Supplementation of parenteral nutrition with fish oil attenuates acute lung injury in a rat model

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Fish oil rich in *n*-3 polyunsaturated fatty acids has diverse immuno-modulatory properties and attenuates acute lung injury when administered in enteral nutrition. However, enteral nutrition is not always feasible. Therefore, we investigated the ability of parenteral nutrition supplemented with fish oil to ameliorate acute lung injury. Rats were infused with parenteral nutrition solutions (without lipids, with soybean oil, or with soybean oil and fish oil) for three days. Lipopolysaccharide (15 mg/kg) was then administered intratracheally to induce acute lung injury, characterized by impaired lung function, polymorphonuclear leukocyte recruitment, parenchymal tissue damage, and upregulation of mRNAs for inflammatory mediators. Administration of parenteral nutrition supplemented with fish oil prior to lung insult improved gas exchange and inhibited neutrophil recruitment and upregulation of mRNAs for inflammatory mediators. Parenteral nutrition supplemented with fish oil also prolonged survival. To investigate the underlying mechanisms, leukotriene B_4 and leukotriene B_5 secretion was measured in neutrophils from the peritoneal cavity. The neutrophils from rats treated with fish oil-rich parenteral nutrition released significantly more leukotriene B_5, an anti-inflammatory eicosanoid, than neutrophils isolated from rats given standard parenteral nutrition. Parenteral nutrition with fish oil significantly reduced lipopolysaccharide-induced lung injury in rats in part by promoting the synthesis of anti-inflammatory eicosanoids.

Key Words: omega-3 fatty acids, nutritional support, acute lung injury, rat model, fish oil

P arental nutrition (PN) is important to supply adequate calories to critically ill patients and is particularly important in patients with intestinal failure.[1,2] Lipid emulsions containing soybean oil-based *n*-6 polyunsaturated fatty acids (PUFAs) have been traditionally used as a standard intravenous PN supplement. This practice may not be optimal because it may cause an excess of linoleic acid. Meta-analyses have suggested that inclusion of lipids in PN might be detrimental, at least very ill patients; however, most of the studies included in the meta-analysis used soybean oil-based lipid emulsions.[3] Alternatives to soybean oil include its partial replacement by fish oil, which is rich in *n*-3 PUFAs, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Acute lung injury (ALI) can lead to morbidity and mortality in the intensive care unit (ICU) and is characterized by neutrophilic lung inflammation, permeability, and intravascular and alveolar fibrin deposition.[4] Clinical and experimental studies have demonstrated that enteral nutrition containing fish oil has diverse immunomodulatory properties and attenuates inflammatory events, including ALI, resulting in improved clinical outcomes of severely ill patients.[5–11] Lipid emulsions containing fish oil are well established and already commercially available, however, the emulsions are still undergoing trials for use in different patient populations. Additionally, the clinical efficacies of fish oil administered intravenously (such as in PN) and the mechanism by which fish oil elicits protective effects have not been fully elucidated.[12] Herein, we investigated the efficacy of PN emulsions supplemented with fish oil and determined if supplementing PN with fish oil can ameliorate ALI using an established ALI model.

Materials and Methods

Animals. Five weeks old male Sprague-Dawley rats were kept in individual stainless steel cages in a temperature, humidity and light-controlled room ($23 \pm 3°C$, $55 \pm 15\%$; 12-h light-dark cycle) for 3 weeks before the experiments. During this period, all animals were provided standard food (AIN-93G diet; Oriental Kobo Corporation, Tokyo, Japan) and free access to water. All procedures involving rats were conducted in accordance with the guidelines of the Animal Care and Use Committees of the Hyogo College of Medicine and Otsuka Pharmaceutical Factory, Inc. and complied with the National Research Council's Guide for the Humane Care and Use of Laboratory Animals.

Animal model. Under general anesthesia, catheters for PN were placed into the rats' external jugular veins. Thereafter, rats were then infused with PN solution (380 ml/kg/day; 270 kcal/kg/day) for 3 days. Basic PN solutions consisted of 11% (w/v) glucose, 2.9% (w/v) amino acids, electrolytes and vitamins; lipid emulsions were supplemented according to the experimental protocol. During PN administration, the animals were fasted and deprived of water. Because the most common cause of ALI in humans is sepsis, intratracheal administration of gram-negative bacterial endotoxin lipopolysaccharide (LPS) was used as an animal model of sepsis-related lung injury in this study. To create a lung injury, 15 mg/kg of lipopolysaccharide (LPS, *Escherichia coli*. O111: B4, Sigma, St. Louis, MO) was administered intratracheally after 3 days of PN. Preliminary studies revealed that topical instillation of 30 µg LPS into the trachea induced consistent and maximal alveolar cell injury in the instilled lung causing mortality within 24 h. Sham control rats were administered saline instead of LPS. Two hours before the administration of LPS, the infusion of PN was ceased and the lactate ringer's solution (LR) was infused at 10 ml/kg/h. LR infusion was continued after LPS administration. Rats were sacrificed 3 h or 24 h after LPS administration, and lung tissue and blood samples were harvested. Separate rats were used for analysis of survival. At sampling, the lungs were excised

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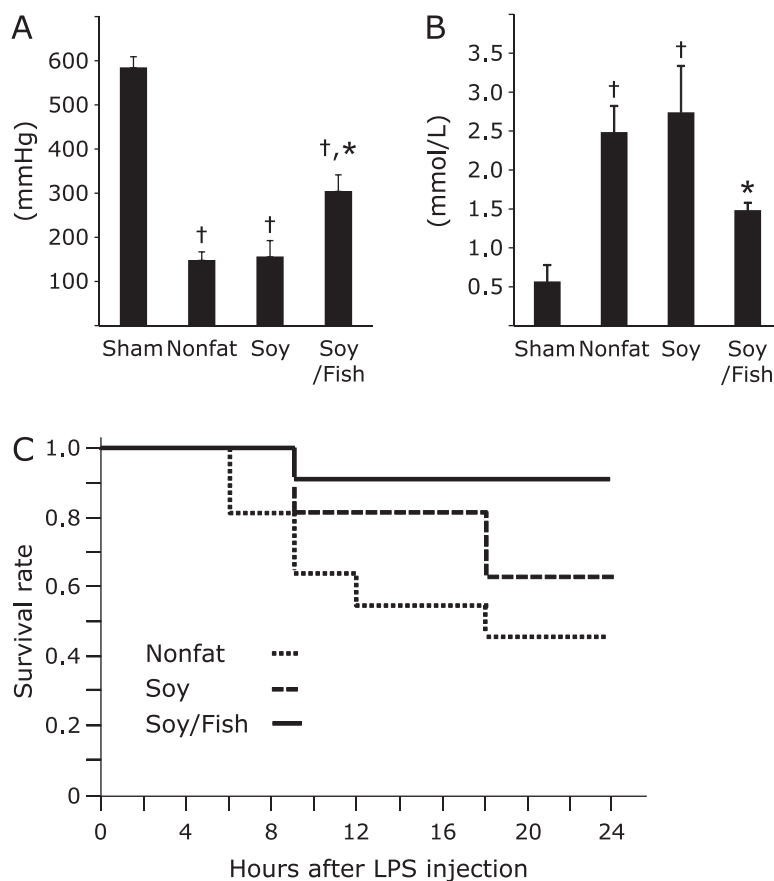
Fig. 1. (A) Blood oxygenation levels in blood drawn from the abdominal aorta of rats after 24 h of exposure to LPS ($n = 6$ for each group, *$p<0.05$ vs nonfat; †$p<0.05$ vs sham), (B) Blood lactate levels in blood after 24 h of exposure to LPS ($n = 6$ for each group, *$p<0.05$ vs nonfat; †$p<0.05$ vs sham), (C) Animal survival after LPS treatment ($n = 11$ for each group, Log-Rank $p = 0.0445$ vs nonfat, Wilcoxon $p = 0.0469$ vs nonfat).

and divided into two sections. The right lobe was snap frozen immediately to liquid nitrogen for further analysis. The left lobe of the lungs was used for histologic examination and immuno-staining.

Experimental groups. The rats were assigned to four experimental groups. One group (nonfat) was given PN solution without lipids (lipids calories were compensated by the increase of glucose). The second group (soybean oil) was given PN containing 2.7% (w/v) soybean oil (Intralipos®, Otsuka, Tokyo, Japan). The third group (soy/fish) was given PN containing 1.6% (w/v) soybean oil and 1.1% (w/v) fish oil emulsion (Omegaven®, Fresenius-Kabi, Bad Homburg, Germany) (mixed n-6 PUFAs and n-3 PUFAs; ratio 1.4:1). The control group (control/sham) was given PN supplemented with soy/fish oil but was given sham treatment (saline administration) instead of LPS administration. The PN solutions were prepared to contain the same calories.

Assessment of gas exchange function and blood lactate levels. Partial pressure of oxygen (PO2) and blood lactate levels were assessed by analysis of blood gases (Rapidpoint 500, Siemens Healthcare Diagnostics Inc., Frimley, Camberley, UK) on a fraction of inspired oxygen of 1.0 in blood drawn from the abdominal aorta 5 min after oxygen inspiration was initiated.

Histopathology. Recruitment maneuvers were performed immediately after thoracotomy to insure that the lungs were free of macroscopic atelectasis. After preparation, the lungs were fixed by inflation with buffered 4% paraformaldehyde for 6 h. After embedding in paraffin, the sections were prepared and stained with hematoxylin and eosin (H&E). Polymorphonuclear neutro-

phils (PMNs) were stained using a naphthol AS-D chloroacetate esterase staining kit (Sigma Diagnostics, St. Louis, MO) and identified by nuclear morphology stained in bright red. Acute lung injury was blindly scored according to previously described criteria,[13] specifically 1) thickness of the alveolar wall, 2) infiltration or aggregation of neutrophils in air space, alveolar wall, or vessel wall, and 3) alveolar congestion. Each item was graded on a four-point scale (0 to 3), with higher scores indicating more severe damage. ALI scores were assessed in 10 high-power fields per sample.

SYBR green real-time RT-PCR. Rat mRNAs were quantified in duplicate using SYBR Green two-step, real-time RT-PCR, as previously described.[14–16] The following mRNAs were quantitated: rat tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM)-1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Optimized and validated primer sets were obtained from realtimeprimers.com (Elkins Park, PA).

Culture of peritoneal exudative neutrophils and measurement of leukotrienes. To measure the leukotriene (LT)-synthesizing capacity of the PMNs, neutrophils from the peritoneal cavity (>90%) were isolated from rats that received each type of PN (without fat emulsion, soybean oil, and soybean and fish oil) for 3 days.[17] PMNs were resuspended in Hanks' balanced salt solution (HBSS, ~4×10^7 cells/ml) and kept for 10 min at 37°C. The calcium ionophore A23187 was added to a final concentration of 12.5 μM, and the mixture was incubated for 10 min. The sample was then immediately centrifuged to remove the cells, and LTs in the supernatants were quantified by using a previously
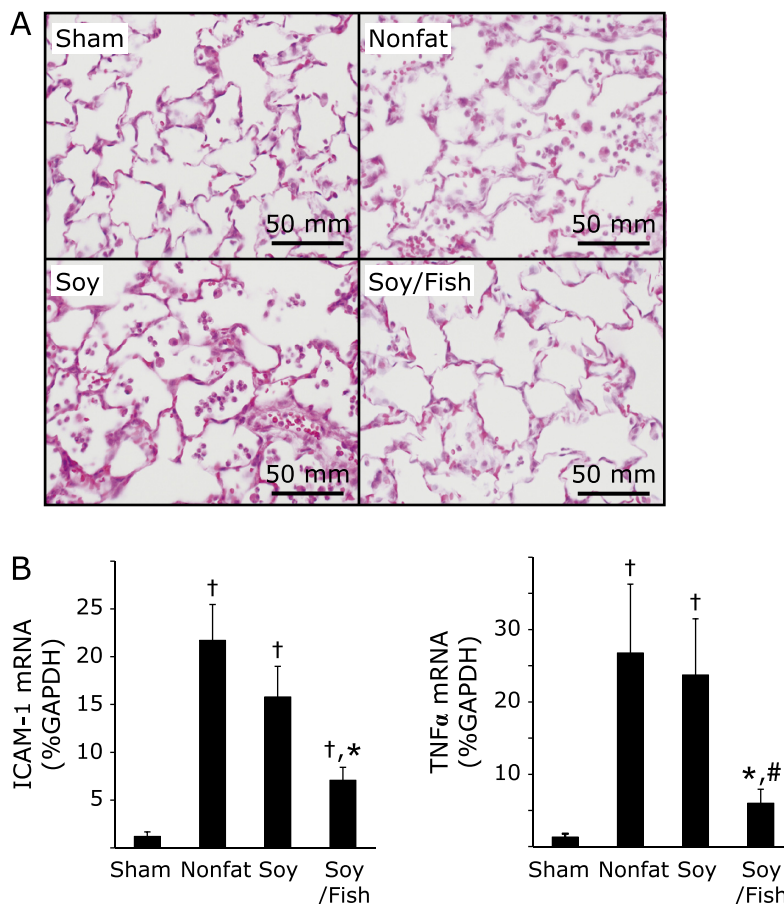
Fig. 2. (A) Representative images of H&E staining in the lung. Inflammatory cells have migrated into the alveolar spaces and there are destructive changes in the alveolar walls 24 h after LPS instillation in the sham, nonfat and soy groups. Images are representative of 5 different sections examined from independent animals, (B) Real time RT-PCR determination of ICAM-1 and TNFα mRNA levels in the lungs 3 h after LPS administration ($n = 5$, *$p<0.05$ vs nonfat; †$p<0.05$ vs sham; #$p<0.05$ vs soybean).

reported method.[17] Briefly, 13,14-dihydro-15-keto-PGE$_2$ (100 ng/sample) was added to the supernatant as an internal standard and citrate buffer was added to adjust the pH to 3.0. The solution was applied to a C18-silica column (GL Sciences Inc., Tokyo), and the LTs were eluted with ethyl acetate:methanol (9:1, vol/vol). The eluent was concentrated under nitrogen, and an aliquot of the residual mixture was used for the quantitation of LTs by high-performance liquid chromatography (HPLC).

Statistical analysis. Results are expressed as mean ± SEM. Parametric data were analyzed with one-way analysis of variance followed by *post hoc* analysis with the Bonferroni correction. The lung injury score was analyzed with the Mann-Whitney *U* test with the Bonferroni correction. The cell count was analyzed using Tukey-Kramer methods. JMP version 8 (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

Fish oil supplementation alleviated acute lung injury as determined by blood gas analysis. Exposure to LPS impaired lung function, as evident by a remarkable decrease in PO$_2$ 24 h after LPS administration in rats given PN (with or without soybean oil) as compared with sham control rats. Supplementation of PN with fish oil significantly increased PO$_2$ levels 24 after LPS injection (Fig. 1A). There was no significant difference in the partial pressure of carbon dioxide (PCO$_2$) or blood pH between the groups (data not shown).

Fish oil supplementation reduced blood lactate concentration. Blood lactate levels of the rats given PN without lipids notably increased 24 h after LPS treatment. While supplementation of the PN with soybean fat emulsion did not change blood lactate levels elevated by LPS administration, blood lactate levels did not increase in LPS-treated rats receiving PN supplemented with soy and fish oils (Fig. 1B).

Fish oil supplementation enhanced survival. Supplementation of the PN with fish oil was remarkably effective in prolonging animal survival. Overall survival 24 h after LPS administration was 45.5% (5/11) in rats that received PN without lipids. While soybean oil supplementation did not significantly change survival (63.6% after 24 h, 7/11), supplementation with soy and fish oil significantly prolonged animal survival with 91% (10/11) surviving 24 h after LPS treatment (Fig. 1C).

Fish oil supplementation reduced ALI as determined by histologic analysis. In histologic analysis, the lungs of rats treated with intratracheal LPS showed marked cellular infiltration, edema in the interstitial area, and associated thickening of the alveolar septum. Supplementation of PN with fish oil/*n*-3 PUFA reduced both edema and inflammatory cell infiltration (Fig. 2A). The mean ALI score of the rats given PN without lipids was 7.8 ± 1.0, and mean ALI score of the rats given PN with soybean oil was 7.7 ± 0.6. In contrast, the ALI score of the rats given PN supplemented with soybean oil and fish oil was 4.7 ± 0.6, and was significantly lower than in the other experimental groups (*p*<0.05 vs without lipids and soybean).
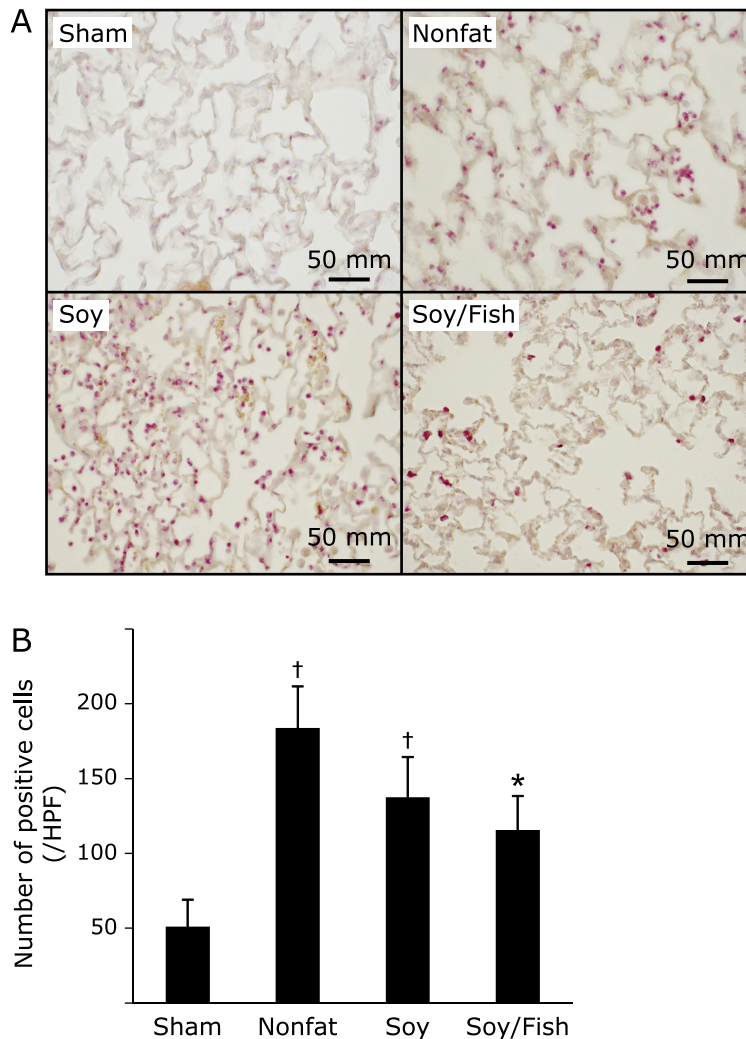
Fig. 3. Upper panels indicate representative images of naphthol AS-D chloroacetate esterase staining of lungs 24 h after LPS administration. Infiltrated neutrophils are stained red (original magnification ×400). Histogram indicates the number of positive cells ($n = 5$, *$p<0.05$ vs nonfat; †$p<0.05$ vs sham).

Fish oil inhibited upregulation of proinflammatory mediators in the lungs. Next, we evaluated the effects supplementing PN with fish oil on proinflammatory cytokine expression in the lung. Three hours after LPS inoculation, the mRNAs for TNFα and ICAM-1 were significantly upregulated. PN supplemented with soybean oil did not alter the expression of these genes; however, supplementation with fish oil significantly reduced the peak expression of the transcripts for these inflammatory mediators (Fig. 2B).

Fish oil supplemented PN reduced PMN infiltration after ALI. PMNs are the predominant infiltrating cells in the lung during LPS-induced ALI. While there were scarce PMNs in the sham-treated lungs, intratracheal administration of LPS increased PMN infiltration in the lung within 24 h. Massive neutrophil accumulation in the alveolar space, alveolar capillary congestion, and exudation in the lungs was also seen in the rats given PN with soybean oil emulsion and then treated with LPS. In contrast, PN supplemented with fish oil reduced neutrophil infiltration into the alveolar space after LPS administration (Fig. 3).

Fish oil increased neutrophil LTB₅ production. To further investigate the mechanisms underlying the protective effects of supplementing PN with fish oil, the concentrations of LTB$_4$ and LTB$_5$ secreted by the migrated neutrophils were measured using neutrophils isolated from the peritoneal cavity of rats given PN. The neutrophils isolated from rats treated with fish oil-rich PN released significantly more LTB$_5$, which is a known anti-inflammatory eicosanoid, when exposed to the calcium ionophore A23187 than neutrophils isolated from rats given PN with soybean oil or PN without lipids (Fig. 4). In contrast, the production of LTB$_4$, a proinflammatory product of n-6 PUFAs, in neutrophils isolated from rats treated with PN supplemented with fish oil was similar to that seen in rats given PN without lipids or PN supplemented with soybean oil.

Discussion

Our study demonstrated that supplementing PN with fish oil significantly reduced LPS-induced ALI in a rat model, as determined by reduction of lung inflammation and improvement of gas exchange, at least in part by increasing the ability of PMNs to secrete anti-inflammatory eicosanoids. Because unbalanced host defense mechanisms result in complications, such as sepsis, the development of immunomodulatory supportive strategies is required despite considerable progress in prophylaxis with modern antibiotics. An "immune-enhancing" enteral diet is a promising option.[18] In fact, clinical and experimental studies have demon-
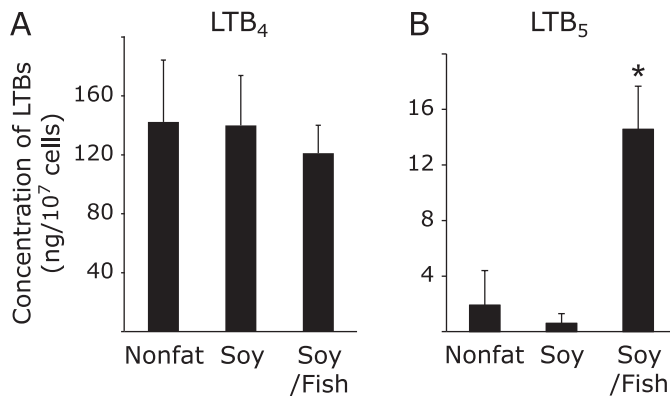
Fig. 4. The production of LTB$_4$ (A) and LTB$_5$ (B) in response to calcium ionophore treatment in neutrophils isolated from rats given different types of PN ($n = 7$ for each group, $*p < 0.05$ vs nonfat and vs soy).

strated that oral intake of fatty acids had beneficial effects for ICU patients and was associated with attenuation of ALI.[12,19–21] However, enteral nutrition is sometimes not feasible, particularly in patients with intestinal failure or impaired gut absorbance function. Intravenously injected fatty acids (such as those used to supplement PN) may directly reach their site of action without loss during digestion and may be clinically advantageous for patients in the ICU.

Several recent clinical studies provided evidence of the beneficial effects of supplementation of fish oil-based, long-chain *n*-3 PUFAs in PN for critically ill patients.[6,22,23] Archad *et al.*[24] reported beneficial effects of an intravenous, omega-3-rich infusion for patients receiving chemotherapy with advanced pancreatic cancer. However, there are also conflicting reports demonstrating that enteral feeding of fish oil does not improve the clinical outcomes of acute lung disease in humans.[21,25] Some studies even suggest that fish oil may lead to serious complications such as cholestasis, occurring particularly in infants and children with intestinal failure.[26,27] Thus, further studies are warranted to determine the efficacy of fish oil, the optimal ratio of soybean/fish oil, and molecular mechanisms involved in the beneficial effects conferred by fish oil supplemented into either PN or enteral nutrition.

PUFAs are classified into *n*-3 and *n*-6 systems based on the position of the first double bond from the methyl group end and become the base of various bioactive lipid mediators.[28] PUFAs are taken in by the cell membrane, and are metabolized to LTs that exert dual functions. LTs are known potent inflammatory lipid mediators, are involved in hypersensitivity and respiratory disorders, and exert immunoregulatory activities. *n*-6 PUFA products, including LTB$_4$, generally promote inflammation, function as potent chemotactic factors for PMN leukocytes, and induce superoxide generation and degranulation of neutrophils.[29] On the other hand, *n*-3 PUFAs products, including LTB$_5$, which compete with *n*-6 PUFAs products for access to the cyclooxygenase and lipoxygenase enzymes, cause biological responses opposite to those caused by the *n*-6 PUFAs.[30] The proinflammatory biological activities of LTB$_5$ are very minor, less than 100-fold in comparison to LTB$_4$.[31,32] Fish oil decreases production of *n*-6 PUFA-derived mediators, such as PGE$_2$ and LTB$_4$, as a result of the reduced *n*-6 PUFA content of cell membranes, and increases production of anti-inflammatory eicosanoid mediators such as LTB$_5$ from EPA.[12] We previously demonstrated that *n*-3 PUFA-rich PN regulated neutrophil apoptosis and prevented synthesis of pro-inflammatory eicosanoids, partially explaining the protective effects seen in the clinical setting.[33] Our results are also consistent with previous studies suggesting that utilizing fish oil to partly replace soybean oil in PN may both decrease the amount of linoleic acid and increase the amount of biologically-active *n*-3 PUFA. In clinical studies of patients with sepsis who were intolerant of EN and received PN with either standard soybean oil-based emulsion or an emulsion containing fish oil, blood leukocyte counts and serum C-reactive protein concentrations were lower and production of LTB$_5$ by stimulated neutrophils was much higher in patients receiving fish oil.[9,34]

Acute inflammation is often noted during ALI and acute respiratory distress syndrome. The pathophysiology of ALI involves inflammation with diffuse alveolar damage, increased capillary permeability, interstitial and alveolar edema, structural damage of the type II alveolar epithelial cells, and influx of circulating inflammatory cells. Reduction of the lamellar structure in the osmiophilic bodies may impact the release of pulmonary surfactant and, thus, affect lung function. The pathogenesis of ALI may result from massive activation of the proinflammatory response, including release of inflammatory mediators, such as eicosanoids.[35] LPS, the main component of the cell wall of gram-negative bacteria, can induce marked acute pulmonary inflammation and lead to further systemic inflammatory response syndrome or sepsis. Low-dose exposure to LPS activates macrophages and PMN cells, presumably to eliminate the toxic agents, whereas higher doses on LPS cause tissue injury.[36] Experimental administration of LPS, often through intratracheal injection, is used to create acute lung inflammation in animal models.[37,38] Although TNFα and ICAM-1 are not in the final common pathway leading to shock and tissue injury, both ICAM-1 and TNFα are activated in LPS-induced ALI.[39–42] Our data clearly demonstrated that supplementation of PN with fish oil significantly inhibited the upregulation of mRNAs for ICAM-1 and TNFα in the lung with LPS-induced ALI.

In conclusion, using an LPS-induced rat lung inflammation model, this study demonstrated that fish oil supplemented PN reduces ALI. Fish oil may act as an anti-inflammatory agent, at least in part, by reducing the expression of pro-inflammatory cytokines, such as TNFα and ICAM-1, and altering the balance of LTB$_4$ and LTB$_5$.

Abbreviations

ALI	acute lung injury
DHA	docosahaenoic acid
EPA	eicosapentaenoic acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
H&E	hematoxylin and eosin
ICAM	intercellular adhesion molecule
ICU	intensive care unit
LPS	lipopolysaccharide
LT	leukotriene
PMNs	polymorphonuclear neutrophils
PN	parental nutrition
PO$_2$	partial pressure of oxygen
PUFAs	polyunsaturated fatty acids
TNF	tumor necrosis factor

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 McClave SA, Martindale RG, Vanek VW, *et al*. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr* 2009; **33**: 277–316.

2 Singer P, Shapiro H, Bendavid I. Behind the ESPEN Guidelines on parenteral nutrition in the ICU. *Minerva Anestesiol* 2011; **77**: 1115–1120.

3 Heyland DK, MacDonald S, Keefe L, Drover JW. Total parenteral nutrition in the critically ill patient: a meta-analysis. *JAMA* 1998; **280**: 2013–2019.

4 Bernard GR. Acute respiratory distress syndrome: a historical perspective. *Am J Respir Crit Care Med* 2005; **172**: 798–806.

5 Carpentier YA, Hacquebard M, Portois L, Dupont IE, Deckelbaum RJ, Malaisse WJ. Rapid cellular enrichment of eicosapentaenoate after a single intravenous injection of a novel medium-chain triacylglycerol:fish-oil emulsion in humans. *Am J Clin Nutr* 2010; **91**: 875–882.

6 Köller M, Senkal M, Kemen M, König W, Zumtobel V, Muhr G. Impact of omega-3 fatty acid enriched TPN on leukotriene synthesis by leukocytes after major surgery. *Clin Nutr* 2003; **22**: 59–64.

7 Schrepf R, Limmert T, Claus Weber P, Theisen K, Sellmayer A. Immediate effects of *n*-3 fatty acid infusion on the induction of sustained ventricular tachycardia. *Lancet* 2004; **363**: 1441–1442.

8 König D, Berg A, Weinstock C, Keul J, Northoff H. Essential fatty acids, immune function, and exercise. *Exerc Immunol Rev* 1997; **3**: 1–31.

9 Mayer K, Meyer S, Reinholz-Muhly M, *et al*. Short-time infusion of fish oil-based lipid emulsions, approved for parenteral nutrition, reduces monocyte proinflammatory cytokine generation and adhesive interaction with endothelium in humans. *J Immunol* 2003; **171**: 4837–4843.

10 Heller AR, Rössler S, Litz RJ, *et al*. Omega-3 fatty acids improve the diagnosis-related clinical outcome. *Crit Care Med* 2006; **34**: 972–979.

11 Linseisen J, Hoffmann J, Lienhard S, Jauch KW, Wolfram G. Antioxidant status of surgical patients receiving TPN with an omega-3-fatty acid-containing lipid emulsion supplemented with alpha-tocopherol. *Clin Nutr* 2000; **19**: 177–184.

12 Calder PC. *n*-3 fatty acids, inflammation, and immunity—relevance to post-surgical and critically ill patients. *Lipids* 2004; **39**: 1147–1161.

13 Kawamura T, Huang CS, Peng X, *et al*. The effect of donor treatment with hydrogen on lung allograft function in rats. *Surgery* 2011; **150**: 240–249.

14 Nakao A, Kimizuka K, Stolz DB, *et al*. Carbon monoxide inhalation protects rat intestinal grafts from ischemia/reperfusion injury. *Am J Pathol* 2003; **163**: 1587–1598.

15 Kohmoto J, Nakao A, Kaizu T, *et al*. Low-dose carbon monoxide inhalation prevents ischemia/reperfusion injury of transplanted rat lung grafts. *Surgery* 2006; **140**: 179–185.

16 Itoh T, Itoh A, Pleasure D. Bcl-2-related protein family gene expression during oligodendroglial differentiation. *J Neurochem* 2003; **85**: 1500–1512.

17 Hagi A, Nakayama M, Shinzaki W, Haji S, Ohyanagi H. Effects of the omega-6:omega-3 fatty acid ratio of fat emulsions on the fatty acid composition in cell membranes and the anti-inflammatory action. *JPEN J Parenter Enteral Nutr* 2010; **34**: 263–270.

18 Moore FA, Moore EE, Kudsk KA, *et al*. Clinical benefits of an immune-enhancing diet for early postinjury enteral feeding. *J Trauma* 1994; **37**: 607–615.

19 Singer P, Shapiro H. Enteral omega-3 in acute respiratory distress syndrome. *Curr Opin Clin Nutr Metab Care* 2009; **12**: 123–128.

20 Mancuso P, Whelan J, DeMichele SJ, Snider CC, Guszcza JA, Karlstad MD. Dietary fish oil and fish and borage oil suppress intrapulmonary proinflammatory eicosanoid biosynthesis and attenuate pulmonary neutrophil accumulation in endotoxic rats. *Crit Care Med* 1997; **25**: 1198–1206.

21 Rice TW, Wheeler AP, Thompson BT, deBoisblanc BP, Steingrub J, Rock P; NIH NHLBI Acute Respiratory Distress Syndrome Network of Investigators. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 2011; **306**: 1574–1581.

22 Grimm H, Mertes N, Goeters C, *et al*. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. *Eur J Nutr* 2006; **45**: 55–60.

23 Morlion BJ, Torwesten E, Lessire H, *et al*. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. *Metabolism* 1996; **45**: 1208–1213.

24 Arshad A, Chung W, Isherwood J, Steward W, Metcalfe M, Dennison A. Restoration of mannose-binding lectin complement activity is associated with improved outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous ω-3 fish oil. *JPEN J Parenter Enteral Nutr* 2013; in press.

25 Stapleton RD, Martin TR, Weiss NS, *et al*. A phase II randomized placebo-controlled trial of omega-3 fatty acids for the treatment of acute lung injury. *Crit Care Med* 2011; **39**: 1655–1662.

26 Koletzko B, Goulet O. Fish oil containing intravenous lipid emulsions in parenteral nutrition-associated cholestatic liver disease. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 321–326.

27 de Meijer VE, Gura KM, Le HD, Meisel JA, Puder M. Fish oil-based lipid emulsions prevent and reverse parenteral nutrition-associated liver disease: the Boston experience. *JPEN J Parenter Enteral Nutr* 2009; **33**: 541–547.

28 Wanten GJ, Calder PC. Immune modulation by parenteral lipid emulsions. *Am J Clin Nutr* 2007; **85**: 1171–1184.

29 Gaudreault E, Thompson C, Stankova J, Rola-Pleszczynski M. Involvement of BLT1 endocytosis and Yes kinase activation in leukotriene B4-induced neutrophil degranulation. *J Immunol* 2005; **174**: 3617–3625.

30 Rola-Pleszczynski M, Stanková J. Leukotriene B4 enhances interleukin-6 (IL-6) production and IL-6 messenger RNA accumulation in human monocytes *in vitro*: transcriptional and posttranscriptional mechanisms. *Blood* 1992; **80**: 1004–1011.

31 Lee TH, Menica-Huerta JM, Shih C, Corey EJ, Lewis RA, Austen KF. Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B5 and the double lipoxygenase product. *J Biol Chem* 1984; **259**: 2383–2389.

32 Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010; **68**: 280–289.

33 Terashima M, Aoyama-Ishikawa M, Ueda T, *et al*. The effects of *n*-3 polyunsaturated fatty acid-rich total parenteral nutrition on neutrophil apoptosis in a rat endotoxemia. *J Clin Biochem Nutr* 2013; **52**: 154–159.

34 Mayer K, Fegbeutel C, Hattar K, *et al*. Omega-3 vs. omega-6 lipid emulsions exert differential influence on neutrophils in septic shock patients: impact on plasma fatty acids and lipid mediator generation. *Intensive Care Med* 2003; **29**: 1472–1481.

35 Pontes-Arruda A, Aragão AM, Albuquerque JD. Effects of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in mechanically ventilated patients with severe sepsis and septic shock. *Crit Care Med* 2006; **34**: 2325–2333.

36 Lentsch AB, Czermak BJ, Bless NM, Van Rooijen N, Ward PA. Essential role of alveolar macrophages in intrapulmonary activation of NF-kappaB. *Am J Respir Cell Mol Biol* 1999; **20**: 692–698.

37 Altemeier WA, Matute-Bello G, Gharib SA, Glenny RW, Martin TR, Liles WC. Modulation of lipopolysaccharide-induced gene transcription and promotion of lung injury by mechanical ventilation. *J Immunol* 2005; **175**: 3369–3376.

38 Gharib SA, Liles WC, Matute-Bello G, Glenny RW, Martin TR, Altemeier WA. Computational identification of key biological modules and transcription factors in acute lung injury. *Am J Respir Crit Care Med* 2006; **173**: 653–658.

39 Coimbra R, Melbostad H, Loomis W, *et al*. LPS-induced acute lung injury is attenuated by phosphodiesterase inhibition: effects on proinflammatory mediators, metalloproteinases, NF-kappaB, and ICAM-1 expression. *J Trauma* 2006; **60**: 115–125.

40 Simpson SQ, Casey LC. Role of tumor necrosis factor in sepsis and acute lung injury. *Crit Care Clin* 1989; **5**: 27–47.

41 Fisher CJ Jr, Opal SM, Dhainaut JF, *et al*. Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. The CB0006 Sepsis Syndrome Study Group. *Crit Care Med* 1993; **21**: 318–327.

42 Wachtler P, Konig W, Senkal M, Kemen M, Köller M. Influence of a total parenteral nutrition enriched with omega-3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. *J Trauma* 1997; **42**: 191–198.