistent inflammatory response, one also needs to induce vasodilatory effects to optimize the improvements in protein permeability and oxygenation. Recent animal (7–10, 12–14) and clinical (1) studies have shown that nutrition support with diets containing EPA, DHA, or GLA allow for a means to decrease levels of arachidonic acid in immune cells, thereby reducing the production of inflammatory mediators and improving clinical outcomes.

Several caveats merit discussion with regard to our findings. The intent of this study was to explore the hypothesis that enteral feeding with EPA+GLA could reduce alveolar-capillary membrane protein permeability and the production of BALF inflammatory mediators using a subset of patients from the original multicenter trial (1). One limitation with this approach is that this subanalysis was not specifically powered to fully explore the potential mechanisms underlying the outcome benefits observed in the above trial. Although not all changes in BALF measurements reached statistical significance, all indices of pulmonary inflammation and alveolar-capillary membrane protein permeability were consistently lower in patients fed EPA+GLA as compared with the control diet. The importance of these findings were further brought to light with the significant correlation analyses showing the relationship between pulmonary inflammation and measures of oxygenation and alveolar-capillary membrane permeability that is consistent with other studies. Due to the small sample size, it was also not our intent to perform subanalyses on specific patients or attempt to identify inflammatory markers that can predict outcome. A larger clinical trial specifically designed and powered for those outcomes would be required. Although it is reasonable to postulate that patients fed EPA+GLA reduced pulmonary inflammation via reductions in IL-8 and $LTB_4$, we did not further assess the complex balance between proinflammatory and anti-inflammatory mediators by measuring other cy-

tokines, such as epithelial cell-derived neutrophil activator-78, monocyte chemoattractant protein-1, macrophage inhibitory factor, and IL-10. Therefore, we cannot make conclusive statements that EPA+GLA modulated all aspects of bioactivity of BALF inflammatory mediators. It would be beneficial in future studies to include these cytokines and their naturally occurring inhibitors or antagonists.

Although our data suggest anti-inflammatory benefits of EPA+GLA, this diet may have modulated proinflammatory gene expression and certain transcription factors such as activator protein-1 and nuclear factor kappa-β. Nuclear factor kappa-β is an essential transcription factor that regulates gene expression of IL-6, IL-8, and TNF-α (61). The activation of nuclear factor kappa-β has been associated with lung injury in lipopolysaccharide-treated rats (62) and ARDS patients (63). There exists accumulating evidence suggesting that n-3 fatty acids (EPA, DHA) can regulate gene expression via activation of transcription factors, such as peroxisome proliferator-activated receptor-α and sterol regulatory element-binding proteins (64), or by decreasing the expression of cyclooxygenase COX-2, IL-1, and TNF-α messenger RNAs (65). Whether these transcription factors are affected by adding GLA and elevated antioxidants to EPA and DHA (EPA+GLA diet) remains to be elucidated. Therefore, assessing the effects of EPA+GLA on molecular events such as modulation of proinflammatory gene expression and certain transcription factors (activator protein-1 and nuclear factor kappa-β) would expand our knowledge base.

## CONCLUSIONS

Collectively, we have presented novel information on the physiologic mechanisms whereby enteral feeding with a diet supplemented with EPA, DHA, GLA and elevated antioxidants (EPA+GLA) in patients with acute lung injury/ARDS, can modulate pulmonary inflammation through a decrease in BALF levels of IL-8 and $LTB_4$. The associated reduction in BALF neutrophils is a reasonable explanation for the observed improvements in alveolar-capillary membrane protein permeability and gas exchange seen in EPA+GLA patients. A larger clinical trial specifically designed to further examine the above conclusions is warranted. However, the linkage of these biochemical markers of lung injury with the previously described clinical improvements in ARDS patients fed EPA+GLA (1) suggests that this enteral nutrition formula would be a useful adjuvant therapy in the clinical management of patients with lung inflammation.

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