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I am submitting herewith a dissertation written by Peter Mancuso entitled "Anti-inflammatory effects of n-3 and n-6 fatty acids in endotoxin-induced lung injury." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

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ANTI-INFLAMMATORY EFFECTS OF N-3 AND N-6 FATTY ACIDS IN ENDOTOXIN-INDUCED LUNG INJURY

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Peter Mancuso
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DEDICATION

This dissertation is dedicated to my family
for their support and encouragement during the pursuit of this degree.

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ABSTRACT

Proinflammatory eicosanoids and cytokines are important mediators of inflammation in the adult respiratory distress syndrome. We investigated the effect of enteral nutrition with anti-inflammatory fatty acids, eicosapentaenoic acid and γ-linolenic acid from fish and borage oil as compared with corn oil on 1) lung microvascular permeability, mean arterial blood pressure, intrapulmonary synthesis of proinflammatory eicosanoids and cytokines, neutrophil accumulation, and platelet and white blood cell counts in endotoxin-induced lung injury, 2) alveolar macrophage prostaglandin and leukotriene synthesis, 3) liver, lung, and alveolar macrophage phospholipid fatty acid composition, 4) in a model of endotoxin-induced acute lung injury. Rats were randomized into four dietary treatment groups and fed nutritionally complete diets (300 $kcal \cdot kg^{-1} \cdot day^{-1}$) containing 55.2% of the total calories from fat with either 97% corn oil, 20% fish oil, 20% fish and 5% borage oil, or 20% fish and 20% borage oil for 21 days. On day 22, lung microvascular permeability, mean arterial blood pressure, and white blood cell and platelet counts were determined 2 hours following iv injection of *Salmonella enteritidis* endotoxin (10 mg/kg). In a second group of rats, bronchoalveolar lavage was performed 2 hours following an iv injection of *Salmonella enteritidis* endotoxin (10 mg/kg) or saline. In a third group of rats alveolar macrophages were harvested from bronchoalveolar lavage fluid and stimulated *in vitro* with a calcium ionophore (A23187). Bronchoalveolar lavage fluid and alveolar macrophage cell culture

media were analyzed for leukotrienes, thromboxane, and prostaglandins. Lung myeloperoxidase activity (a marker for neutrophil accumulation) and lung, liver and alveolar macrophage phospholipid fatty acids were also determined. Lung permeability was greatest with corn oil and significantly attenuated with 20% fish oil and 20% fish and 5% borage oil. Early and late hemodynamic insufficiency was attenuated with 20% fish oil and 20% fish and borage oil (5 and 20%) as compared with corn oil. Intrapulmonary leukotrienes B_4 and C_4/D_4 and TXB_2 and 6-keto-$PGF_{1\alpha}$ were significantly elevated with corn oil as compared with saline. In contrast, leukotrienes B_4, and C_4/D_4, and TXB_2, did not increase above those of saline in rats with 20% fish oil and 20% fish and 5% borage oil. Leukotriene B_4, PGE_2 and TXB_2 released from A23187 stimulated alveolar macrophages were significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. Lung myeloperoxidase activity was significantly elevated with endotoxin treated rats as compared with their respective saline controls. Lung myeloperoxidase was significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil after endotoxin. Lung, liver, and alveolar macrophage levels of arachidonic acid were lower and the levels of eicosapentaenoic acid and docosahexaenoic acid were higher with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. Dihomo-γ-linolenic acid was elevated with 20% fish and 20% borage oil as compared with corn oil and 20% fish oil. These results indicate that dietary fish oil and fish and borage oil as compared with corn oil may have

provided protection against endotoxin-induced increases in lung microvascular protein permeability and hemodynamic insufficiency by reducing the synthesis of intrapulmonary arachidonic acid derived proinflammatory eicosanoids and attenuating lung neutrophil accumulation.

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PART 1

INTRODUCTION

1. INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a clinical condition that is characterized by pulmonary edema, hypoxemia, and decreased pulmonary compliance (1,2). In the United States, 150,000 people are diagnosed with ARDS each year with a mortality rate of greater than 50% (3). The leading cause of ARDS is gram-negative sepsis (1-3). Treatment of ARDS consists largely of supportive therapy which includes nutritional support (4-6). Enteral diets formulated for patients with respiratory distress contain high levels of fat and low levels of carbohydrate. These diets are formulated to decrease CO_2 production and the respiratory quotient and have been shown to decrease the time needed for ventilatory support in patients with respiratory distress (5,6). However, these diets usually contain high levels of linoleic acid which is desaturated and elongated to arachidonic acid and incorporated into tissue phospholipids. Arachidonic acid is the precursor of the proinflammatory eicosanoids which have been shown to be important mediators of ARDS (7-9).

The 5-lipoxygenase metabolites of arachidonic acid have been shown to be elevated in the bronchoalveolar lavage fluid of ARDS patients (9,10). The cysteinyl-leukotrienes (LTC_4, LTD_4, and LTE_4) increase lung microvascular permeability and constrict pulmonary vascular and bronchial smooth muscle (9,11-13). LTB_4 is a potent chemoattractant of neutrophils which accumulate in the lungs and damage the alveolar-capillary barrier in ARDS patients (14,15).

In addition, TXA_2, a constrictor of vascular and airway smooth muscle has been shown to be an important causative agent of pulmonary hypoxemia and edema in sepsis and acute lung injury (16-20).

Along with proinflammatory eicosanoids, the cytokines TNF-α and MIP-2 are key mediators of endotoxin-induced lung injury in experimental animal models of ARDS (21-25). TNF-α is elevated in the plasma and bronchoalveolar lavage fluid of patients with ARDS and has been shown to induce acute lung injury in experimental animals (24-27). MIP-2 is a member of the α-chemokine family of cytokines which play a significant role in attracting neutrophils to the lung in animal models of acute lung injury (21,22). In response to chemoattractants produced by macrophages and other cells in the lung, neutrophils accumulate in alveolar capillaries, adhere to endothelium, traverse the endothelium and damage vascular endothelial tissue by releasing proteolytic enzymes, reactive oxygen species, and hypochlorous acid during adherence and diapedesis (28).

Previous studies have shown that decreasing the content of arachidonic acid in tissue phospholipids suppresses proinflammatory eicosanoid formation in animal models of acute lung injury (29,30). Dietary fish oil which contains the n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid have been shown to decrease arachidonic acid and increase n-3 fatty acid content of tissue membrane phospholipids (31-34). These n-3 fatty acids inhibit proinflammatory eicosanoid (2-series prostaglandins and thromboxanes)

formation by competing with arachidonic acid for 5-lipoxygenase and cyclooxygenase enzymes (33,35,36). The 5-series leukotrienes and 3-series thromboxanes and prostaglandins generated from eicosapentaenoic acid are less biologically active than the eicosanoids formed from arachidonic acid (37,38). Moreover, dietary fish oil has been shown to decrease the severity of acute lung injury in animal models of endotoxin-induced lung injury (30,39,40).

Although dietary fish oil has been shown to suppress eicosanoid biosynthesis and inflammation, it has also been shown to increase TNF-α biosynthesis in rodent peritoneal macrophages *in vitro* (41-44). In contrast, fish oil supplementation has also been shown to decrease TNF-α biosynthesis in human peripheral blood monocytes and rat kupffer cells *in vitro* (45,46). The effect on dietary n-3 fatty acids on the in vivo production of TNF-α in endotoxic rats has not been adequately addressed in the literature.

Borage oil is a plant seed oil that contains γ-linolenic acid which has been shown to suppress arachidonic acid metabolism and inflammation (47,48,50). γ-linolenic acid does not accumulate in tissues but is elongated to dihomo-γ-linolenic acid which competes with arachidonic acid for cyclooxygenase and lipoxygenase binding sites reducing the 2-series prostaglandins and thromboxanes and 4-series leukotrienes (47,51,52). Dihomo-γ-linolenic acid, which is not a substrate for 5-lipoxygenase, is the precursor of the 1-series prostaglandins (i.e. PGE_1) and the 15-lipoxygenase metabolite 15-HETrE. The iv infusion of liposomal PGE_1 has been shown to

beneficially increase cardiac output and systemic oxygen delivery while reducing pulmonary artery pressure and vascular resistance in patients with the acute respiratory distress syndrome (53). In addition, 15-HETrE has been shown to inhibit LTB_4 formation in human neutrophils (47,52).

Since dietary fish oil and borage oil have been shown to have beneficial effects on various inflammatory disease state in humans and animal models, the use of these anti-inflammatory fatty acids, in high fat, low carbohydrate diets designed for patients with ARDS may also be warranted.

II. RATIONALE

High fat, low carbohydrate enteral formulas designed to lower CO_2 production, the respiratory quotient, and reduce time the patient spends on ventilatory support typically contain corn oil. Corn oil contains high levels of linoleic acid which is a percussor of arachidonic acid . The oxygenated metabolites of arachidonic acid are important mediators in ARDS. Since n-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) from fish oil decrease tissue phospholipid arachidonic acid content and modulate the production of proinflammatory eicosanoids, the substitution of a portion of the fat in these diets with fish oil may attenuate pulmonary inflammation in endotoxin-induced lung injury in rats (an animal model of ARDS). Moreover, the addition of borage oil to a diet containing fish oil may further depress tissue arachidonic acid levels and arachidonic acid derived eicosanoids and modulate the production of 1,2 and 3-series prostaglandins and 4- and 5-series leukotrienes.

Animal models of human sepsis have been useful in studying the mechanisms of the disease process. The intravenous infusion of gram-negative endotoxin in the rat induces septic shock and produces the same functional and morphological pulmonary pathology associated with ARDS in humans. Feeding rats high fat, low carbohydrate diets enriched with fish oil alone or in combination with borage oil as compared with a standard corn oil based diet for

21 days should significantly decrease tissue phospholipid arachidonic acid and increase n-3 fatty acids and dihomo-γ-linolenic acid content. This will provide a useful model needed to determine if altering tissue phospholipid fatty acid composition significantly influences eicosanoid and cytokine production and ultimately physiologic responses to endotoxin-induced lung injury.

III. OBJECTIVES

The purpose of this dissertation was to determine the effect of enteral nutrition for 21 days with diets containing fatty acids from fish oil alone and in combination with borage oil as compared with corn oil (represents standard commercially available diet) on: 1) lung microvascular protein permeability, arterial blood pressure, intrapulmonary eicosanoid and cytokine production, neutrophil accumulation, and platelet and white blood cell counts in a model of endotoxin-induced lung injury 2) alveolar macrophage prostaglandin and leukotriene synthesis in vitro, and 3) liver, alveolar macrophage and lung phospholipid fatty acid composition.

REFERENCES

1. Bernard GR, Artigas A, Brigham KL, et al: Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. *Inten Care Med* 1994; 20:225-232

2. Kollef MH, Schuster DP: The acute respiratory distress syndrome. *N Eng J Med* 1995; 332:27-37

3. Flick MR: Pulmonary edema and acute lung injury. *In*: Murray JF, Nadel JA., eds. Textbook of Respiratory Medicine. Philadelphia: W.B. Saunders Company, 1994, 1725-1777

4. Pinard B, Geller E. Nutritional support during pulmonary failure. *Crit Care Clin* 1995; 11:705-715

5. Askanazi J, Nordenstrom J, Rosenbaum SH, et al: Nutrition for the patient with respiratory failure: Glucose vs. fat. *Anesthesiol* 1981; 54:373-377

6. Al-Sandy NM, Blackmore CM, Bennett ED: High fat, low carbohydrate, enteral feeding lowers $PaCO_2$ and reduces the period of ventilation in artificially ventilated patients. *Inten Care Med* 1989; 15:290-295

7. Turner CR, Quinlan MF, Schwartz LW, et al: Therapeutic intervention in a rat model of ARDS: I. Dual inhibition of arachidonic acid metabolism. *Circ Shock* 1990; 32:231-242

8. Turner CR, Lackey MN, Quinlan MF, et al: Therapeutic intervention in a rat model of adult respiratory distress syndrome: III. Cyclooxygenase pathway inhibition. *Circ Shock* 1991; 34:270-277

9. Stephenson AH, Lonigro AJ, Hyers TM, et al: Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* 1988; 138:714-719

10. Antonelli M, Bufi M, DeBlasi RA, et al: Detection of leukotrienes B_4, C_4 and of their isomers in arterial, mixed venous blood and bronchoalveolar lavage fluid from ARDS patients. *Intensive Care Med* 1989; 15:296-301

11. Cohn SM, Kruithoff KL, Rothchild HR, et al: Beneficial effects of LY203647, a novel leukotriene C_4/D_4 antagonist, on pulmonary function and mesenteric perfusion in a porcine model of endotoxic shock and ARDS. *Circ Shock* 1991; 33:7-16

12. Bursztein S, D'Attellis NP, Askanazi J: Nutrition and the respiratory system. *In*: Hall JB, Schmidt GA, Wood LDH., eds. Principles of critical care. New York: McGraw-Hill, 1992, 1093-1097

13. Abela A, Daniel EE: Neural and myogenic effects of leukotrienes C_4, D_4, and E_4, on canine bronchial smooth muscle. *Am J Physiol* 1994; 266:L414-L425

14. Rosengren S, Olofsson AM, Von Andrian UH, et al: Leukotriene B_4-induced neutrophil-mediated endothelial leakage in vitro and in vivo. *J Appl Physiol* 1991; 71:1322-1330

15. Li EJ, Cook JA, Wise WC, et al: Effect of LTB_4 receptor antagonists in endotoxic shock in the rat. *Circ Shock* 1991; 34:385-392

16. Bernard GR, Reines HD, Halushka PV, et al: Prostacyclin and thromboxane A_2 formation is increased in human sepsis syndrome: Effects of cyclooxygenase inhibition. *Am Rev Respir Dis* 1991; 144:1095-1101

17. Peterson MB, Huttemeier PC, Zapol WM, et al: Thromboxane mediates acute pulmonary hypertension in sheep extracorporeal perfusion. *Am J Physiol* 1982; 243:H471-H479

18. Wisner D, Sturm J, Sutter G, et al: Thromboxane receptor blockade in an animal model of ARDS. *Surgery* 1988; 104:91-97

19. Winn R, Harlan J, Nadir B, et al: Thromboxane A_2 mediates lung vasoconstriction but not permeability after endotoxin. *J Clin Invest* 1983; 72:911-918

20. Santoli D, Phillips PD, Colt TL, et al: Zurier RB. Suppression of interleukin 2-dependent human T cell growth in vitro by prostaglandin E and their precursor fatty acids. *J Clin Invest* 1990; 85:424-432

21. Schmal H, Shanley TP, Jones ML, et al: Role of macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J Immunol* 1996; 156:1963-1972

22. Strieter RM, Standiford TJ, Huffnagle GB, et al: The good, the bad, and the ugly: The role of chemokines in models of human disease. *J Immunol* 1996; 156:3583-3586

23. Parsons PE, Moore FA, Moore EE, et al: Studies on the role of tumor necrosis factor in adult respiratory distress syndrome. *Am Rev Respir Dis* 1992; 146:694-700

24. Wheeler AP, Jesmok G, Brigham KL: Tumor necrosis factor's effects on lung mechanics, gas exchange, and airway reactivity in sheep. *J Appl Physiol* 1995; 68:2542-2549

25. Ferrari-Balliviera E, Mealy K, Smith RJ, et al: Tumor necrosis factor induces adult respiratory distress syndrome in rats. *Arch Surg* 1989; 124:1400-1405

26. Johnson J, Brigham KL, Jesmok G, et al: Morphologic changes in lungs of anesthetized sheep following intravenous infusion of recombinant tumor necrosis factor α. *Am Rev Respir Dis* 1991; 144:179-186

27. Suter PM, Suter S, Girardin E, et al: High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992; 145:1016-1022

28. Hafstrom I, Palmblad J, Malmsten CL, et al: Leukotriene B4- a specific stimulator for release of lysosomal enzymes from neutrophils. *FEBS Lett* 1981; 130:146-148

29. Ball HA, Cook JA, Spicer KM, et al: Essential fatty acid-deficient rats are resistant to oleic acid-induced pulmonary injury. *J Appl Physiol* 1989; 57:811-816

30. Murray MJ, Svingen BA, Yaksh TL, et al: Effects of endotoxin on pigs prefed omega-3 vs. omega-6 fatty acid-enriched diets. *Am J Physiol* 1993; 265:E920-E927

31. Whelan J, Broughton KS, Kinsella JE: The comparative effects of dietary alpha-linolenic acid and fish oil on 4- and 5-series leukotriene formation in vivo. *Lipids* 1991 ;26:119-126

32. Chilton FH, Patel M, Fonteh AN, et al: Dietary n-3 fatty acid effects on neutrophil lipid composition and mediator production. Influence of duration and dosage. *J Clin Invest.* 1993; 91:115-122

33. Leaf A, Weber PC: Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 1988; 318:549-557

34. von Schacky C, Fischer S, Weber PC: Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest* 1985; 76:1626-1631

35. Kinsella JE, Lokesh B, Broughton S, et al: Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* 1990; 6:24-44

36. Lands WEM: Biochemistry and physiology of n-3 fatty acids. *FASEB J* 1992; 6:2530-2536

37. Lee TH, Mencia-Huerta J, Shih C, et al: Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J Biol Chem* 1984; 259:2383-2389

38. Fischer S, Weber PC: Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature* 1984; 307:165-168

39. Murray MJ, Kumar M, Gregory TJ, et al: Enteral diets enriched in eicosapentaenoic acid and gamma-linolenic acid attenuate cardiopulmonary dysfunction in a porcine model of acute lung injury. *Am J Physiol* 1995; 269:H2090-H2099

40. Murray MJ, Svingen BA, Holman RT, et al: Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *J Parenter Enter Nutr* 1991; 15:152-158

41. Carrick JB, Schnellmann RG, Moore JN: Dietary source of Omega-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. *Shock* 1994; 2:421-426

42. Hardardottir I, Kinsella JE: Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* 1991; 1095:187-195

43. Hardardottir I, Whelan J, Surette ME, et al: The effects of dietary n-3 polyunsaturated fatty acids and cyclic AMP-elevating agents on tumor necrosis factor production by murine-resident and thioglycollate-elicited peritoneal macrophages. *J Nutr Biochem* 1993; 4:534-542

44. Hardardottir I, Kinsella JE: Increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. *J Nutr* 1992; 122:1942-1951

45. Billiar TR, Bankey PE, Svingen BA, et al: Fatty acid intake and kuppfer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* 1988; 104:343-349

46. Endres S, Ghorbani R, Kelley VE, et al: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989; 320:265-271

47. Chilton-Lopez T, Surette ME, Swan DD, et al: Metabolism of gamma-linolenic acid in human neutrophils. *J Immunol* 1996; 156:2941-2947

48. Kies C. Evening primrose oil: a source of gamma linolenic acid. *CFW* 1989; 34:1016-1020

49. Tate G, Mandell, BF, Laposta, M, et al: Suppression of acute and chronic inflammation by dietary gamma linolenic acid. *J Rheumatol* 1989; 16:729-734

50. Tate GA, Mandell BF, Karmali RA, et al: Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. *Arthrit Rheumat* 1988; 31:1543-1551

51. Manku MS, Morse-Fisher N, Horrobin DF: Changes in human plasma essential fatty acid levels as a result of administration of linoleic acid and gamma-linolenic acid. *Eur J Clin Nutr* 1988; 42:55-60

52. Ziboh VA, Fletcher MP: Dose-response effects of dietary τ-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B_4. Am J *Clin Nutr* 1992; 55:39-45

53. Abraham E, Park YC, Covington P, et al: Liposomal prostaglandin E_1 in acute respiratory distress syndrome: A placebo-controlled, randomized, double-blind, multicenter clinical trial. *Crit Care Med* 1996; 24:10-15

PART 2

LITERATURE REVIEW

I. ACUTE RESPIRATORY DISTRESS SYNDROME

A. Definition and Epidemiology of the Acute Respiratory Distress Syndrome

The acute respiratory distress syndrome (ARDS) was first described by Asbaugh and associates (1) in 1967 as acute respiratory distress following trauma, sepsis, or certain drug overdoses. Sepsis is the most common cause of acute lung injury and it develops in 10 to 35% of all patients with this condition (2). Acute lung injury has been defined by the European-American Consensus Conference on the adult respiratory distress syndrome (ARDS) as a condition with the following characteristics: acute onset (72 hours following initial insult), poor efficiency of gas exchange as reflected by the ratio of arterial oxygen concentration to the concentration of oxygen in inspired air (PaO_2/FiO_2 200 - 300 mmHg) (3), pulmonary edema (bilateral pulmonary infiltrates as revealed by a chest radiograph), and no evidence of left atrial hypertension (pulmonary arterial occlusion pressure $\leq$ 18 mmHg). The same characteristics of acute lung injury apply to the ARDS, however, the PaO_2/FiO_2 is $\leq$ 200 mmHg indicating a more severe form of acute lung injury (4). The incidence of ARDS in the US has been estimated at 150,000 cases per year with a high degree of mortality (>50%) (3).

B. Pathophysiology of ARDS

The development of ARDS can occur through two pathways: one

pathway is initiated by direct injury to pulmonary cells and the other occurs through an indirect activation of a systemic inflammatory response. The direct pathway can be initiated by aspiration, pulmonary infection (bacterial, viral or pneumocystis infection), near-drowning, toxic inhalation, or lung contusion. ARDS caused by an indirect systemic inflammatory response can be initiated by sepsis, severe non-thoracic trauma, hypertransfusion of blood for emergency resuscitation, or cardiopulmonary bypass (4).

Regardless of the initiating insult, the most prominent feature of the onset of acute lung injury is the disruption of the alveolar-capillary interface and subsequent increases in lung microvascular permeability. According to the Starling equation:

$$Q_f = K [(P_c + \pi_i) - (P_i + \pi_p)]$$

where the net transvascular fluid movement (Q_f) is equal to the conductance (permeability) of the microvascular barrier (K), multiplied by the sum of the capillary hydrostatic pressure (P_c) plus the interstitial fluid oncotic pressure (π_i) minus the of sum the interstitial fluid hydrostatic pressure (P_i) plus the plasma protein oncotic pressure (π_p). When the number for Q_f is positive, fluid filtration occurs (a net movement of fluid out of the vascular space and into the extravascular space) and if it is negative, fluid is absorbed from the extra vascular space into the vascular space (5).

Increases in lung microvascular permeability in ARDS or after endotoxin or live bacterial infusion may be due to increases in K and increases in capillary

hydrostatic pressure (P_i). Changes in K are probably due to the digestion of the basement membrane by proteolytic enzymes and reactive oxygen species released from neutrophils which accumulate in alveolar capillaries of the microvasculature during acute lung injury (6-8). However, ARDS does occur in neutropenic patients and increases in lung microvascular permeability have been observed in animal models independent of neutrophil participation (2). LTC_4, LTD_4, histamine, and platelet activating factor (PAF) have been shown to directly increase lung microvascular permeability (9-13). In addition, endotoxin has been shown to increase human vascular endothelial cell permeability *in vitro* by increasing the size of the intercellular junctions (14). Finally, increases in lung microvascular permeability due to increases in capillary hydrostatic pressure are due to vasoactive mediators such as TXA_2 and LTC_4, LTD_4, and LTE_4. Increased levels of these mediators have been shown to accumulate in the serum and bronchoalveolar lavage fluid of ARDS patients (13,15-18).

Under normal circumstances, fluid and plasma proteins leak out of the alveolar capillary and into the interstitial space of the lungs which includes the alveolar wall interstitium (perimicrovascular) and the loose connective tissue spaces around the bronchi and blood vessels (peribronchovascular). The pressure gradient favors the movement of fluid and protein from the perimicrovascular into the peribronchovascular spaces which prevents the build up of fluid (edema) near the air-blood interface. A hydrostatic pressure gradient and breathing movements drive fluid and protein into the lymphatic capillaries at

the level of the bronchioles via the peribronchovascular space. The lymphatic capillaries drain into lymphatic ducts which eventually empty into the thoracic ducts which deliver lymph into the superior vena cava (2). Pulmonary edema occurs when vascular fluid leaks into the extravascular space and accumulates faster than it is removed by the lymphatic system (2).

Edema impairs normal respiratory function by increasing the distance by which diffusion of O_2 and CO_2 occurs which can result in decreasing levels of arterial oxygen concentration (PaO_2) and inadequate O_2 delivery. Edema may also influence lung mechanics by reducing lung compliance (the volume of lung expansion per unit of pressure needed to inflate the lungs) and decreasing the diameter of the bronchioles (2).

Alveolar fluid accumulation is also a prominent feature of ARDS. However, the processes by which fluid leaks into the alveoli have not been fully elucidated. The alveolus is composed of epithelial cells which form tight junctions that are normally impermeable to fluid, protein, and electrolytes. It is unlikely that the epithelial barrier to fluid and protein becomes more permeable as does the microvascular endothelium in ARDS in the first few hours following the onset of lung injury. Fluid and protein are thought to move into the alveoli by leaking through the area of cell type transition between alveolar epithelial cells and squamous cells of the respiratory bronchioles. The accumulation of fluid and protein in the peribronchovascular space provides a pressure gradient which drives fluid through this transitional area of tissue (2). Normally,

surfactant maintains a lower surface tension inside the alveolus preventing alveolar collapse. Alveolar flooding impairs gas exchange efficiency by disrupting the surfactant coating of the alveolus which increases surface tension resulting in alveolar collapse (atelectasis). In addition, the accumulation of fluid in the perimicrovascular and peribronchovascular spaces can also cause alveolar collapse by increasing the pressure outside of the terminal airways (2).

The development of hypoxemia in ARDS is a complex sequence of events and is usually the result of diffusion impairment and right-to-left shunt (19). Pulmonary edema can cause hypoxemia by impairing gas exchange between the alveolar space and red blood cells in the pulmonary capillaries. The transfer of gas through a sheet of tissue is described by Fick's law which states:

$$Vgas \propto A/T \cdot D(P_1-P_2)$$

where the rate of transfer of a gas (Vgas) through a sheet of tissue is proportional to the area (A), a diffusion constant (D), the difference in partial pressure (P_1-P_2), and is inversely proportional to the thickness (T) (19). Gas exchange between the red blood cells and the alveolar air occurs through what has been described as the respiratory membrane which consists of the layer of fluid and surfactant lining the alveolar membrane, the alveolar epithelium, the epithelial basement membrane, the interstitial space between the alveolar epithelium and the capillary membrane (perimicrovascular space), the capillary basement membrane, and the capillary endothelial membrane (20). The

average thickness of the respiratory membrane averages 0.6 micrometers. Edema fluid in the interstitial space can severely interfere with gas exchange since it increases the thickness of the respiratory membrane. Since the thickness of the respiratory membrane is inversely proportional to the rate of diffusion, an increase in the thickness to two or three times normal can significantly interfere with respiratory gas exchange (20).

Alveolar collapse decreases pulmonary gas exchange by reducing the total number of respiratory units. Pulmonary edema and alveolar collapse ultimately increase the right-to-left shunting of blood or shunt fraction which refers to blood that perfuses the lungs without going through ventilated areas of the lungs (19). In patients with ARDS, the perfusion of areas of the lungs where the alveoli have collapsed or are surrounded by fluid (as with edema) severely compromises gas exchange between the alveolar space and blood flowing through the alveolar capillaries and increases the shunt fraction. Severe hypoxemia develops when a large amount of fluid has accumulated in the pulmonary extravascular spaces and a large number of alveoli have collapsed (large shunt) (2).

In addition to the shunt fraction, ventilation perfusion inequality is also thought to contribute to hypoxemia in the ARDS patient. Under normal conditions, hypoxic pulmonary vasoconstriction prevents the perfusion of unventilated respiratory units. However, in sepsis (a leading cause of ARDS) there may be a loss of pulmonary vascular contractility. As a result, the loss of

hypoxic vasoconstriction will allow unventilated respiratory units to receive blood flow leading to ventilation-perfusion mismatching (21).

While the disruption of the alveolar-capillary interface and increases in lung microvascular permeability are prominent features of the acute onset of ARDS, changes in lung morphology leading to diffuse alveolar damage occur as the disease process develops over the next few weeks. In general, there are three overlapping phases of lung remodeling that occur in patients with ARDS: the exudative phase, the proliferative phase, and the fibrotic phase. The exudative phase occurs in the first week after the initial onset of ARDS. It is characterized by capillary congestion, interstitial and alveolar edema, and intra-alveolar hemorrhage (22). The breakdown of the alveolar-capillary barrier is due to the disruption of the vascular endothelial cells and alveolar epithelial cells. The endothelial cells become swollen and the intercellular junctions widen. Changes to the pulmonary capillary endothelial barrier include endothelial cell necrosis and denuding of the capillary basement membrane with intravascular neutrophil and fibrin accumulation (22-24). The alveolar septa widen due to interstitial edema, fibrin deposition and extravasated erythrocytes (25,26). The alveolar epithelial barrier becomes denuded as both type I and type II alveolar epithelial cells undergo necrosis (22,27). As the cellular barriers to fluid flux breakdown, interstitial fluid moves freely into the alveolar space (22,23,25,26). A striking feature of the exudative phase is the deposition of hyaline membranes along the alveolar duct and tips of the

alveolar septa. Hyaline membranes are composed of condensed plasma proteins (immunoglobulins, fibrinogen and complement) which have leaked into the alveolar spaces (22,26). The diffuse alveolar barrier damage, edema formation, and deposition of hyaline membranes contributes to hypoxemia (by increasing diffusion distance for gas exchange) and the decrease in lung compliance.

The proliferative phase, which can begin as early as three days from the onset of ARDS, is marked by cellular reorganization and proliferation. Under normal conditions, the alveolar epithelium which is separated from the capillary endothelium by a basement membrane, is predominantly composed of two functionally and histologically distinct epithelial cell types. Type I epithelial cells which cover the majority of the surface area inside the alveolus are extremely thin and have a high surface to volume ratio which facilitates gas exchange. The cuboidal type II cell which secretes surfactant make up a much smaller portion of the intra-alveolar surface area. Since the type I epithelial cells are more vulnerable to necrosis during the exudative phase, a large number of the surviving cells lining the partially denuded intra-alveolar surface are type II epithelial cells. During the proliferative phase, type II alveolar epithelial cells regenerate and cover the previously denuded alveolar basement membrane. However, type II cells are not well suited for gas exchange (22). Fibroblasts and myofibroblasts from the alveolar wall proliferate and migrate through breaks in the alveolar basement membrane into the intra-alveolar space where they

convert the exudate (which leaked in the alveolus previously) into a dense fibrous tissue by the deposition of collagen (22,28,29). Partial or completely collapsed alveoli can become sealed completely or partially following the deposition of layers of epithelium and fibrin (22,30). The net result of the proliferative phase is a decrease in the number of functional respiratory units which contributes to hypoxemia.

During the fibrotic stage of lung remodeling, nonfunctional connective tissue replaces functional lung tissue and the lung is transformed to a non-compliant end stage organ. This process can occur in ventilator-dependent patients three to four weeks after the onset ARDS (22). Fibrosis by accretion is the most important process of lung remodeling where alveolar ducts and alveolar walls thicken following the deposition of collagen. Chronic interstitial edema is a potent stimulus for alveolar wall fibrosis (22,31).

Finally, remodeling of the pulmonary vasculature is also an important characteristic of lung remodeling in ARDS. Following the acute onset of ARDS, pulmonary arterial pressure rises due to vasoconstriction, thromboembolism and interstitial edema (22). Several weeks after the onset of ARDS, permanent changes in vascular remodeling leading to pulmonary hypertension include vascular intimal fibrosis, capillary obliteration and vascular smooth muscle hypertrophy (22).

C. Sepsis

Sepsis, which is the leading cause of ARDS, is the systemic response to the presence of invading organisms in the normally sterile tissue of the host (32). Sepsis is the thirteenth leading cause of death in the United States and has been estimated to account for 100,000 deaths per year (33). Sepsis can be caused by a number of microorganisms including gram-negative and gram-positive bacteria, and fungi (34). This review of the literature will focus on gram-negative sepsis. Under normal conditions, gram-negative bacteria reside in the large bowel of the host. Bacterial translocation is the process by which gram-negative organisms can enter the systemic circulation of the host resulting in bacteremia (presence of live bacteria in blood). This occurs when there is bacterial overgrowth, immune dysfunction or increased permeability across the epithelial barrier of the large intestine (33). In addition, gram-negative bacteria can also enter the systemic circulation through bowel perforation which would occur as a result of penetrating trauma (i.e. gun shot or knife wound to the abdomen).

When gram-negative bacteria are killed the cell wall breaks loose and travels throughout the circulatory system. The cell wall of gram-negative bacteria is an endotoxin which contains lipopolysaccharide (LPS). LPS is toxic to cells and tissues and causes the release of inflammatory mediators from monocytes, tissue macrophages and other cells (35,36). In addition, LPS activates the cytokine, complement, coagulation and kinin and ACTH/endorphin

systems (33,37). These mediators have a profound influence on a number of cells and tissue throughout the host. Vasodilation reduces peripheral vascular resistance and decreases mean arterial blood pressure (38). TNF-α, a cytokine released by primarily by monocytes in response to LPS in septic patients, depresses myocardial performance by reducing left ventricular ejection fraction. The increase in the levels of intravascular bradykinin, PAF, LTC_4, and histamine increase microvascular permeability and edema (3). The increase in intravascular mediators of coagulation leads to disseminated intravascular coagulation. As this process occurs, organs and tissues are inadequately perfused with blood and become ischemic. Eventually septic shock leads to multi-organ system dysfunction which includes acute lung injury and liver and kidney failure (36).

D. Endotoxin Signal Transduction

The cardiovascular, inflammatory, and metabolic abnormalities associated with sepsis can be attributed to the LPS, a component of the cell wall of gram-negative bacteria (36). The general structure of gram-negative lipopolysaccharide consists of a polysaccharide covalently linked to a lipid component (39). The polysaccharide portion consists of two different parts; an O-specific chain and a polysaccharide chain with several repeating units of oligosaccharide containing 3-deoxy-D-manno-octulosonate (Kdo) (39-41). The phospholipid component, referred to as lipid A, causes the in vivo

manifestations of endotoxemia including fever, hypotension, leukocytopenia, and widespread intravascular coagulation (40). The lipid A component is composed of a phosphoglycolipid containing a bisphosphorylated β-(1,6)-linked D-gluco-hexosamine disaccharide with six acyl groups containing 4 (R)-3-hydroxy fatty acids (C_{10} to C_{16}) and two (R)-3-acyloxyacyl groups (39). The presence of two hexosamine residues, two phosphoryl groups, and six fatty acids containing saturated and (R)-3-hydroxylated fatty acids must be present for full endotoxic activity (40). Variations in the structure of the lipid A component such as the number of acyl chains, molecules containing different but chemically related constituents, or analogues with different arrangements will have less endotoxicity *in vivo* (40).

LPS interacts with peripheral blood monocytes and tissue macrophages through its association with a lipopolysaccharide binding protein (LBP) (39). LBP is an acute phase protein produced by the liver that is present in the plasma of both animals and humans (42). Plasma LBP increases in response to trauma or the presence of LPS in septic patients (43). LBP, which binds LPS and has a high affinity for the lipid A portion of the LPS molecule, enhances the association of LPS with peripheral monocytes and macrophages (42-44). The LPS/LBP complex interacts with monocytes, macrophages, and activated neutrophils through the CD14 receptor. The CD14 receptor is primarily expressed on the surface of myeloid cells (45) and is believed to be the most important receptor in LPS signal transduction. The CD14 receptor is

attached to the cell membrane by a glycosylphosphatidylinositol linked anchor (46) which does not traverse the cell membrane. In addition, a smaller soluble CD14 receptor that lacks a cell membrane anchor is shed *in vivo* in septic patients from monocytes and macrophages and may potentiate LPS activation of cell types that do not express the membrane bound CD14 receptor (46-48). Since CD14 lacks a cytoplasmic tail, it is believed to associate with another cell surface protein with a cytoplasmic transcellular domain that transmits an intercellular signal through a cascade of protein phosphorylations leading to changes in cell function in response to LPS (47, 49).

LPS activates diverse intracellular signal transduction pathways leading to transcriptional activation of genes that encode cytokines (IL-1, IL-6 and TNF-α) and phospholipase enzymes that play important roles in inflammatory cascades (46). The complete identification of the steps in the signal transduction pathway following CD14 binding of the LPS/LBP complex leading to cell activation have not been completely elucidated at this time. The following description of events in the LPS signal transduction pathway represents a reasonable sequence of events based on the current literature. Immediately after the CD14 receptor binds its ligand (LPS/LBP), tyrosine phosphorylation of cellular proteins including the *src* family p53/56^{lyn} occurs (50-52). Along with tyrosine phosphorylation, activation of Raf-1 and mitogen-activated protein kinases (MAP-kinases) have also been reported following LPS stimulation (53,54). Raf proteins are serine/threonine-specific protein kinases

that transmit mitogenic signals from the ligand-activated receptors at the cell membrane to the cell nucleus (55). Raf-1, a member of the *raf* oncogene family, may function down stream of the *src* family kinases on the pathway culminating in the synthesis of cytokines (56). Upon LPS-stimulation and tyrosine phosphorylation, Raf-1 may associate with *ras* (a small molecular weight G protein) (47). This activated complex may phosphorylate MEK (MAP kinase kinase) which in turn can phosphorylate several different MAP kinases including three members of the ERK supergene family ERK1 (p44), ERK 2 (p41) and p38 (57). These MAP kinases can activate nuclear transcription factors (NF-κB and AP-1) which migrate from the cytosol to the cell nucleus to initiate cytokine transcription. In addition, MAP kinases (ERK 1 and ERK 2) have been shown to phosphorylate cytosolic phospholipase A_2 enzymes at the Ser^{505} which increases PLA_2 activity (58,59).

Phospholipase A_2 activation following exposure to endotoxin has been shown to occur *in vivo* and *in vitro* and is the initial enzymatic step in the arachidonic acid cascade (47). The isoforms of phospholipase A_2 include two calcium-dependent forms (secretory or $sPLA_2$ and cytosolic or $cPLA_2$) and a calcium-independent form ($iPLA_2$) (47). There are two groups of low molecular weight (12 to 20 kDa) $sPLA_2$: mammalian pancreatic $sPLA_2$ is a group I enzyme and group II $sPLA_2$ has been identified in platelets in serum and inflammatory exudates of patients with septic shock and rheumatoid arthritis (59). Prior to its release from secretory granules, $sPLA_2$ is in an inactive state. Following

release from secretory granules and exposure to calcium in the extracellular space, activated $sPLA_2$ may cleave arachidonic acid from the outer plasma membrane phospholipids (47,59). Extracellular arachidonic acid may act as a second messenger since it can easily traverse cell membranes (47). The high molecular weight (85kDa) $cPLA_2$ resides in the cytosol of cells isolated from its substrate. After cellular activation and increases in intracellular calcium, calcium binds to the amino-terminal domain of $cPLA_2$ which migrates to membrane phospholipids. Previous studies using A23187 (calcium ionophore) stimulated mast cells and chinese hamster ovary cells over expressing $cPLA_2$, indicate that $cPLA_2$ translocates to the endoplasmic reticulum and nuclear envelope (59-61). In addition, $cPLA_2$ co-localizes to the nuclear envelope with 5-lipoxygenase, 5-lipoxygenase activating protein (FLAP), and cyclooxygenase-2 (59). $cPLA_2$ then catalyses the hydrolysis of phosphotidylcholine and phosphatidylethanolamine releasing arachidonic acid from the sn-2 position leading to eicosanoid formation (47). Finally, a calcium-independent $iPLA_2$ has been identified in myocardial cells and in the $P388D_1$ macrophage cell line (59). $iPLA_2$ is a 40 kDa protein that is associated with a molecular mass complex which includes a regulatory subunit (80 kDa). $iPLA_2$ is regulated by ADP and is thought to be responsible for arachidonic acid release in smooth muscle and pancreatic islet cells (47,59).

The activity of phospholipase C (PLC) has also been implicated in LPS signal transduction as well. Isoforms of PLC can be activated by G proteins

(PLCβ) or tyrosine phosphorylation (PLCγ). Previous studies have shown that PLC may contribute to LPS-mediated cellular activation (47). When activated, PLC cleaves phosphatidylinositol trisphosphate yielding diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diacylglycerol activates protein kinase C (PKC) (a neutral lipid and calcium dependent kinase) which is an important serine/threonine kinase that plays a significant role in cytokine gene transcription and the oxidative burst of neutrophils and macrophages (47,48,62,63). IP_3 activates the opening of calcium channels in intracellular compartments leading to increases in the concentration of cytosolic calcium which can activate PKC and PLA_2 (47,59). The role of calcium in LPS-stimulated intracellular signal transduction is controversial, however, since not all investigators have observed increases in cytosolic calcium following LPS-stimulation (47,64, 65).

Phospholipase D, which hydrolyses phosphatidylcholine releasing choline and phosphatidic acid, may also play an important role in LPS-stimulated signal transduction (47). While the increase in intracellular choline is not significant and does not seem to perform any role in cellular activation, the accumulation of phosphatidic acid is significant for many reasons. First, phosphatidic acid may directly activate NADPH oxidase in the respiratory burst of leukocytes (47,66). Second, phosphatidic acid hydrolase converts phosphatidic acid to diacylglycerol which as mentioned earlier can activate PKC. Finally, phosphatidic acid is also a substrate of PLA_2 which converts it to

lysophosphatidic acid (LPA). The role of intracellular LPA is not well established although exogenous LPA has been shown to play an important role in platelet activation (47).

PKC has been implicated as a critical factor in transcriptional regulation of TNF-α in LPS-stimulated macrophages and monocytes (62,67). PKC is a calcium-diacylglycerol dependent serine/threonine kinase that has been shown to phosphorylate the NF-κB transcription factor inhibitor IκB (68). The inactive NF-κB/IκB complex resides in the cytoplasm of unstimulated macrophages and monocytes (68). Phosphorylation of the IκB subunit causes it to dissociate from NF-κB which can then migrate to the nucleus (68). NF-κB is a heterodimeric protein that binds to specific binding sites on the TNF-α and IL-8 gene and activates transcription (69). Since many protein kinases have been shown to mediate NF-κB activation, PKC inhibitors used in LPS signal transduction experiments may inhibit a wide variety of protein kinases. Thus, the exact role of PKC in LPS mediated macrophage and monocyte cytokine production is still under investigation (47).

II. MECHANISMS OF ACUTE LUNG INJURY

A. The Role of Macrophages, Neutrophils, and Platelets in ARDS

The alveolar macrophage, which resides in the surfactant-rich alveolar fluid in contact with the alveolar epithelial space, is the first line of defense against invading organisms and inhaled inorganic particles (70). The alveolar macrophage is derived from peripheral blood monocytes that migrate to the alveolar space of the lung where they differentiate and increase in size and metabolic activity in the process of becoming an alveolar macrophage (7,71). In addition to monocytes maturation, alveolar macrophages are capable of replication and proliferation within the alveolus (71). In addition to the alveolar macrophage, pulmonary interstitial and intravascular populations of macrophages have been identified in humans (71).

The alveolar macrophage plays a central role in mediating the pulmonary inflammatory response in septic patients. First, the alveolar macrophage orchestrates the host's response to LPS in septic patients by releasing TNF-α and IL-1 which are key mediators in endotoxin-induced lung injury in animal models, sepsis, and ARDS (72-76). In sepsis or endotoxin-induced lung injury, the alveolar macrophage is the primary cell type that recognizes and responds to LPS in the lung since it expresses the CD14 cell surface antigen (43,44,47,51). As lung microvascular permeability increases following acute lung injury, LBP accumulates in the lungs and regulates the alveolar

macrophage response to LPS (43). In addition, cytokines (TNF-α and IL-1) produced by intravascular and interstitial pulmonary macrophages and peripheral blood monocytes can leak into the alveolar space and stimulate the alveolar macrophage. TNF-α and IL-1 have been shown to initiate inflammatory cascades in adjacent alveolar cells in the alveolar epithelium, vascular endothelium, and fibroblasts of the alveolar wall (77). Second, the alveolar macrophage is a phagocyte which engulfs invading pathogenic organisms, LPS and inorganic particles (7). Subsequently, it removes them from the alveolar space (71). Following activation, the alveolar macrophage releases reactive oxygen species and proteases that are the primary means of killing pathogenic organisms and damaging lung tissue in the alveolar space (7).

An important feature of pulmonary inflammation in acute lung injury is the recruitment of leukocytes to the interstitium of the lungs. The alveolar macrophage plays a central role in recruiting systemic neutrophils and monocytes to the interstitial spaces of the lung during acute lung injury by releasing chemoattractants, TNF-α and IL-1. These cytokines activate surrounding tissues such as the vascular endothelium and the alveolar epithelium which in turn release chemoattractants and express adhesion molecules. IL-8, CINC (cytokine-induced neutrophil chemoattractant), MIP-2, and GROα), LTB_4, C5a, and PAF and monocyte chemoattractants MCP-1, MIP-1, and RANTES have all been implicated as important mediators of leukocyte

accumulation in acute lung injury (78,79,80,81). While IL-8 has been shown to increase in the BAL fluid in human ARDS patients, the α-chemokines, MIP-2 and CINC, are thought play the role of IL-8 in rodents (6,82). In addition, a number of studies have shown that both MIP-2 and LTB_4 contribute to neutrophil chemotaxis and transendothelial migration in pulmonary inflammation in the rat (83-89). After neutrophils and monocytes are attracted to the lung in sepsis-induced lung injury, they adhere to the vascular endothelium, become activated, release granular oxidants and proteolytic enzymes, traverse the capillary endothelium and move through the interstitium to the alveolar space causing increases in lung microvascular permeability.

The recruitment of neutrophils to extravascular sites of the lung is an important means of host defense against infection (90). Neutrophils play an essential role in phagocytizing and killing pathogens. In sepsis, the neutrophil has little intrinsic ability to differentiate antigens of pathogens and the host (91). As a result, neutrophil accumulation in the lungs of septic patients leads to the destruction of the alveolar-capillary barrier increasing lung microvascular permeability (92-95). The neutrophil constitutes 50-70% of the circulating white cells and is a major contributor to sepsis and endotoxin-induced lung injury (94-98). The total number of neutrophils that are in the pulmonary vasculature at any given time exceeds the number in the systemic circulation by 35-100 times (99). These neutrophils represent the marginated pool in the lungs. This is due to the additional time required by the neutrophils to squeeze

through the pulmonary vasculature. The diameter of 38% of the vascular segments in the human lung are smaller than the diameter of the neutrophil indicating that the neutrophil must deform to pass through the lungs (100). In addition, neutrophils that become activated by TNF-α, IL-1, and LTB_4 swell and become more rigid in response to mediators of sepsis and acute lung injury. This further increases the transit time of the neutrophil through the pulmonary vasculature.

In addition to the size limitations that slow the neutrophils passage through the lungs, adhesion molecules also contribute to the stasis of neutrophils in the pulmonary circulation. Under normal conditions the neutrophil and the endothelium do not interact. However, in endotoxin-induced lung injury, the initial interaction of the endothelium and neutrophils is mediated by the selectins (101). L-selection is an adhesion molecule that is expressed constituitively on the surface of quiescent neutrophils (102). E-selectin is an adhesion molecules expressed by the endothelium in response to TNF-α, IL-1 or endotoxin (90,103). P-selectin, which is stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells, is mobilized to the cell surface in response to histamine and thrombin (90,103). Selectins bind to carbohydrate ligands through their lectin-like amino-terminal domains (L-selectin binds to sulfated glycoproteins on the endothelium and P- and E-selectin binds to sialyl-$Lewis^x$-like molecules) on leukocyte-surface glycoprotein and glycolipid carbohydrate groups (104,105). The first step in the neutrophil-endothelium

interaction is the selectin mediated rolling of the neutrophil in the pulmonary vasculature which is transient and reversible. The next step is the firm adhesion of neutrophils to the endothelium which is mediated by the integrins (90). The integrins are composed of heterodimeric noncovalently associated α and β subunits which are expressed on the surface of quiescent neutrophils in an inactive conformation (105). The neutrophil expresses CD11a/18 (LFA-1), CD11b/18 (Mac-1), CD11c/18 (P150,95) integrins which become activated in response to LPS, LTB_4, C5a, and IL-8. Following activation, the neutrophil shape changes from a spherical to a flattened shape (104-106). After activation, L-selection is shed from the neutrophil through proteolytic cleavage (106,107). The activated CD11/18 integrins binds to ICAM-1 and ICAM-2 (intercellular adhesion molecules) which are members of the immunoglobulin superfamily of adhesion receptors expressed by the vascular endothelium (105). These molecules are constituitively expressed on the surface of endothelial cells. However, the expression of ICAM-1 can be upregulated by LPS, TNF-α, IL-1, γ-interferon and oxidants (96,108,109).

Following the firm adhesion of the neutrophil to the pulmonary vascular endothelium, the neutrophils migrate out of the vascular space into the pulmonary interstitium. The transmigration of neutrophils across the endothelium requires a chemotactic gradient that is provided by IL-8 (and other α-chemokines in rodents), LTB_4, and C5a released by the alveolar macrophage (106,110,111). Transendothelial migration may be mediated by platelet-

endothelial cell adhesion molecule-1(PECAM-1), a member of the immunoglobulin superfamily, which is expressed in the junction between endothelial cells (106). PECAM-1 may act as a guide to facilitate neutrophil diapedesis between endothelial cells (106,112). Neutrophil transendothelial migration is also facilitated by endotoxin and chemokines which increase the size of the endothelial intercellular junctions (14,106).

The vascular endothelium is the primary target of neutrophil mediated lung injury. During the process of transmigration, the neutrophil must pass between endothelial cells and digest its way through the basement membrane (96). The activated neutrophil releases proteolytic enzymes from primary (azurophil) and secondary (specific) secretory granules which damage endothelial cells and digest the basement membrane of the endothelium (7,90,110,113). The proteolytic enzymes consist of elastase, cathepsins B, D, and G (a chymotrypsin like enzyme in neutrophils), collagenase, plasminogen activator, gelatinase and heparinase (7). These enzymes are responsible for lung matrix destruction observed in the lungs of patients with ARDS (7).

Another important means of neutrophil mediated pathogen killing is the generation of reactive oxygen species (7,63,96,114). Neutrophil activation results in the assembly and activation of the membrane associated NADPH oxidase complex which generates superoxide anions (O_2-) (respiratory burst) (109). Most of the O_2- produced by the neutrophil is spontaneously or in the presence of superoxide dismutase converted to hydrogen peroxide (H_2O_2).

H_2O_2 is slightly more toxic to cells than O_2- and can easily diffuse across cell membranes (115). In the presence of an iron catalyst (Fe^{+2}), O_2- and H_2O_2 can be converted to a hydroxyl radical (•OH) which is the most toxic and destructive of all the reactive oxygen species generated by neutrophils (115). Finally, the reaction of H_2O_2 with Cl-, catalyzed by myeloperoxidase, leads to the formation of hypochlorous acid (HOCl) (63,96,115,116). Neutrophils have the ability to generate large quantities of HOCl which is an extremely powerful oxidant (91). All cells in the lungs of the host contain intrinsic antioxidant systems to counteract the formation of reactive oxygen species, however. Reduced glutathione (GSH) detoxifies lipid hydroperoxides and H_2O_2. Catalase reduces the toxicity of H_2O_2 by converting it to H_2O which prevents the formation of •OH and HOCl (115).

Neutrophil generated reactive oxygen species cause lipid peroxidation and DNA strand breaks of pathogens and tissues of the host. Although oxidant mediated endothelial cell lipid peroxidation alone may not induce increases in lung microvascular permeability in sepsis-induced ARDS, the reactive oxygen species play a significant role in inactivating anti-protease enzymes and augmenting proteolytic digestion of lung tissue (63,91,109,115). HOCl may not cause tissue damage in the lungs of ARDS patients by itself but has been shown to inactivate α_1-proteinase inhibitor (a potent elastase inhibitor) (91,115). Moreover, all anti-protease enzymes present in the lungs (α_1-proteinase inhibitor, α_2-macroglobulin, leukoproteinase inhibitor) which limit neutrophil

mediated damage in the lung by inactivating elastase, can be inactivated by oxidation (91). Therefore, the HOCl may contribute to lung injury by deactivating anti-protease enzymes allowing neutrophil liberated elastase to attack susceptible tissues in the ARDS patients (91).

Platelets are thought to play a significant role in sepsis-induced ARDS. Endotoxin activates macrophages, monocytes, neutrophils and endothelial cells which release platelet-activating factor (PAF) and other aggregating substances such as TXA_2. Platelets are sequestered in the lung of ARDS patients (117). During aggregation, platelets release TXA_2 and serotonin which causes pulmonary vascular and bronchial smooth muscle constriction which contributes to hypoxemia (117,118). Thrombocytopenia occurs in 50% of patients with non-traumatic ARDS and the severity of thrombocytopenia correlates with the degree of hypoxia (117). The TXA_2 induces increases in pulmonary capillary hydrostatic pressure and augments increases in lung microvascular permeability (15,119-122). In septic shock or severe sepsis, platelet aggregation occurs in many vascular beds (disseminated intravascular coagulation (DIC)). Platelet aggregates impede the perfusion of the lungs increasing hypoxic vasoconstriction and vascular resistance (37).

B. The Eicosanoids: Important Mediators of ARDS

The presence of endotoxin or other stimuli activates the arachidonic acid cascade leading to the formation of eicosanoids which are key mediators of

acute lung injury and inflammation (38,59). Arachidonic acid (20:4n-6) is a fatty acid that contains 20 carbons and 4 double bonds and occupies the sn-2 position of membrane phospholipids. It can be cleaved from this site by phospholipase A_2 and oxygenated in three different enzymatic pathways (38). The cyclooxygenase and lipoxygenase pathways are the primary enzymes which oxygenate arachidonic acid. At this time, it is unknown if the cytochrome P-450 monooxygenase pathway plays a significant role in the formation of eicosanoids in endotoxin-induced acute lung injury (123).

Cyclooxygenase mediates the stereospecific insertion of molecular oxygen (O_2) in the C_9 and C_{15} positions in arachidonic acid (and other fatty acids) (38) forming prostaglandin G_2 (PGG_2). In addition to catalyzing the insertion of molecular oxygen, the peroxidase activity of cyclooxygenase reduces PGG_2 to PGH_2 (123). Two isoforms of cyclooxygenase (or PGH_2 synthase) have been identified that are expressed in lung tissue; COX-1 which is the constitutive form and COX-2 is the inducible form which is upregulated by inflammatory or mitogenic stimuli (127,128). PGH_2 can be converted to a number of different prostanoids depending on the tissue where it is produced (38). PGH_2 can be converted to thromboxane A_2 (TXA_2) in platelets, macrophages, neutrophils and other cell types by the enzyme thromboxane synthase (123). TXA_2 is a potent platelet aggregator and constrictor of vascular and bronchial smooth muscle (38). It also has been shown to be an important mediator of pulmonary hypertension (122), hypoxemia and increased airway

resistance in acute lung injury (129). The spontaneous hydrolysis of TXA_2 results in the formation of TXB_2 which is a stable metabolite that is measured in biological samples.

Prostacyclin (PGI_2) is a major PGH_2 metabolite of vascular endothelial cells, and vascular and airway smooth muscle cells formed by the activity of prostacyclin synthase. PGI_2 is a dilator of vascular smooth muscle and an inhibitor of platelet aggregation and plays an important role in regulating pulmonary blood flow. PGI_2 undergoes spontaneous hydrolysis forming 6-keto-prostaglandin $F_{1\alpha}$. 6-keto-prostaglandin $F_{1\alpha}$ is used as an indicator of PGI_2 in biological samples since the half-life of PGI_2 is brief (minutes)(123).

The isomerization of PGH_2 to prostaglandin E_2 (PGE_2) is catalyzed by a microsomal isomerase which is found in a number of different cell types. PGE_2 has both pro- and anti-inflammatory properties. PGE_2 is a potent vasodilator and has been shown to potentiate the effects of histamine and bradykinin on vascular permeability (123,130). It has been shown to inhibit T-cell proliferation at concentrations $>10^{-8}$M and is thought to be immunosuppressive (131). PGE_2 has also been shown to down regulate TNF-α synthesis by raising intracellular cyclic adenosine monophosphate levels (132-134) *in vitro*.

PGD_2 is the main product of cyclooxygenase activity in human and rat mast cells. It is formed by the isomerization of PGH_2 and its formation is catalyzed by PGD_2 isomerase. Biological properties of PGD_2 include inhibiting platelet aggregation, pulmonary vasoconstriction, bronchoconstriction, and

hyperreactivity (123).

$PGF_{2\alpha}$, a potent vasoconstrictor, is formed by a process that has yet to be identified. It may be formed by a double electron reduction of PGH_2 catalyzed by a PGF synthase and uses NADPH as an electron donor (135). It is an important mediator in asthma since it has been shown to stimulate airway sensory nerve endings and cause reflex bronchoconstriction (123).

The lipoxygenase enzymes are capable of inserting one molecule of oxygen into the 5-, 8-,12- or 15- position of arachidonic acid or other 20 carbon polyunsaturated fatty acids in mammalian cells forming 5-, 8-, 12-, or 15-hydroperoxy, 8, 11, 14-eicosatetraenoic acid (HPETE) (136). Although the products of 8-, 12- and 15-lipoxygenase have been shown to play important roles in pulmonary diseases, more is known about the 5-lipoxygenase metabolites (38). 5-lipoxygenase is a soluble cytosolic protein that translocates to the nuclear envelope where it associates with 5-lipoxygenase-activating protein (FLAP) following cellular activation (137). 5-lipoxygenase converts arachidonic acid to 5-hydroperoxy, eicosatetraenoic acid (5-HPETE). The same enzyme converts 5-HPETE to leukotriene A_4 (136). The hydrolysis of LTA_4 yields LTB_4 and the conjugation of reduced glutathione and LTA_4 catalyzed by LTC_4 synthase yields LTC_4 (38,123). Both LTB_4 and LTC_4, which are transported out of the cell by a carrier-mediated process, undergo further metabolism in the extracellular space (123,138). LTB_4 is metabolized to 20-hydroxy-LTB_4 by cytochrome P-450 (123). The removal of glutamic acid from

LTC_4 yields LTD_4 (mediated by γ-glutamyl transpeptidase) and the subsequent removal of glycine by a dipeptidase yields LTE_4 (123). LTC_4, LTD_4 and LTE_4 are collectively referred to as the slow-reacting substances of anaphylaxis, cysteinyl-leukotrienes or peptido-leukotrienes (136,139).

While 5-lipoxygenase is expressed mainly in myeloid cells, LTA_4 hydrolase and LTC_4 synthase are expressed in a wide variety of cells types (136). LTA_4 that is produced in neutrophils can be exported to the vascular endothelium, erythrocytes or platelets and converted to LTB_4 or LTC_4, LTD_4, and LTE_4 (136,140,141).

The leukotrienes are important mediators of ARDS and have been shown to be significantly elevated in the bronchoalveolar lavage fluid and plasma of ARDS patients (18,110,114). LTB_4 is a potent chemoattractant of neutrophils and monocytes and is formed primarily in macrophages, monocytes, and neutrophils (110,136,142). It has been suggested that the primary role of LTB_4 in ARDS is the recruitment of neutrophils and monocytes which adhere to and damage the endothelium increasing microvascular permeability (110). However, LTB_4 also stimulates neutrophils to synthesize and release IL-8, TNF-α, oxidants, proteases and leukotrienes (114,136,142,143). LTB_4 has also been shown to increase leukocyte adhesion to vascular endothelium in post-capillary venules (13,144-146). Thus, LTB_4 plays a significant role in neutrophil adhesion and activation following leukocyte accumulation within the vasculature of the lungs in ARDS. The cysteinyl-leukotrienes, which have also been shown

to increase in the bronchoalveolar lavage fluid of ARDS patients, play a significant role in mediating lung microvascular permeability and vascular and bronchial smooth muscle constriction in ARDS (9,11,17,18,93,147,148). LTC_4, LTD_4, and to a lesser extent LTE_4 are potent constrictors of vascular and bronchial smooth muscle (123,147,148). Albert and co-workers showed that the perfusion of excised rabbit lungs with LTC_4 and LTD_4 (3×10^{-9} M) significantly increased lung microvascular permeability (11). Using the hamster cheek pouch preparation, Dahlen and others demonstrated that the direct application of LTC_4 and LTD_4 (0.3 to 20 nM) induced arteriole constriction and increased macromolecular (fluorescein isothiocyanate conjugated-dextran) permeability in postcapillary venules (13). Furthermore, the use of a LTC_4/D_4 receptor antagonist in a porcine model of endotoxin and ARDS significantly improved arterial oxygenation and attenuated lung extravascular water accumulation (9). However, not all investigators have shown that LTC_4 and LTD_4 are important mediators of microvascular permeability in the lung. Noonan and others, showed that LTC_4 and LTD_4 (1×10^{-7} M) failed to increase albumin permeability across cultured bovine endothelial cells *in vitro*. They also showed that LTD_4 failed to increase K (capillary filtration coefficient) in an isolated-perfused guinea pig lung (16).

III. MODULATION OF THE INFLAMMATORY RESPONSE WITH ANTI-INFLAMMATORY FATTY ACIDS

A. Dietary Fatty Acids and Membrane Phospholipid Manipulation

Linoleic acid (18:2n-6) is the principle polyunsaturated fatty acid in plant seed oils and accounts for 7% of the calories in the average American diet (149,150). Linoleic acid is an n-6 or ω-6 fatty acid since the first double bond from the methyl end of the fatty acid is at the sixth carbon atom (149,150). It is an essential fatty acid since humans can not synthesize fatty acids with a double bond more distal from the carboxyl end than the ninth carbon atom (150). Linoleic acid can be oxidized as a source of energy, stored in tissue phospholipids or undergo desaturation (by Δ6-desaturase), elongation (by elongase), and desaturation (by Δ5- desaturase) to form arachidonic acid (149,150). The rate limiting step in the conversion of linoleic acid to arachidonic acid is the Δ6-desaturase (151). Arachidonic acid (20:4n-6) is essential for the development of the nervous system and vision in human infants (152). Arachidonic acid is also the precursor of the proinflammatory eicosanoids which play an important role in inflammation and ARDS (15,17,87,136).

α-Linolenic acid (18:3n-3) is also an essential fatty acid that can only be made in plant chloroplasts (150). In humans, α-linolenic acid can undergo

elongation and desaturation forming eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). However, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) make up a very small percentage of the total tissue phospholipid content as compared with arachidonic acid in humans (153,154). This may be due to the high levels of n-6 fatty acids in Western diets which interfere with the formation of eicosapentaenoic acid and docosahexaenoic acid from the desaturation of α-linolenic acid since n-3 and n-6 fatty acids are competitive substrates for the same desaturase and elongase enzymes (150,155).

A number of studies have shown that tissue phospholipid fatty acid composition is significantly influenced by dietary fatty acid intake in humans and animals (154,156-159). Consumption of fish oil which contains the n-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, has been shown to significantly alter tissue phospholipid composition by decreasing tissue phospholipid arachidonic acid and increasing eicosapentaenoic acid and docosahexaenoic acid (150,154,160-162). Eicosapentaenoic acid and docosahexaenoic acid decrease tissue phospholipid arachidonic acid content by depressing Δ6-desaturase activity which reduces arachidonic acid formation from linoleic acid and by competing for acyltransferase enzymes which preferentially incorporate n-3 fatty acids into tissue phospholipids over n-6 fatty acids (149,163). In addition to n-3 fatty acids, γ-linolenic acid, a fatty acid found in high concentrations in borage oil and evening primrose oil, has also

been shown to displace arachidonic acid from tissue phospholipids (164-166). γ-Linolenic acid does not accumulate in tissues but is rapidly elongated to dihomo-γ-linolenic acid which is incorporated into tissue phospholipids (165-168).

B. Effect of Dietary Fish Oil and Borage Oil on the Arachidonic Acid Cascade

Manipulation of membrane phospholipids with dietary n-3 fatty acids from fish oil and γ-linolenic acid from borage oil significantly decreases proinflammatory eicosanoid formation *in vitro* and *in vivo* (154,162,166,168-170). Decreasing tissue arachidonic acid decreases the substrate for 2-series prostaglandin (PGE_2, PGI_2) and 4-series leukotriene (LTB_4, LTC_4, LTD_4 and LTE_4) formation which are key mediators of inflammation in ARDS (38,149,171,172). In addition, the incorporation of eicosapentaenoic acid, docosahexaenoic acid, and dihomo-γ-linolenic acid into tissue phospholipids further suppresses proinflammatory eicosanoid formation by altering the substrate for eicosanoid formation and by competing for 5-lipoxygenase and cyclooxygenase binding sites (149,150,166). The cyclooxygenase metabolites of eicosapentaenoic acid (3-series prostaglandins and 3-series thromboxanes) are less biologically active than the same 2-series metabolites formed from arachidonic acid (150,173,174). Moreover, the 5-series lipoxygenase metabolite of eicosapentaenoic acid, LTB_5, has been shown to be less potent than LTB_4 in recruiting neutrophils (158,175). Dihomo-

γ-linolenic acid is not a substrate for 5-lipoxygenase but it can undergo oxygenation by cyclooxygenase forming PGE_1 and by 15-lipoxygenase forming 15-hydroxyeicosatrienoic acid (15-HETrE) (168,176,177).

C. Effect of Dietary Fish Oil on TNF-α Synthesis

TNF-α is an important mediator of septic shock and ARDS since it has been shown to induce ARDS-like symptoms when injected into experimental animals (178). Furthermore, elevated levels of TNF-α have been found in the serum and bronchoalveolar lavage fluid of ARDS patients (77). Although dietary fish oil has been shown to attenuate inflammation by inhibiting proinflammatory eicosanoid production in animals and humans, a number of studies have shown that TNF-α production increases after dietary fish oil supplementation in rodent macrophages *in vitro* (132,179-181). It has been suggested that n-3 fatty acid supplementation enhances TNF-α production by suppressing PGE_2 and PGI_2 synthesis. PGE_2 and PGI_2 inhibit TNF-α mRNA accumulation and protein release by raising intracellular cAMP in endotoxin stimulated peritoneal macrophages in rodents *in vitro* (133,134,179). In contrast, dietary fish oil has also been shown to decrease TNF-α and IL-1 production in endotoxin stimulated rat kupffer cells and human peripheral blood monocytes (153,182). The significance of TNF-α and IL-1 production in macrophages and monocytes stimulated *in vitro* from humans and animals fed n-3 fatty acids is unknown. However, the anti-inflammatory effects of n-3 fatty

acids are well established *in vivo* (human inflammatory diseases and in experimental animal models of inflammation) (183-186).

D. Effect of Dietary Fish Oil and Borage Oil on Inflammatory Disease and Models of Sepsis and Lung Injury

In the mid-1970's, epidemiological studies revealed that Greenland Eskimos, who consumed large quantities of marine foods rich in n-3 fatty acids, had a much lower incidence of cardiovascular and inflammatory diseases (150,162,183). Subsequent studies have shown that dietary n-3 fatty acid supplementation attenuated the severity of various inflammatory diseases such as rheumatoid arthritis, lupus, and psoriasis (184-186). The attenuation of inflammation by n-3 fatty acid supplementation has been attributed to reducing tissue arachidonic acid, inhibition of proinflammatory eicosanoid formation and altered neutrophil function (149,153,158,169,187). Dietary n-3 fatty acid supplementation in humans has been shown to decrease neutrophil and monocyte migration in response to various chemoattractants (153,158,169).

Dietary n-3 fatty acids have been shown to have beneficial effects in experimental models of sepsis and lung injury (188-191). In a study conducted by Soichiro and associates, dietary eicosapentaenoic acid as compared with olive oil (3 weeks of feeding), significantly attenuated endotoxin-induced microcirculatory damage in rats (188). Murray and co-workers have shown that dietary fish oil as compared with corn oil (8 days of feeding) significantly

attenuated lung water accumulation, improved arterial oxygenation and mean arterial blood pressure (189,190). These improvements observed in pigs fed fish oil were associated with decreases in the levels of TXB_2 (stable metabolite of TXA_2), PGE_2, and 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2). Pomposelli et al. (192) and others showed that prefeeding diets containing menhaden oil (fish oil) as compared with safflower oil for six weeks significantly ameliorated lactic acidosis and tissue hypoperfusion in endotoxic guinea pigs.

Dietary enrichment with γ-linolenic acid from borage oil or blackcurrent seed oil has been reported to ameliorate clinical symptoms associated with rheumatoid arthritis and atopic eczema (193,194). In addition, γ-linolenic acid has been shown to suppress inflammation in animal models of acute and chronic inflammation (195-197). γ-Linolenic acid does not accumulate in tissues but is rapidly converted to dihomo-γ-linolenic acid which is incorporated into tissue phospholipids and accumulates faster than it can be converted to arachidonic acid by Δ5-desaturase (164,166,168,177,198). Dihomo-γ-linolenic acid may attenuate inflammation by competitively inhibiting eicosanoid biosynthesis since it competes with arachidonic acid for phospholipase A_2, lipoxygenase and cyclooxygenase enzymes (168,177).

Dihomo-γ-linolenic acid is not a substrate for 5-lipoxygenase but can be oxygenated by 15-lipoxygenase and cyclooxygenase to form 15-HETrE and PGE_1, respectively, which have been reported to possess anti-inflammatory effects (168,176,177,199). PGE_1 has been shown to improve arterial

oxygenation in ARDS patients and suppress adjuvant arthritis (200,201). 15-HETrE has been shown to inhibit LTB_4 formation in neutrophils (176,177). In addition, dietary supplementation with γ-linolenic acid has been shown to suppress TXB_2 (stable metabolite of TXA_2) formation and increase or not significantly affect 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2) formation in rats (166,202). This result would be desirable in sepsis and inflammatory lung diseases since TXA_2 mediates vascular constriction and platelet aggregation and PGI_2 opposes the actions of TXA_2 (120,203).

Studies on the effectiveness of dietary γ-linolenic acid as compared with other fatty acids in attenuating sepsis and endotoxin-induced lung injury in experimental animals have had mixed results (191,204,205). In a study by Murray et al. (191), dietary supplementation with fish oil and borage oil (8 days of feeding) as compared with corn oil significantly improved gas exchange and arterial oxygen delivery in endotoxic pigs (191). In contrast, Hirschberg and others observed no significant beneficial effects of intravenous administration of γ-linolenic acid from blackcurrent seed oil as compared with soybean oil in endotoxic guinea pigs (204). In addition, Larsson-Backstrom and associates showed that dietary γ-linolenic acid from borage oil (38% of total fatty acids in the diet) as compared with α-linolenic acid from linseed oil (43% of total fatty acids in the diet) or linoleic acid from soybean oil (40% of total fatty acids in the diet) actually decreased survival in endotoxic rats (205).

IV. NUTRITIONAL SUPPORT FOR CRITICALLY ILL PATIENTS

A. The Metabolic Response to Sepsis

Critical illness and sepsis have profound effects on energy expenditure and substrate metabolism (206,207). Resting energy expenditure may increase 60% in patients with severe sepsis (206). Associated with the rise in energy expenditure is tissue catabolism and mobilization of protein, carbohydrates, and fat (187,206,207). Septic patients have increased levels of glucagon, cortisol, insulin and catecholamines which orchestrate the metabolic response to sepsis (187,206,207). Glucagon promotes gluconeogenesis from lactate (Cori cycle) and alanine in the liver which are the primary sources of glucose in septic patients since glycogen stores are limited (207). The catabolism of glycogen, however, is actually lower in septic patients as compared with normal patients consuming the same diet (206). Septic patients are hyperglycemic and hyperinsulinemic and may develop insulin resistance (187). High levels of blood glucose provide the brain and other non-insulin dependent tissues with energy in insulin-resistant septic patients (206).

Fatty acid and glycerol turnover have been shown to increase in septic patients indicating increased lipid oxidation (187,206-208). The increase in lipolysis in septic patients is probably mediated by catecholamines which increase the activity of hormone-sensitive lipase (187). The mobilization of free

fatty acids provides a readily available source of energy to skeletal and cardiac muscle while sparing glucose for the central nervous system (187).

Protein catabolism and muscle wasting are common features of the hypermetabolic state in septic patients (187,206-208). Cortisol and other stress hormones mobilize amino acids from skeletal muscle and other tissues for utilization in gluconeogenesis (207,208). If untreated, protein catabolism can lead to a net loss of 15g of nitrogen per day accounting for a 0.5kg loss of lean body mass each day (187).

B. Clinical Nutrition for the ARDS Patient

The goals of nutritional support for the hypermetabolic critically ill or patient at risk of developing ARDS include the provision of caloric needs, prevention of muscle wasting, and avoidance of increased carbon dioxide production (209). Nutritional support is appropriate in patients who: have lost ≥ 10% of their usual body weight, have had no nutrient intake for 5 to 7 days, or are expected to go without oral nutrient intake for more than ten days (210). Patients should be fed enterally whenever possible (211). The administration of enteral nutrition as compared with parenteral nutrition reduces septic complications (210-213). In addition, previous studies have demonstrated that patients who are fed parenteral are at risk of bacterial translocation (210,214). Nutritional support can be administered parenterally when the patient requires short-term supplementation of deficient oral or enteral nutrition or is intolerant of

enteral nutrition (210).

Septic patients have an increased energy expenditure which causes increased oxygen consumption, carbon dioxide production and respiratory load (215). The loss of lean body mass and muscle wasting is a very serious complication of sepsis that is directly related to diaphragm muscle catabolism and the loss of respiratory muscle contractile force and reduced vital capacity and minute ventilation (209,215). However, the provision of nutritional support to septic patients with diets high in carbohydrate and protein spare skeletal muscle but also increases the ventilatory demand (209,215-217). The increased demand for ventilation may cause respiratory acidosis and acute respiratory failure in patients with reduced ventilatory reserves (215). However, high fat, low carbohydrate enteral formulas have been shown to satisfy the metabolic demand of artificially ventilated patients without maximizing the ventilatory drive (215-217). The consumption of diets with a large percentage of calories from fat designed for patients with respiratory distress decreases CO_2 production, the respiratory quotient and ventilator dependency.(216,217).

In conclusion, sepsis initiates an inflammatory cascade that leads to the disruption of homeostasis and a systemic inflammatory response which may cause the acute respiratory distress syndrome. The alveolar macrophage along with other pneumocytes orchestrates the inflammatory response in the lungs. This process involves the release of the proinflammatory metabolites of arachidonic acid and the cytokines which mediate pulmonary inflammation and

promote neutrophil mediated lung injury. Anti-inflammatory fatty acids (eicosapentaenoic acid, docosahexaenoic acid and γ-linolenic acid) modulate tissue phospholipid composition, arachidonic acid metabolism, and attenuate the severity of inflammatory disease in humans and experimental animals. Therefore, it may be possible to attenuate proinflammatory eicosanoid synthesis and lung injury in an animal model of the acute respiratory distress syndrome by prefeeding high fat, low carbohydrate enteral formulas containing anti-inflammatory fatty acids from fish oil alone or in combination with borage oil as compared with corn oil (represents standard diet).

REFERENCES

1. Ashbaugh DG, Bigelow DB, Petty TL, et al: Acute respiratory distress in adults. *Lancet* 1967; 2:319-323

2. Flick MR: Pulmonary edema and acute lung injury. In: Murray JF, Nadel JA., eds. Textbook of Respiratory Medicine. Philadelphia: W.B. Saunders Company; 1994: 1725-1777

3. Kollef MH, Schuster DP: The acute respiratory distress syndrome. *N Eng J* Med. 1995; 332:27-37

4. Bernard GR, Artigas A, Brigham KL, et al: Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. *Intensive Care Medicine*. 1994; 20:225-232

5. Berne RM, Levy MN: The microcirculation and lymphatics. In: Berne RM, Levy MN., eds. Physiology. St. Louis: C.V. Mosby Company; 1988: 495-507

6. Schmal H, Shanley TP, Jones ML, et al: Role of macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J Immunol* 1996; 156:1963-1972

7. Sibille Y, Reynolds HY: Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 1990; 141:471-501

8. Mulligan MS, Polley MJ, Bayer RJ, et al: Neutrophil-dependent acute lung injury. Requirement for P-selectin (GMP-140). *J Clin Invest* 1992; 90:1600-1607

9. Cohn SM, Kruithoff KL, Rothchild HR, Wang H, Antonsson JB, Fink MP. Beneficial effects of LY203647, a novel leukotriene C_4/D_4 antagonist, on pulmonary function and mesenteric perfusion in a porcine model of endotoxic shock and ARDS. *Circ Shock* 1991; 33:7-16

10. Rabinovici R, Esser KM, Lysko PG, et al: Priming by platelet-activating factor of endotoxin-induced lung injury and cardiovascular shock. *Circ Res* 1991; 69:12-25

11. Albert RK, Greenberg GM, Henderson W. Leukotriene C_4 and D_4 increase pulmonary vascular permeability in excised rabbit lungs. Chest 1983; 83:85s-86s

12. Terashita Z, Shibouta Y, Imura Y, et al: Endothelin-induced sudden death and the possible involvement of platelet activating factor (PAF). *Life Sci* 1989; 45:1911-1918

13. Dahlen S, Bjork J, Hedqvist P, et al: Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: *In vivo* effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci USA* 1981; 78:3887-3891

14. Meyrick B, Berry LCJ, Christman BW: Response of cultured human pulmonary artery endothelial cells to endotoxin. *Am J Physiol* 1995; 268:239-244

15. Schuster DP, Stephenson AH, Holmberg S, et al: Effect of eicosanoid inhibition on the development of pulmonary edema after acute lung injury. J Appl Physiol. 1996; 80:915-923

16. Noonan TC, Selic WM, Kern DF, et al: Mechanism of peptidoleukotriene-induced increase in pulmonary transvascular fluid filtration. *J Appl Physiol* 1986; 61:1928-1934

17. Stephenson AH, Lonigro AJ, Hyers TM, et al: Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* 1988; 138:714-719

18. Antonelli M, Bufi M, DeBlasi RA, et al: Detection of leukotrienes B_4, C_4 and of their isomers in arterial, mixed venous blood and bronchoalveolar lavage fluid from ARDS patients. *Intensive Care Med* 1989; 15:296-301

19. West JB: Respiratory physiology - the essentials. Baltimore : Williams & Wilkins; 1990:21-30

20. Guyton AC, Hall JE: Textbook of medical physiology. Philedelphia: W.B. Saunders Company; 1996:501-509

21. McCormack DG: Control of vascular reactivity. *Crit Care Med* 1995; 3:248-256

22. Tomashefski JFJ: Pulmonary pathology of the adult respiratory distress syndrome. *Clin Chest Med* 1990; 11:593-619

23. Albertine KH: Ultrastructural abnormalities in increased-permeability pulmonary edema. *Clin Chest Med* 1985; 6:345-366

24. Kistler G, Caldwell PRB, Weibel ER: Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lungs. *J Cell Biol* 1967; 33:605-628

25. Bachofen M, Weibel ER: Alterations of the gas exchange apparatus in adult respiratory insufficiency associated with septicemia. *Am Rev Respir Dis* 1977; 116:589-615

26. Nash G, Foley FD, Langlinais PL: Pulmonary interstitial edema and hyaline membranes in adult burn patients: Electron microscope observations. *Hum Pathol* 1974; 5:149-160

27. Adamson IYR, Bowden DH: The type 2 cell as progenitor of alveolar epithelial regeneration: A cytodynamic study in mice after exposure to oxygen. *Lab Invest* 1974; 30:35-42

28. Riede UN, Joachim H, Hassenstein J, et al: The pulmonary air blood barrier of human shock lungs (a clinical, ultrastructural and morphometric study). *Pathol Res Pract* 1978; 162:41-72

29. Fukuda Y, Ishizaki M, Masuda Y, et al. The role of intraalveolar fibrosis in the process of pulmonary structural remodeling in patients with diffuse alveolar damage. *Am J Pathol* 1987; 126:171-182

30. Spencer H: Pathology of the Lung. Oxford: Pergamon Press; 1985:261-267

31. Burkhadt A: Alveolitis and collapse in the pathogenesis of pulmonary fibrosis. *Am Rev Respir Dis* 1989; 140:513-524

32. Bone RC: The sepsis syndrome: Definition and general approach to management. *Clin Chest Med* 1996; 17:175-181

33. Marsh CB, Wewer MD. The pathogenesis of sepsis: Factors that modulate the response to gram-negative bacterial infection. *Clin Chest Med* 1996; 17:183-197

34. Parrillo JE: Shock related to sepsis. In: Bennett JC, Plum F., eds. Cecil: Textbook of Medicine. Philadelphia: W. B. Saunders; 1996:496-501

35. Hinshaw LB: Overview of endotoxin shock. In: Adams Cowley R, Trump BF., eds. Pathophysiology of shock, anoxia, and ischemia. Baltimore: Wiliams and Wilkins; 1982: 219-235

36. Cotran RS, Kumar V, Robbins SL: Hemodynamic disorders, thrombosis, and shock. In: Schoen FJ., ed. Robbins: Pathologic basis of disease. Philadelphia: W. B. Saunder; 1994:93-121

37. Sheagren JN. Shock syndromes related to sepsis. In: Bennett JC, Plum F., eds. Cecil: Textbook of Medicine. Philadelphia: W. B. Saunders; 1992: 1584-1588

38. Makhlouf M, Durando M, Cook JA, et al: Role of eicosanoids in endotoxin-induced lung dysfunction. In: Brigham KL., ed. Endotoxin and the Lungs. New York City: Marcel Dekkar, Inc.; 1994: 153-169

39. Anders Sonesson HR, Zahringer U, Grimmecke HD, et al: Bacterial Endotoxin: Chemical structure and biological activity. In: Brigham KL., ed. Endotoxin and the Lung. New York: Marcel-Dekker; 1994:1-20

40. Rietschel ET, Kirikae T, Ulrich Schade F, et al: Bacterial endotoxin: molecular relationship of structure to activity and function. *FASEB* 1994; 8:217-225

41. Tobias PS, Mathison J, Mintz D, et al: Participation of lipopolysaccharide-binding protein in lipopolysaccharide-dependent macrophage activation. *Am J Respir Cell Mol Biol* 1992; 7:239-245

42. Watson RWG, Redmond HP, Boycheir-Hayes D: Role of endotoxin in mononuclear phagocyte-mediated inflammatory responses. *J Leuk Biol* 1994; 56:95-103

43. Martin TR, Mathison JC, Tobias PS, et al: Lipopolysaccharide binding protein enhances the responsiveness of alveolar macrophages to bacterial lipopolysaccharide: Implications for cytokine production in normal and injured lungs. *J Clin Invest* 1992; 90:2209-2219

44. Wright SD, Tobias PS, Ulevich RJ, et al: Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *J Exp Med* 1989; 170:1231-1241

45. Ferrero E, Hsieh C, Francke U, et al: CD14 is a member of the family of leucine-rich proteins and is encoded by a gene syntenic with multiple receptor genes. *J Immunol* 1990; 145:331-336

46. Delude RL, Fenton MJ, Saverdra R, et al: CD14-mediated translocation of nuclear factor-KB induced by lipopolysaccharide does not require tyrosine kinase activity. *J Biol Chem* 1994; 269:22253-22260

47. Chow CW, Grinstein S, Rotstein OD: Signaling events in monocytes and macrophages. *New Horizons* 1995; 3:342-351

48. Yang Z, Breider MA, Carroll RC, et al: Soluble CD14 and lipopolysaccharide-binding protein from bovine serum enable bacterial lipopolysaccharide-mediated cytotoxicity and activation of bovine vascular endothelial cells in vitro. *J Leuk Biol* 1996; 59:241-247

49. Novogrodsky A, Vanichkin A, Patya M, et al: Prevention of lipopolysaccharide-induced lethal toxicity by tyrosine kinase inhibitors. Science 1994; 264:1319-1322

50. Geng Y, Zhang B, Lotz M: Protein tyrosine kinase activation is required for lipopolysaccharide induction of cytokines in human blood monocytes. *J Immunol* 1993; 151:6692-6700

51. Weinstein SL, June CH, DeFranco AL: Lipopolysaccharide-induced protein tyrosine phosphorylation in human macrophages is mediated by CD14. *J Immunol* 1993; 151:3829-3838

52. Stefanova I, Corcoran ML, Horak EM, et al: Lipopolysacchardie induces activation of CD14-associated protein tyrosine kinase $p53/56^{lyn}$. *J Biol Chem* 1993; 268:20725-20728

53. Weinstein SL, Sanghera JS, Lemke K, et al: Bacterial lipopolysaccharide induces tyrosine phosphorylation and activation of mitogen-activated protein kinases in macrophages. *J Biol Chem* 1992; 267:14955-14962

54. Hambleton J, McMahon M, DeFranco AL: Activation of Raf-1 and mitogen-activated protein kinase in murine macrophages partially mimics lipopolysaccharide-induced signaling events. *J Exp Med* 1995; 182:147-154

55. Heidecker G, Kolch W, Morrison DK, Rapp UR. The role of Raf-1 phosphorylation in signal transduction. *Adv Cancer Res* 1992; 58:53-73

56. Reimann T, Buscher D, Hipskind RA, et al: Lipopolysaccharide induces activation of the Raf-1/MAP kinase pathway: A putative role for Raf-1 in the induction of IL-1B and the TNF-α genes. *J Immunol* 1994; 153:5740-5749

57. Willis SA, Nisen PD: Differential induction of the mitogen-activated protein kinase pathway by bacterial lipopolysaccharide in cultured monocytes and astrocytes. *Biochem J* 1996; 313:519-524

58. Lin L, Wartmann M, Lin AY, et al: $cPLA_2$ is phosphorylated and activated by MAP kinase. *Cell* 1993;72:269-278

59. Serhan CN, Haeggstrom JZ, Leslie CC: Lipid mediator networks in cell signaling: update and impact of cytokines. *FASEB J* 1996;10:1147-1158

60. Glover S, de Carvalho MS, Bayburt T, et al: Translocation of the 85-kDa phospholipase A_2 from cytosol to the nuclear envelope in rat basophilic leukemia cells stimulated with calcium ionophore or IgE/antigen. *J Biol Chem* 1995; 270:15359-15367

61. Schievella AR, Regier MK, Smith WL, et al: Calcium-mediated translocation of cytosolic phospholipase A_2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* 1995; 270:30749-30754

62. Tschaikosky K: Protein kinase C inhibitors supress LPS-induced TNF production in alveolar macrophages and in whole blood: the role of encapsulation into liposomes. *Bichim et Biophys Acta* 1994; 1222:113-121

63. Wong C, Flynn J, Demling RH: Role of oxygen radicals in endotoxin-induced lung injury. *Arch Surg* 1987; 119:77-82

64. Hotchkiss RS, Karl IE: Calcium: A regulator of the inflammatory response in endotoxemia and sepsis. *New Horiz* 1996; 4:58-71

65. Leaver HA, Janah S, Yap PL, et al: Pathways controlling the superoxide response during phagocyte differentiation: Involvement of arachidonic acid and calcium in the response to endotoxin. *FEMS Microbiol Immunol* 1992; 5:261-270

66. Bauldry SA, Bass DA, Cousart SL, et al: TNF priming of phospholipase D in human neutrophils: Correlation between phosphatidic acid production and the respiratory burst. *J Biol Chem* 1991; 266:4173-4179

67. Bankey P, Carlson A, Ortiz M, et al: Tumor necrosis factor production by kupffer cells requires protein kinase C activation. *J Surg Res* 1990; 49:256-261

68. Ghosh S, Baltimore D: Activation *in vitro* of NF-kB by phosphorylation of its inhibitor IkB *Nature*. 1990; 344:678-682

69. Hawiger J: Lipopolysaccharide-induced signal transduction and gene transcription. In: Brigham KL., ed. Endotoxin in the Lung. New York: Marcel Dekker; 1994 :69-82

70. Fels AOS, Cohn ZA: The alveolar macrophage. *J Appl Physiol* 1986; 60:353-369

71. Goya T, Abe M, Shimura H, et al: Characteristics of alveolar macrophages in experimental septic lung. *J Leuk Biol* 1992; 52:236-243

72. Parsons PE, Moore FA, Moore EE, et al: Studies on the role of tumor necrosis factor in adult respiratory distress syndrome. *Am Rev Respir Dis* 1992; 146:694-700

73. Nhieu JT, Misset B, Lebargy F, et al: Expression of tumor necrosis factor-α gene in alveolar macrophages from patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1993; 147:1585-1589

74. Hyers TM, Tricomi SM, Dettenmeier PA, Fowler AA: Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991; 144:268-271

75. Tracey KJ, Lowry SF, Cerami A: Cachetin/TNF-α in septic shock and septic adult respiratory distress syndrome. *Am Rev Respir Dis* 1988; 138:1377-1379

76. Johnson J, Brigham KL, Jesmok G, Meyrick B: Morphologic changes in lungs of anesthetized sheep following intravenous infusion of recombinant tumor necorsis factor α. *Am Rev Respir Dis* 1991; 144:179-186

77. Suter PM, Suter S, Girardin E, et al: High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992; 145:1016-1022

78. Strieter RM, Koch AE, Anthony VB, et al: The immunopathology of chemotactic cytokines: The role of interleukin-8 and monocyte chemoattractant protein-1. *J Lab Clin Med* 1994; 123:183-197

79. Xing Z, Jordana M, Kirpalani H, et al: Cytokine expression by neutrophils and macrophages *in vivo*: Endotoxin induces tumor necrosis factor-α, macrophage inflammatory protein-2, interleukin-1β, and interleukin-6 but not RANTES or transforming growth factor-β mRNA expression in acute lung inflammation. *Am J Respir Cell Mol Biol* 1994; 10:148-153

80. Villard J, Dayer-Pastore F, Hamacher J, et al: GRO alpha and interleukin-8 in *pneumocystis carinii* or bacterial pneumonia and adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1995; 152:1549-1554

81. Strieter RM, Standiford TJ, Huffnagle GB, et al: The good, the bad, and the ugly: The role of chemokines in models of human disease. *J Immunol* 1996; 156:3583-3586

82. Donnelly SC, Strieter RM, Kunkel SL, et al: Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups. *Lancet* 1993; 341:643-647

83. Huang S, Paulauskis JD, Godleski JJ, et al: Expression of macrophage inflammatory protein-2 and KC mRNA in pulmonary inflammation. *Amer J Pathol* 1992; 141:981-988

84. Driscoll KE, Hassenbein DG, Carter J, et al: Macrophage inflammatory proteins 1 and 2: Expression by rat alveolar macrophages, fibroblasts, and epithelial cells and in rat lung after mineral dust exposure. *AmerJ Respir Cell and Mol Biol* 1993; 8:311-318

85. Driscoll KE, Hassenbein DG, Howard BW, et al: Cloning, expression, and functional characterization of rat MIP-2: a neutrophil chemoattractant and epithelial cell mitogen. *J Leuk Biol* 1995; 58:359-364

86. Brigham KL, Serafin W, Zadoff A, et al: Prostaglandin E_2 attenuation of sheep lung responses to endotoxin. *J Appl Physiol* 1988; 64:2568-2574

87. Li EJ, Cook JA, Wise WC, et al: Effect of LTB_4 receptor antagonists in endotoxic shock in the rat. *Circ Shock* 1991; 34:385-392

88. Turner CR, Quinlan MF, Schwartz LW, et al: Therapeutic intervention in a rat model of ARDS: I. Dual inhibition of arachidonic acid metabolism. *Circ Shock* 1990; 32:231-242

89. Turner CR, Lackey MN, Quinlan MF, et al: Therapeutic intervention in a rat model of adult respiratory distress syndrome: II. Lipooxygenase pathway inhibition. *Circ Shock* 1991; 34:263-269

90. Sharar SR, Winn RK, Harlan JM: Endotoxin-induced interactions of inflammatory cells with the lungs. In: Brigham KL., ed. Endotoxin and the lungs. New York: Marcel Dekker, Inc.; 1994:229-265

91. Weiss SJ: Tissue destruction by neutrophils. *N Engl J Med* 1989; 320:365-375

92. Kubes P, Suzuki M, Granger DN: Modulation of PAF-induced leukocyte adherence and increased microvascular permeability. *Am J Physiol* 1990; 259:G859-G864

93. Cook JA, Li EJ, Spicer KM, et al: Effect of leukotriene receptor antagonists on vascular permeability during endotoxic shock. *Circ Shock* 1990; 32:209-218

94. Weiland JE, Davis WB, Holter JF, et al: Lung neutrophils in the adult respiratory distress syndrome. Clinical and pathophysiologic significance. *Am Rev Respir Dis* 1986; 133:218-225

95. Yoshimura K, Nakagawa S, Koyama S, et al: Leukotriene B_4 induces lung injury in the rabbitt: role of neutrophils and effect of indomethacin. *J Appl Physiol* 1993; 74:2174-2179

96. Varani J, Ward PA: Mechansims of endothelial cell injury in acute inflammation. *Shock* 1994; 2:311-319

97. Repine JE: Scientific perspectives on adult respiratory distress syndrome. *Lancet* 1992; 339:466-472

98. Kuby J. Immunology. New York: W. H. Freeman; 1992:39.

99. Doerschuk CM, Allard MF, Martin BA, et al: Marginated pool of neutrophils in the lungs of rabbits. *J Appl Physiol* 1987 ;63:1806-1815

100. Doerschuk CM, Beyers N, Coxson HO, et al: Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung. *J Appl Physiol* 1993; 74:3040-3045

101. Read MA, Meyrick BO. Effects of Endotoxin on Lung Endothelium. In: Brigham KL., ed. Endotoxin and the Lung. New York, NY: Marcel Dekker, Inc.; 1994:83-110

102. Ley K, Gaehtgens P, Fennie C, et al: Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. *Blood* 1991; 77:2553-2555

103. Springer TA: Adhesion receptors of the immune system. *Nature.* 1990; 346:425-434

104. Malik AB: Endothelial cell interactions and integrins. *New Horizons.* 1993; 1:37-51

105. Donwey GP, Fialkow L, Fukushima T, et al: Initial interaction of leukocytes within the microvasculature: deformability, adhesion and transmicgration. *New Horiz* 1995; 3:219-228

106. Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. *FASEB J* 1994; 8:504-512

107. Ryan US, Worthington RE: Cell-cell contact mechanisms. *Curr Op Immunol* 1992; 4:33-37

108. Morita Y, Clemens MG, Miller LS, et al: Reactive oxidants mediate TNF-α-induced leukocyte adhesion to rat mesenteric venular endothelium. *Am J Physiol* 1995; 269:H1833-H1842

109. Kinnula VL, Crapo JD, Raivio KO: Biology of disease. Generation and disposal of reactive oxygen metabolites in the lung. *Lab Invest* 1995; 73:3-19

110. Rosengren S, Olofsson AM, Von Andrian UH, et al: Leukotriene B_4-induced neutrophil-mediated endothelial leakage in vitro and in vivo. *J Appl Physiol* 1991; 71:1322-1330

111. Goldstein IM, Shak S: Host defenses in the lung: neutrophils, complement and other humoral mediators. In: Murray JF, Nadel JA., eds. Textbook of respiratory medicine. Philadelphia: W.B. Saunders Company; 1994: 402-418

112. Muller WA, Weigl SA, Deng X, et al: PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993; 178:449-460

113. Gee MH, Albertine KH: Neutrophil-endothelial cell interactions in the lung. *Ann Rev Physiol.* 1993; 55:227-248

114. Yoshimura K, Nakagawa S, Koyama S, et al: Roles of neutrophil elastase and superoxide anion in leukotriene B_4-induced lung injury in rabbit. *J Appl Physiol* 1994; 76:91-96

115. Repine JE, Parsons PE: Oxidant-antioxidant balance in endotoxin-induced oxidative injury and tolerance to oxidative injury. In: Brigham KL., ed. Endotoxin and the lung. New York: Marcel-Dekker, Inc.; 1994:207-227

116. Murakami H, Nakao A, Kishimoto W, et al: Detection of O_2- generation and neutrophil accumulation in rat lungs after acute necrotizing pancreatitis. *Surgery.* 1995; 118:547-554

117. Heffner JE, Sahn SA, Repine JE: The role of platelets in the adult respiratory distress syndrome: Cluprits or bystanders?. *Am Rev Respir Dis* 1987; 135:482-492

118. Thies SD, Corbin RS, Goff CD, et al: Thromboxane receptor blockade improves oxygenation in an experimental model of acute lung injury. *Ann Thorac Surg* 1996; 61:1453-1457

119. Needleman P, Minkes M, Raz A: Thromboxanes: selective biosynthesis and distinct biological properties. *Science* 1976; 193:163-165

120. Hales CA, Sonne L, Peterson MB, et al: Role of thromboxane and prostacyclin in pulmonary vasomotor changes after endotoxin in dogs. *J Clin Invest.* 1981; 68:497-505

121. Peterson MB, Huttemeier PC, Zapol WM: Thromboxane mediates acute pulmonary hypertension in sheep extracorporeal perfusion. *Am J Physiol* 1982; 243:H471-H479

122. Wisner D, Sturm J, Sutter G, et al: Thromboxane receptor blockade in an animal model of ARDS. *Surgery* 1988; 104:91-97

123. Holtzman MJ: Arachidonic acid metabolism in airway epithelial cells. *Ann Rev Physiol* 1992; 54:303-329

127. O'Neill GP, Ford-Hutchinson AW: Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Letters* 1993; 330:156-160

128. Wilborn J, DeWitt DL, Peters-Golden M: Expression and role of cyclooxygenase isoforms in alveolar and peritoneal macrophages. *Am J Physiol* 1995; 268:L294-L301

129. Turner CR, Lackey MN, Quinlan MF, et al: Therapeutic intervention in a rat model of adult respiratory distress syndrome: III. Cyclooxygenase pathway inhibition. *Circ Shock* 1991; 34:270-277

130. Conary JT, Parker RE, Christman BW, et al: Protection of rabbit lungs from endotoxin injury by in vivo hyperexpression of the prostaglandin G/H synthase gene. *J Clin Invest* 1994; 93:1834-1840

131. Santoli D, Phillips PD, Colt TL, et al: Suppression of interleukin 2-dependent human T cell growth in vitro by prostaglandin E and their precursor fatty acids. *J Clin Invest* 1990; 85:424-432

132. Hardardottir I, Kinsella JE: Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* 1991; 1095:187-195

133. Renz H, Gong J, Schmidt A, et al: Release of tumor necrosis factor-a from macrophages: enhancement and supression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. *J Immunol* 1988; 141: 2388-2393

134. Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Remick D. Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. *J Biol Chem* 1988; 263:5380-5384

135. Smith WL: Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 1992; 263:F181-F191

136. Lewis RA, Austen KF, Soberman RJ: Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med* 1990; 323:645-655

137. Brock TG, McNish RW, Peters-Golden M: Translocation and leukotriene synthetic capacity of nuclear 5-lipoxygenase in rat basophilic leukemia cells and alveolar macrophages. *J Biol Chem* 1995; 270:21652-21658

138. Lam BK, Gagnon L, Austen KF, et al: The mechanism of leukotriene B_4 export from human polymorphonuclear leukocytes. *J Biol Chem* 1990; 265:13438-13441

139. Sjolander A, Gronroos E, Hammarstrom S, et al: Leukotriene D_4 and E_4 induce transmembrane signaling in human epithelial cells. *J Biol Chem* 1990; 265:20976-20981

140. Feinmark SJ, Cannon PJ: Endothelial cell leukotriene C_4 synthesis results from intercellular transfer of leukotriene A_4 synthesized by polymorphonuclear leukocytes. *J Biol Chem* 1986; 261:16466-16472

141. Maclouf JA, Murphy RC: Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets. *J Biol Chem.* 1988; 263:174-181

142. McCain RW, Holden EP, Blackwell TR, et al: Leukotriene B_4 stimulates human polymorphonuclear leukocytes to synthesize and release interleukin-8 *in vitro*. *Am J Respir Cell Mol Biol* 1994; 10:651-657

143. Goldman G, Welbourn R, Kobzik L, et al: Lavage with leukotriene B_4 induces lung generation of tumor necrosis factor-α that in turn mediates neutrophil diapedesis. *Surgery* 1993; 113:297-303

144. Zimmerman BJ, Holt JW, Paulson JC, et al: Molecular determinants of lipid mediator-induced leukocyte adherence an emigration in rat mesenteric venules. *Am J Physiol* 1994; 266:H847-H853

145. Damtew B, Goldsmith G, Tserng K, et al: Human neutrophil adhesion to bovine aortic endothelium. Evidence for endothelial lipoxygenase activity. *J Leukoc Biol* 1993; 53:619-629

146. Bienvenu K, Russell J, Granger DN: Leukotriene B_4 mediates shear rate-dependent leukocyte adhesion in mesenteric venules. *Circ Res* 1992; 71:906-911

147. Davidson D, Drafta D: Prolonged pulmonary hypertension caused by platelet-activating factor and leukotriene C_4 in the rat lung. *J Appl Physiol.* 1992; 73:955-961

148. Abela A, Daniel EE: Neural and myogenic effects of leukotrienes C_4, D_4, and E_4, on canine bronchial smooth muscle. *Am J Physiol.* 1994; 266:L414-L425

149. Kinsella JE, Lokesh B, Broughton S, Whelan J: Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* 1990; 6:24-44

150. Leaf A, Weber PC: Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 1988; 318:549-557

151. Kinsella JE, Lokesh B: Dietary lipids, eicosanoids, and the immune system. *Crit Care Med* 1990; 8:S94-S113

152. Salem NJ, Wegher B, Mena P, et al: Arachidonic and docosaxehaenoc acids are biosynthesized from their 18-carbon precursors in human infants. *Proc Natl Acad Sci* 1996; 93:49-54

153. Endres S, Ghorbani R, Kelley VE, et al: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989; 320:265-271

154. Mori TA, Vandongen R, Mananian F, et al: Plasma lipid levels and platelet and neutrophil function in patients with vascular disease following fish oil and olive oil supplementation. *Metabol* 1992; 41:1059-1067

155. Holman RT: Nutritional and metabolic interrelationships between fatty acids. *Fed Proc* 1964; 23:1062-1067

156. Calder PC, Bond JA, Harvey DJ, et al: Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* 1990; 269:807-814

157. Chapkin RS, Carmichael SL: Effects of dietary n-3 and n-6 polyunsaturated fatty acids on macrophage phospholipid classes and subclasses. *Lipids* 1990; 25:827-834

158. Lee TH, Hoover RL, Williams JD, et al: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 1985; 312:1217-1224

159. Pullman-Mooar S, LaPosata M, Lem D, et al: Alteration of the cellular fatty acid profile and the production of eicosanoids in human monocytes by gamma-linolenic acid. *Arthritis Rheum* 1990; 33:1526-1533

160. Nassar BA, Huang YS, Horrobin DF: Response of tissue phospholipid fatty acid composition to dietary (n-6) and replacement with marine (n-3) and saturated fatty acids in the rat. *Nutr Res* 1986; 6:1397-1409

161. Lands WEM: Biochemistry and physiology of n-3 fatty acids. *FASEB J* 1992;6:2530-2536

162. von Schacky C, Fischer S, Weber PC: Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest.* 1985; 76:1626-1631

163. Stubbs CD, Smith AD: The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim et Biophys Acta* 1984; 779:89-137

164. Das UN, Horrobin DF, Begin ME, et al: Clinical significance of essential fatty acids. *Nutrition* 1988; 4:337-341

165. Carter JP: Gamma-linolenic acid as a nutrient. *Food Techol* 1988;June:72-82

166. Karlstad MD, DeMichele SJ, Leathem WD,et al: Effect of intravenous lipid emulsions enriched with gamma-linolenic acid on plasma n-6 fatty acids and prostaglandin biosynthesis after burn and endotoxin injury in rats. *Crit Care Med.* 1993; 21:1740-1749

167. Manku MS, Morse-Fisher N, Horrobin DF: Changes in human plasma essential fatty acid levels as a result of administration of linoleic acid and gamma-linolenic acid. *Euro J Clin Nutr* 1988; 42:55-60

168. Ziboh VA, Fletcher MP: Dose-response effects of dietary τ-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B_4. *Am J Clin Nutr* 1992; 55:39-45

169. Sperling RI, Benincaso AI, Knoell CT, et al: Dietary Ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J Clin Invest* 1993; 91:651-660

170. Barre DE, Holub BJ, Chapkin RS et al: The effect of borage oil supplementation on human platelet aggregration, thromboxane B_2, prostaglandin E_1 and E_2 formation. *Nutr Res* 1993; 13:739-751

171. Matthay MA, Eschenbacher WL, Goetzl EJ: Elevated concentrations of: leukotriene D4 in pulmonary edema fluid of patients with the adult respiratory distress syndrome. *J Clin Immumol* 1984; 4:479-483

172. Loyd JE, Tagari P, Chapel H, et al: Persistant generation of peptido leukotrienes in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1984; 29:A118

173. Fischer S, Weber PC: Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature* 1984; 307:165-168

174. Needleman P, Raz A, Minkes MS, et al: Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci USA* 1979; 76:944-948

175. Lee TH, Mencia-Huerta J, Shih C, et al: Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J.Biol.Chem* 1984; 259:2383-2389

176. Chapkin RS, Miller CC, Somers SD, et al: Ability of 15-hydroxyeicosatrienoic acid (15-OH-20:3) to modulate macrophage arachidonic acid metabolism. *Biochem Biophys Res Commun* 1988;153:799-804

177. Chilton-Lopez T, Surette ME, Swan DD, et al: Metabolism of gammalinolenic acid in human neutrophils. *J Immunol* 1996; 156:2941-2947

178. Ferrari-Balliviera E, Mealy K, Smith RJ, et al: Tumor necrosis factor induces adult respiratory distress syndrome in rats. *Arch Surg* 1989; 124:1400-1405

179. Hardardottir I, Whelan J, Surette ME, et al: The effects of dietary n-3 polyunsaturated fatty acids and cyclic AMP-elevating agents on tumor necrosis factor production by murine-resident and thioglycollate-elicited peritoneal macrophages. *J Nutr Biochem* 1993; 4:534-542

180. Hardardottir I, Kinsella JE: Increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. *J Nutr* 1992; 122:1942-1951

181. Carrick JB, Schnellmann RG, Moore JN: Dietary source of Omega-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. *Shock* 1994; 2:421-426

182. Billiar TR, Bankey PE, Svingen BA, et al: Fatty acid intake and kuppfer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* 1988; 104:343-349

183. Kromann N, Green A: Epidemiological studies in the Upernavik district, Greenland: Incidence of some chronic diseases. *Acta Scand Med* 1980; 208:401-406

184. Bittiner SB, Cartwright I, Tucker WFG, et al: A double-blind, randomized, placebo-controlled trial of fish oil in psoriasis. *Lancet* 1988; 1:378-380

185. Kremer JM, Jubiz W, Michalek A, et al: Fish oil fatty acid supplementation in active rheumatoid arthritis: a double-blind, controlled, crossover study. *Ann Intern Med.* 1987; 106:497-502

186. Kelley VE, Ferretti A, Izui S, et al: A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites, and supresses lupus in MRL-lpr mice. *J Immunol* 1985; 134:1914-1919

187. Wojnar MM, Hawkins WG, Lang CH: Nutritional support of the septic patient. *Crit Care Clin* 1995;11:717-733

188. Miura S, Imaeda H, Shiozaki H, et al: Attenuation of endotoxin-induced intestinal microcirculatory damage by eicosapentanoic acid. Am J Physiol 1993; 264:G828-G834

189. Murray MJ, Svingen BA, Holman RT, et al: Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *J Parenter Enter Nutr* 1991; 15:152-158

190. Murray MJ, Svingen BA, Yaksh TL, et al: Effects of endotoxin on pigs prefed omega-3 vs. omega-6 fatty acid-enriched diets. *Am J Physiol* 1993; 265:E920-E927

191. Murray MJ, Kumar M, Gregory TJ, et al: Enteral diets enriched in eicosapentaenoic acid and gamma-linolenic acid attenuate cardiopulmonary dysfunction in a porcine model of acute lung injury. Am *J Physiol* 1995; 269:H2090-H2099

192. Pomposelli JJ, Flores EA, Blackburn GL, et al: Diets enriched with N-3 fatty acids ameliorate lactic acidosis by improving endotoxin-induced tissue hypoperfusion in guinea pigs. *Ann Surg* 1991; 213:166-176

193. Leventhal LJ, Boyce EG, Zurier RB: Treatment of rheumatoid arthritis with blackcurrant seed oil. *Br J Rheumatol* 1994; 33:847-853

194. Wright S, Burton JL: Oral evening primrose seed oil improves atopic eczema. *Lancet.* 1982; 2:1120

195. Tate G, Mandell BF, LaPosata M, et al. Suppression of acute and chronic inflammation by dietary gamma linolenic acid. *J Rheumatol* 1989; 16:729-734

196. Kies C. Evening primrose oil: a source of gamma linolenic acid. *CFW* 1989; 34:1016-1020

197. Tate GA, Mandell BF, Karmali RA, et al: Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. *Arthrit Rheumat* 1988; 31:1543-1551

198. Chapkin RS, Somers SD, Schumacher L, et al: Fatty acid composition of macrophage phospholipids in mice fed fish or borage oil. *Lipids* 1988; 23:380-383

199. Fan YY, Chapkin RS: Mouse peritoneal macrophage prostaglandin E_1 synthesis is altered by dietary gamma-linolenic acid. *J Nutr* 1992; 122:1600-1606

200. Abraham E, Park YC, Covington P, et al: Liposomal prostaglandin E_1 in acute respiratory distress syndrome: A placebo-controlled, randomized, double-blind, multicenter clinical trial. *Crit Care Med* 1996; 24:10-15

201. Zurier RB: Effect of prostaglandin E_1 on adjuvant arthritis. *Nature* 1971; 234:304-305

202. Kirtland SJ, Buchanan T, Cowan I, et al: Dihomo-gamma-linolenic acid increases prostacyclin production and reduces platelet thromboxane synthesis in the rat. *Prog Lipid Res* 1986; 25:331-334

203. Pittet JF, Morel DR: Imbalance between plasma levels of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ during subacute endotoxin-induced hyperdynamic sepsis or multiple organ failure syndrome in sheep. *Circ Shock* 1991; 35:65-77

204. Hirschberg Y, Shackelford A, Mascioli EA, et al: The response to endotoxin in guinea pigs after intravenous black currant seed oil *Lipids.* 1990; 25:491-496

205. Larsson-Backstrom C, Lindmark L, Svensson L. Effect of dietary alpha and gamma-linolenic acids on liver fatty acids, lipid metabolism, and survival in sepsis. *Shock* 1995; 4:11-20

206. Elwyn DH, Askanazi J. Fuel utilization in critical illness. In: Hall JB, Schmidt GA, Wood LDH., eds. Principles of critical care. New York: McGraw-Hill; 1992: 1057-1064

207. Jeejeebhoy KN: Nutriton in critical illness. In: Ayres SM, Grenvik A, Holbrook PR, Shoemaker WC., eds. Textbook of critical care. Philadelphia: W.B. Saunders; 1995: 1106-1115

208. Kinney JM. Metabolic responses of the critically ill patient. *Crit Care Clin.* 1995; 11:569-585

209. Pinard B, Geller E: Nutritional support during pulmonary failure. *Crit Care Clin* 1995; 11:705-715

210. Bower RH. Nutrition during critical illness and sepsis. *Crit Care Med.* 1993; 1:348-352

211. Grote AE, Elwyn DH, Takala F, et al: Nutritional and metabolic effects of enteral and parenteral feeding in severely injured patients. *Clin.Nutr* 1987; 6:161-167

212. Kudsk KA, Croce MA, Fabian TC, et al. Enteral versus parenteral feeding: Effects of septic morbidity after blunt and penetrating trauma. *Ann Surg* 1992; 215:503-513.

213. Moore F, Moore E, Jones T, et al. TEN versus TPN following major abdominal trauma: Reduced septic morbidity. *J Trauma.* 1989;29:916-923.

214. Alverdy JC, Aoys E, Moss GS. Total parenteral nutrition promotes bacterial translocation from the gut. *Surgery* 1988;104:185-190.

215. Bursztein S, D'Attellis NP, Askanazi J. Nutrition and the respiratory system. In: Hall JB, Schmidt GA, Wood LDH., eds. Principles of critical care. New York: McGraw-Hill; 1992:1093-1097.

216. Al-Saady NM, Blackmore CM, Bennett ED. High fat, low carbohydrate, enteral feeding lowers $PaCO_2$ and reduces the period of ventilation in artifically ventilated patients. *Intensive Care Med* 1989; 15:290-295

217. Askanazi J, Nordenstrom J, Rosenbaum SH, et al: Nutrition for the patient with respiratory failure: Glucose vs. fat. *Anesthesiology*. 1981;54:373-377.

PART 3

EXPERIMENTAL INVESTIGATIONS OF THE EFFECTS OF N-3 AND N-6 FATTY ACIDS IN ENDOTOXIN-INDUCED LUNG INJURY

I. EFFECTS OF EICOSAPENTAENOIC ACID AND γ-LINOLENIC ACID ON LUNG PERMEABILITY AND ALVEOLAR MACROPHAGE EICOSANOID SYNTHESIS IN ENDOTOXIC RATS[1]

A. Abstract

Proinflammatory eicosanoids (cyclooxygenase and lipoxygenase metabolites of arachidonic acid) released by alveolar macrophages play an important role in endotoxin induced acute lung injury. We investigated the effect of prefeeding rats for 21 days with enteral diets that provided the anti-inflammatory fatty acids, eicosapentaenoic acid and γ-linolenic acid (derived from fish oil and borage oil, respectively), as compared with an n-6 fatty acid enriched diet (corn oil) on 1) lung microvascular protein permeability, arterial blood pressure, and platelet and white blood cells in a model of endotoxin-induced acute lung injury, 2) alveolar macrophage prostaglandin and leukotriene synthesis, and 3) liver and alveolar macrophage phospholipid fatty acid composition. Rats were randomized into four dietary treatment groups and fed nutritionally complete diets (300 $kcal \cdot kg^{-1} \cdot day^{-1}$) containing 55.2% of the total calories from fat with either 97% corn oil, 20% fish oil, 20% fish and 5% borage oil, or 20% fish and 20% borage oil for 21 days. On day 22, lung microvascular protein permeability, mean arterial blood pressure, and platelet and white blood cell counts were determined for 2 hours following an iv injection of *Salmonella Enteritidis* endotoxin (10 mg/kg). In a second group of prefed rats, the phospholipid fatty acid composition was determined in liver and alveolar macrophages. Alveolar macrophages were harvested by

bronchoalveolar lavage and stimulated *in vitro* with a calcium ionophore (A23187) and the concentrations of leukotriene B_4 and B_5 , thromboxane A_2, prostaglandin E_2, and 6-keto-prostaglandin $F_{1\alpha}$ were measured in a third group of prefed rats. Lung permeability was greatest with corn oil and was significantly attenuated with 20% fish oil and 20% fish and 5% borage oil and this effect approached significance with 20% fish and 20% borage oil (P=0.06). The early and late hypotensive effects of endotoxin were attenuated with 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil as compared with corn oil. Leukotriene B_4, prostaglandin E_2, and thromboxane B_2 released from A23187 stimulated alveolar macrophages were significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. The increase in lung microvascular protein permeability with 20% fish and 20% borage oil was not significantly different than 20% fish oil (p=0.20) and 20% fish and 5% borage oil (p=0.31). Alveolar macrophage and liver phospholipid levels of arachidonic acid were lower and the levels of eicosapentaenoic acid and docosahexaenoic acid were higher with 20% fish oil, 20% and 5% borage, and 20% fish and 20% borage oil as compared with corn oil. Dihomo-γ-linolenic acid, the desaturated and elongated intermediate of γ-linolenic acid, was elevated with 20% fish and 20% borage oil as compared with 20% fish oil and 20% fish and 5% borage oil. The severity of pulmonary microvascular protein permeability and the degree of hypotension was reduced with fish or fish and borage oil diets as compared with corn oil in endotoxic rats. The reduced

synthesis of the proinflammatory arachidonic acid derived mediators, leukotriene B_4, thromboxane B_2, and prostaglandin E_2, from stimulated alveolar macrophages was indicative of a decrease in arachidonic acid and an increase in eicosapentaenoic acid and docosahexaenoic acid in cell membrane phospholipids. The modulation of proinflammatory eicosanoid synthesis with anti-inflammatory fatty acids, eicosapentaenoic acid, docosapentaenoic acid and γ-linolenic acid, may have reduced the severity of pulmonary permeability and hypotension after endotoxin administration.

[1]This manuscript has been published in similar form with co-authors Jay Whelan, Stephen J. DeMichele, Carolyn C. Snider, Julie A. Guszcza, Kate J. Claycombe, Gary T. Smith, Timothy J. Gregory, and Michael D. Karlstad in: Critical Care Medicine 1996; in Press.

B. Introduction

Endotoxin-induced acute lung injury is characterized by an increase in pulmonary capillary permeability, pulmonary edema, and hypoxemia (1). The alveolar macrophage is an inflammatory cell that resides in great number in the alveolar spaces of the lung and is thought to play a central role in the pathogenesis of acute lung injury. Alveolar macrophages release proinflammatory eicosanoids derived from the lipoxygenase and cyclooxygenase pathways of arachidonic acid (20:4n-6) metabolism during

endotoxemia. These inflammatory mediators have been implicated in the development of acute lung injury. Levels of the leukotrienes derived from arachidonic acid (4-series leukotrienes) are elevated in bronchoalveolar lavage fluid of patients with the acute respiratory distress syndrome (ARDS) (2). Leukotriene B_4, a potent chemoattractant for neutrophils, is capable of stimulating neutrophils to adhere to pulmonary microvascular endothelial cells (3) and secrete oxygen free radicals and proteolytic enzymes that mediate microvascular endothelial damage and pulmonary edema (4). The cysteinyl-leukotrienes, leukotriene C_4 and leukotriene D_4, have been shown to directly increase pulmonary microvascular permeability and increase pulmonary vascular resistance (5,6). Prostaglandins derived from arachidonic acid (2-series prostaglandins), including prostaglandin E_2 and thromboxane A_2, have been implicated as important causative agents in septic shock and acute lung injury in humans and animals (7).

Previous studies have shown that altering the availability of arachidonic acid in tissue phospholipids through dietary fatty acids can reduce the severity of inflammatory injury (8-10). Dietary fish oil (containing eicosapentaenoic (20:5n-3) and docosahexaenoic acids (22:6n-3)) supplements have been used to modulate eicosanoid biosynthesis in a number of inflammatory states (11,12). Fish oil has been shown to reduce arachidonic acid and increase eicosapentaenoic acid and docosapentaenoic acid in tissue phospholipids (13,14). The elevated levels of eicosapentaenoic acid in tissue phospholipids

can compete with arachidonic acid for cyclooxygenase and 5-lipoxygenase binding sites and reduce eicosanoid formation from arachidonic acid (14,15). Eicosapentaenoic acid can be converted to the 3-series prostaglandins and 5-series leukotrienes (16) which are considered less biologically active and less inflammatory than the arachidonic acid derived 2-series prostaglandins and 4-series leukotrienes (17,18). Borage oil is a plant seed oil that contains a high level of γ-linolenic acid (18:3n-6) and has been shown to affect prostaglandin and leukotriene metabolism and suppress acute and chronic inflammation in animal models of injury (9,12). We recently showed in a rat model of burn and endotoxin injury that parenteral diets supplemented with borage oil modulated 2-series prostaglandin levels in plasma by reducing the ratio of thromboxane B_2 to 6-keto-prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) (19). Dietary γ-linolenic acid does not accumulate in tissue phospholipids because it is rapidly elongated to dihomo-γ-linolenic acid. Dihomo-γ-linolenic acid incorporation in membrane phospholipids has been shown to increase prostaglandin E_1 synthesis in stimulated murine peritoneal macrophages (20) and indirectly decrease leukotriene formation by inhibition of 5-lipoxygenase in macrophages (21). The iv infusion of liposomal prostaglandin E_1 in patients with ARDS was shown to improve oxygenation, increase pulmonary compliance and decrease ventilator dependency as compared with those given a placebo (22).

Since fish and borage oil modulate different aspects of prostaglandin and leukotriene metabolism, the potential exists to modulate the production of 1, 2,

and 3-series prostaglandins and 4- and 5-series leukotrienes by providing fish and borage oils in an enteral formula. Thus, fatty acids with anti-inflammatory properties, such as eicosapentaenoic acid and γ-linolenic acid, may modulate the production of proinflammatory eicosanoids and reduce tissue damage secondary to inflammation in endotoxin-induced acute lung injury. Therefore, we investigated the effect of prefeeding rats for 21 days with enteral diets that provided fatty acids derived from fish oil alone and in combination with borage oil as compared with corn oil on 1) lung microvascular protein permeability, arterial blood pressure, and platelet and white blood cells in a model of endotoxin-induced acute lung injury, 2) alveolar macrophage prostaglandin and leukotriene synthesis, and 3) liver and alveolar macrophage phospholipid fatty acid composition.

C. Materials and Methods

Approval to conduct this study was granted by the Animal Care and Use Committee of the University of Tennessee Graduate School of Medicine in accordance with guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Experimental design. Male Long-Evans rats weighing 150 ± 10 g were purchased from Charles River Laboratories (Wilmington, MA) and used for two separate experiments. Experiment 1 assessed the effects of enteral nutrition for 21 days with four high fat, low carbohydrate enteral diets on the

development of acute lung injury following endotoxin administration. Acute lung injury was assessed by measuring lung microvascular permeability (lung:heart radioactivity ratios). The systemic effects of endotoxin were assessed by measuring mean arterial blood pressure and platelet and white blood cell counts. Experiment 2 assessed the effect of enteral nutrition for 21 days on the fatty acid composition of liver and alveolar macrophage phospholipids and the capacity of alveolar macrophages to generate proinflammatory prostaglandins and leukotrienes in vitro. We studied alveolar macrophages from the dietary treatment groups with the greatest differences in tissue phospholipid fatty acid composition.

Enteral nutrition. Rats in experiments 1 and 2 were randomized prior to study inception with regard to dietary treatment. Rats within the same dietary treatment were housed two per cage in a temperature (21-23°C) and light controlled facility (12 h light/12 h dark). Animals were fed eucaloric (300 $kcal \cdot kg^{-1} \cdot day^{-1}$) and isonitrogenous enteral diets for 21 days containing either corn oil, fish oil or fish oil and increasing levels of borage oil. Enteral diets were provided in sterilized feeding bottles. A separate bottle provided water ad libitum. Diets were changed daily to minimize the potential for bacterial growth. The diets provided 16.7% of calories from protein, 28.1% of calories from carbohydrate, and 55.2% of calories from lipid. The fatty acid composition of the enteral diets were determined by gas chromatography and are outlined in Table 1.

Table 1. Fatty acid content of enteral diets.

FATTY ACID	% OF TOTAL, BY WEIGHT			
	Corn Oil	20% Fish Oil	20% Fish and 5% Borage Oil	20% Fish and 20% Borage Oil
Caproic (6:0)	ND	0.09	0.09	0.09
Caprylic (8:0)	ND	12.56	12.85	13.05
Capric (10:0)	ND	9.54	9.71	9.93
Lauric (12:0)	0.09	0.20	0.24	0.20
Myristic (14:0)	0.15	1.22	1.21	1.24
Palmitic (16:0)	11.3	6.26	6.11	5.95
Palmitoleic (16:1n-6)	0.04	1.70	1.67	1.69
Stearic (18:0)	2.27	1.48	1.69	1.86
Oleic (18:1n-9)	24.59	28.0	27.56	25.96
Linoleic (18:2n-6)	59.35	21.67	20.31	16.68
γ-linolenic (18:3n-6)	ND	0.08	1.25	4.63
α-linolenic (18:3n-3)	1.01	3.68	3.72	3.92
Stearidonic (18:4n-3)	ND	0.70	0.69	0.71
Arachidic (20:0)	0.41	0.31	0.81	0.27
Eicosenoic (20:1n-9)	0.30	1.08	1.82	1.92
Arachidonic (20:4n-6)	ND	0.38	0.38	0.38
Eicosapentaenoic (20:5n3)	ND	5.52	5.35	5.31
Erucic (22:1n-9)	0.04	0.57	0.72	1.12
Docosapentaenoic (22:5n3)	ND	0.55	0.54	0.54
Docosahexaenoic (22:6n3)	ND	2.30	2.32	2.52
Others	0.45	2.11	2.06	2.03
Total n-6/Total n-3	58.76	1.74	1.74	1.67

ND, Not Detected.

In experiment 1, 40 rats were randomly divided into four dietary groups, corn oil, 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil. In experiment 2, 19 rats were randomly divided into three dietary groups, corn oil (n = 6), 20% fish oil (n = 6), and 20% fish and 20% borage oil (n = 7). Based on the results of experiment 1, the 20% fish and 5% borage oil group was not included in experiment 2.

The corn oil diet represents a standard high fat formula enriched with linoleic acid. The 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil diets had nearly identical levels of fish oil and medium-chain triglycerides. To maintain a similar n-6 to n-3 fatty acid ratio between these three diets, linoleic acid was replaced with γ-linolenic acid in the 20% fish and 5% borage oil and 20% fish and 20% borage oil diets. Diets were enriched with vitamins and minerals at or above the recommended daily requirement for humans. Diets were formulated and supplied by Ross Products Division, Abbott Laboratories, Columbus, OH.

Experimental Procedures for Experiment 1.

Surgical Preparation. Following the 21 day feeding period, rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago,IL) for the surgical procedures. Catheters were filled with heparinized saline (10 IU/ml) and placed in the jugular vein (silicone medical grade tubing sized 0.025 I.D. x 0.047 O.D.) for i.v. injections and carotid artery (PE-50) for blood sampling and

measurements of mean arterial blood pressure. Isoflurane anesthesia was terminated after surgery and an intramuscular injection of 100 mg/kg ketamine (Ketalar, Parke-Davis, Morris Plains, NJ) and 10 mg/kg xylazine (Rompun, Mobay Corp., Shawnee, KS) was administered to maintain anesthesia during computerized gamma-scintigraphy. Ketamine and xylazine were given iv as needed to maintain anesthesia during the image acquisition period.

Computerized Gamma-Scintigraphy. Pulmonary microvascular protein permeability was determined using computerized gamma-scintigraphy. Rats were placed on a heating pad to maintain body temperature and then positioned on top of a gamma-camera (ARC 3000, ADAC Laboratories, Milpitas, CA). Lung protein permeability was determined by localization of ^{99m}Tc-HSA (human serum albumin) in the lungs by gamma-scintigraphy. Labeling of ^{99m}Tc to HSA was performed using a kit (MediPhysics, Richmond, CA) and labeling efficiency was determined by paper chromatography to be greater than 95%. An iv injection of 0.2 ml ^{99m}Tc-HSA (700-900 µCi) was administered to each rat 20 min prior to the baseline image acquisition. Following the baseline image acquisition, 10 mg/kg of *Salmonella enteritidis* endotoxin (Difco Laboratories, Detroit, MI) was injected through the jugular vein catheter. Static 60 sec images were acquired on an ADAC DPS 33,000 computer system (ADAC Laboratories, Milpitas, CA) using a 128 x 128 x 16 bit matrix prior to and every 15 min for 2 hours following endotoxin administration. At the end of the two hour experimental period (or prior to death), 0.4 ml of

^{99m}Tc-MAA (macroaggregated albumin) (Macrotec kit, Squib Diagnostics, Princeton, NJ) (500-800 µCi) was administered through the venous catheter to highlight the lungs. Five min after the ^{99m}Tc-MAA injection, a final 60 sec static image was acquired. The images were transferred to a Pegasys work station (ADAC Laboratories, Milpitas, CA) for analysis. Regions of interest were drawn around the right lung using the ^{99m}Tc-MAA image and heart using the initial ^{99m}Tc-HSA image for each rat and superimposed over each image acquired. Regions of interest were normalized for area and lung:heart radioactivity ratios were generated to produce time activity curves.

Arterial Blood Pressure and Hematologic Measurements. Arterial blood pressure was measured at 15 min intervals using a blood pressure monitor (Model #78304A, Hewlett-Packard, Liverpool, NY) for two hours post endotoxin. Total white blood cells were determined at baseline and at 30 min intervals for two hours and counted using a Neubauer hemocytometer. Platelets were determined every 15 min for 2 hours using the Unopette Microcollection System™ (Becton-Dickson, Rutherford, NJ) and counted using a Neubauer hemocytometer. Blood volume removed for these procedures was immediately replaced with an equal volume of heparinized saline (10 IU/ml).

Experimental Procedures for Experiment 2.

Bronchoalveolar Lavage and Tissue Collection. Bronchoalveolar lavage was performed on isoflurane anesthetized rats. The trachea was cannulated with a Jelco™ 14 gauge teflon IV catheter placement unit (Critikon, Tampa,FL)

and secured in place with ligatures. The lungs were lavaged with eight separate 6 ml aliquots of ice cold phosphate buffered saline using a 12 ml syringe. The lavage fluid was pooled for each animal. A 0.5 ml aliquot of bronchoalveolar lavage fluid was diluted with an equal volume of phosphate buffered saline and half of this was concentrated on a microscope slide using a Cytospin 2 cytocentrifuge at 500 rpm for 10 min (Shandon Southern Instruments, Sewickley, PA). The cytocentrifuge preparation was stained with Wright's Stain and a differential count was performed. The remaining bronchoalveolar lavage diluent was used for a total cell count using a Neubauer hemocytometer. The pulmonary artery and portal vein were isolated and perfused separately with 12 ml cold phosphate buffered saline to flush the lung and liver of blood, respectively, prior to removal for phospholipid analysis.

Alveolar Macrophage Stimulation, In Vitro. The bronchoalveolar lavage fluid from each rat was centrifuged at 400 x g for 10 min at 4°C to pellet the cells. The cells from three animals in the same dietary treatment group were pooled and washed once with phosphate buffered saline. The cells were resuspended in Dulbecco's Modified Eagle Medium (GIBCO Laboratories, Life Technologies Inc., Grand Island, NY), and counted on a Neubauer hemocytometer. Cell concentrations were adjusted to 1 x 10^6 cells/ml and plated at 2 x 10^6 cells/well in a Multiwell™ Tissue Culture Plate (Falcon, Franklin Lakes, NJ). After two hours of incubation at 37°C and 5% CO_2, the non-adherent cells were removed by aspirating the Dulbecco's Modified Eagle

Medium. The adherent cells were washed twice with Dulbecco's Modified Eagle Medium and then incubated for 15 min with 10 μM of the calcium ionophore A23187 suspended in 2 ml of Dulbecco's Modified Eagle Medium. In addition, alveolar macrophages incubated without A23187 served as the control. Following the incubation period, Dulbecco's Modified Eagle Medium was collected from each well and diluted 1:1 (v/v) with a 20% methanol solution containing 3.0 mM formic acid. Eicosanoids were isolated by solid phase extraction using a C-18 cartridge (Burdick and Jackson, Muskegon, MI), washed with distilled water and eluted off the cartridge with methanol (14).

Prostaglandin and Leukotriene Analysis. Eicosanoids were analyzed as described previously with the following modifications (14). Ten percent of the eluent was evaporated to dryness under nitrogen and resuspended in phosphate buffered saline containing 0.1% (w/v) gelatin. Each sample was analyzed for thromboxane B_2, prostaglandin E_2, and 6-keto-prostaglandin $F_{1\alpha}$ by RIA according to the manufacturer's instructions (Advanced Magnetics, Cambridge, MA). The remaining 90% of the eluent was evaporated and the residue was reconstituted in the high performance liquid chromatography solvent system of methanol/water (65:35, v/v), pH 5.6, containing 5 mM ammonium acetate and 1 mM ethylene diamine tetra-acetic acid. Leukotrienes were separated by reverse phase high performance liquid chromatography on C-18 column (10 mm x 4.6 cm) (Hewlett-Packard, Kennett Square, PA) with a flow rate of 0.9 ml/min and quantified based on their molar extinction

coefficients (23) using the internal standard prostaglandin B_1, with a Hewlett-Packard (Liverpool, NY) 1090M Photodiode Array scanning spectrophotometer, monitoring at 280 nm. All compounds were identified by their distinct ultraviolet (UV) absorption spectra, their absorption maxima, and retention times as compared to known standards.

Alveolar Macrophage and Liver Phospholipid Fatty Acid Analysis. Liver samples were perfused and homogenized with cold saline. Nonstimulated alveolar macrophages were collected from the bronchoalveolar lavage fluid as described above. The tissue lipids were extracted as described previously (24). Briefly, livers (100mg) were homogenized in cold saline (0.9%). Liver homogenates and isolated alveolar macrophages were resuspended in 3 ml methanol-chloroform (2:1, v/v) , extracted once with chloroform-saline (1:1, v/v), and twice with chloroform. The pooled chloroform fractions were evaporated to dryness under nitrogen, redissolved in chloroform, and the phospholipids were separated by thin layer chromatography using a chloroform:methanol (8:1 v/v) solvent system. The phospholipids were recovered by scraping the appropriate band from the thin layer chromotography plate and suspending the scrapings in toluene. The phospholipids were then saponified with 0.5N potassium hydroxide in methanol for eight min at 86°C. The free fatty acids were then acidified in 0.7N HCL in methanol, extracted twice with hexane, and evaporated to dryness under nitrogen. After methylating with ethereal diazomethane, the fatty acid methyl esters were resuspended in hexane and analyzed by gas

chromatography (Hewlett-Packard Model #5890 Series II) using a DB 23 capillary column (0.25mm x 30m) (J & W Chromatography, Folsum, CA) with hydrogen as the carrier gas. Free fatty acids were quantified using pentadecanoic acid as an internal standard and expressed as mol percent.

Statistical Analysis. Baseline values for the study variables were compared across treatments using a one-way analysis of variance. Lung:heart radioactivity ratios, mean arterial blood pressure, and platelets were analyzed with a repeated measures analysis of variance with treatment, time and the treatment by time interaction serving as effects. To facilitate the interpretation of a significant main effect, a number of individual contrasts were carried out among group means. White blood cells, alveolar macrophage and liver fatty acid analyses, and alveolar macrophage eicosanoid analyses were evaluated with a one way analysis of variance (ANOVA) and the *Student-Newmann-Kuels Test* for means separation. Data are expressed as mean ± SEM. $P \leq 0.05$ was considered significant.

D. Results

Lung Microvascular Protein Permeability. The effect of an iv injection of endotoxin on lung microvascular protein permeability (lung:heart ratio) after 21 days of feeding in the four dietary treatment groups is shown in Figure 1. Baseline lung:heart ratios were not significantly different between diet treatment groups indicating that the lung microvascular protein permeability was the same

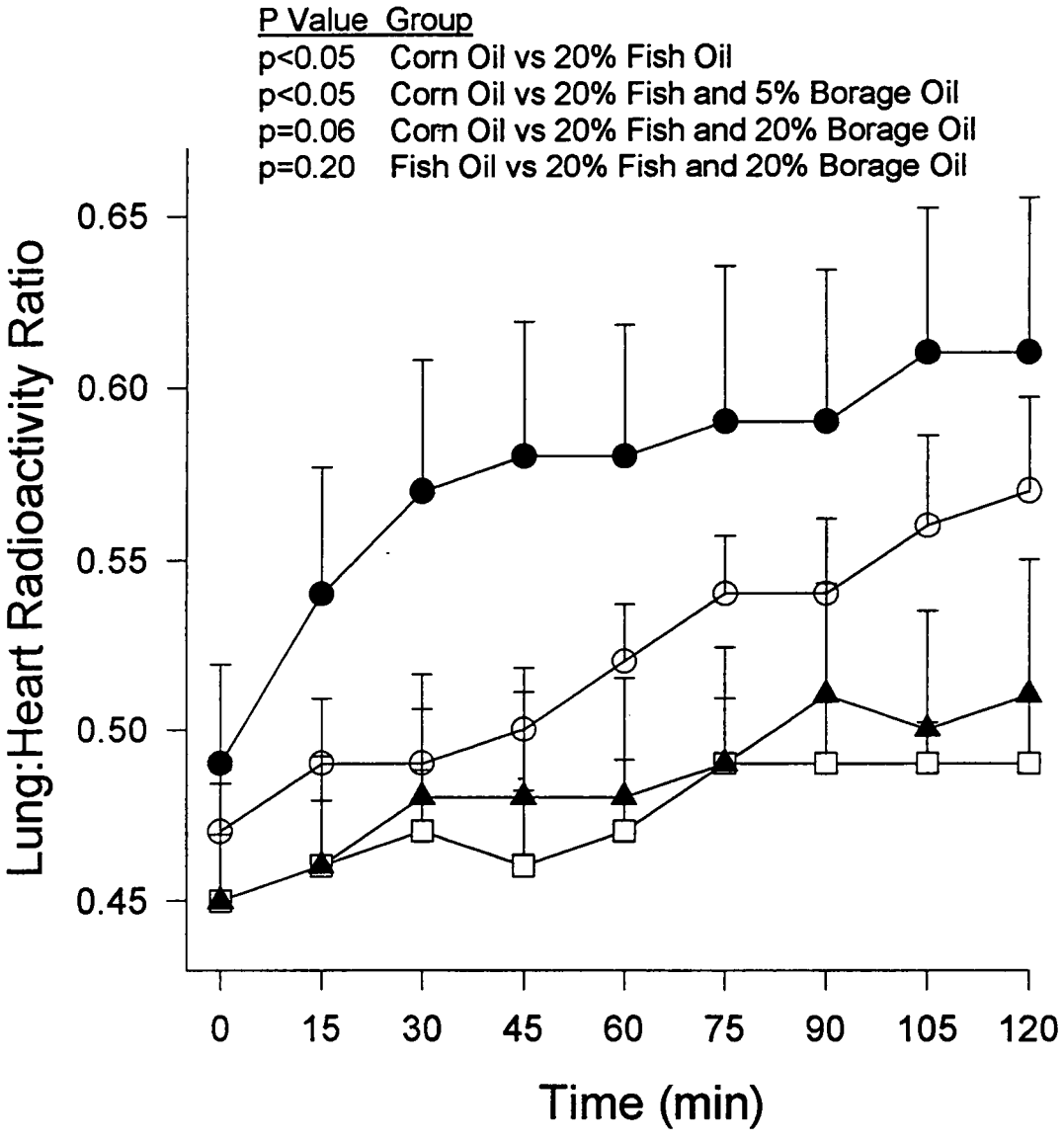


Figure 1. Lung:heart radioactivity ratios following an iv bolus injection of *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) in rats fed diets supplemented with corn oil (●), 20% fish oil (□), 20% fish and 5% borage oil (▲), or 20% fish and 20% borage oil (○) for 21 days. Values are means ± SEM for 9-11 rats. Error bars are shown in one direction for clarity.

for all groups following surgery. There was an increase in the lung:heart ratio beginning at 15 min post-endotoxin and continuing for the duration of the 120 min experimental time period for all dietary treatment groups. Repeated measures ANOVA demonstrated that the increase in lung microvascular protein permeability was significantly higher with corn oil as compared with 20% fish oil and 20% fish and 5% borage oil and approached significance with 20% fish and 20% borage oil ($p = 0.06$). The increase in lung microvascular protein permeability with 20% fish and 20% borage oil was not significantly different than 20% fish oil ($p=0.20$) and 20% fish and 5% borage oil ($p=0.31$).

Mean Arterial Blood Pressure. The effect of prefeeding diets on mean arterial blood pressure after endotoxin administration is shown in Figure 2. Mean arterial blood pressure exhibited the steepest drop with corn oil immediately following endotoxin administration. The decline in blood pressure reached the lowest levels at 30 min followed by an increase toward baseline levels for all groups. Although no overall differences among the dietary groups were observed (repeated measures ANOVA), further analysis demonstrated that the reduction in mean arterial blood pressure was significantly attenuated at early (15 min) and late (120 min) time points post-endotoxin with 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil as compared with corn oil.

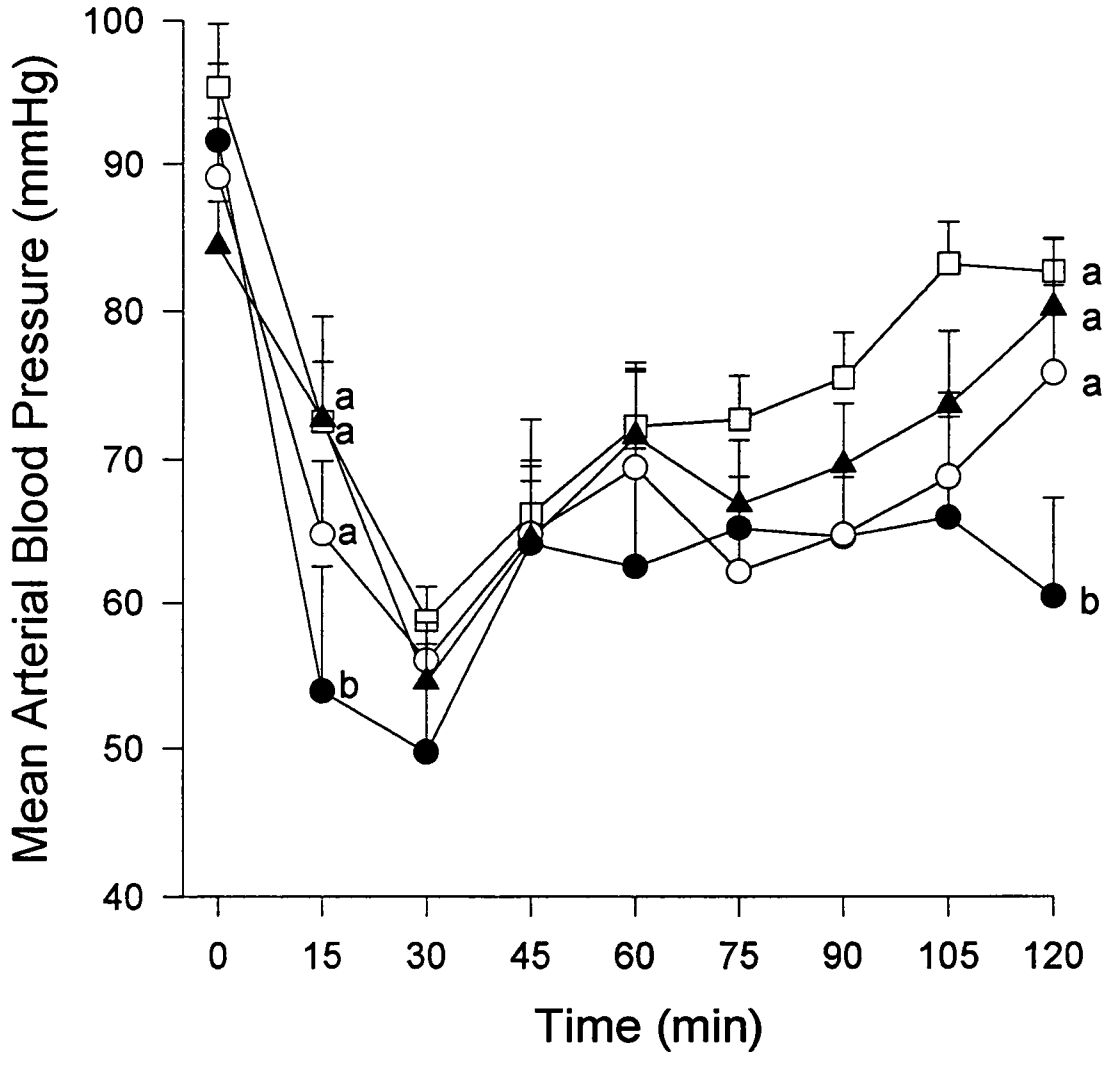


Figure 2. Mean arterial blood pressure following an iv bolus injection of *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) in rats fed diets supplemented with corn oil (●), 20% fish oil (□), 20% fish and 5% borage oil (▲), or 20% fish and 20% borage oil (○) for 21 days. Values are means ± SEM for 9-11 rats. Error bars are shown in one direction for clarity. Symbols with different letters are significantly different ($P \leq 0.05$).

Total White Blood Cells and Platelet Counts. There was a substantial decrease in total white blood cell counts at 60 min which continued to 120 min following endotoxin administration for all dietary treatment groups (Figure 3a). There were no significant differences in total white blood cell counts between dietary treatments. Platelet counts following endotoxin administration are shown in Figure 3b. For all dietary treatment groups there was a decrease in platelet number at 15 min post endotoxin followed by a slight increase over the next 105 min. While platelet counts were consistently highest with 20% fish and 20% borage oil and consistently lowest with 20% fish oil, these differences were only significant at 15 min post-endotoxin administration.

Liver Phospholipid Fatty Acid Composition. The fatty acid composition of liver phospholipids was determined after 21 days of feeding the four different diets in experiment 1 (Table 2). Linoleic acid levels in liver phospholipids were significantly lower with 20% fish and 5% borage oil and 20% fish and 20% borage oil as compared with corn oil. As the level of borage oil increased from 0% to 5% and 20% in the diets containing fish oil, linoleic acid levels progressively decreased to approximately 65% (20% fish and 20% borage oil) of that with corn oil. Dihomo-γ-linolenic acid levels were significantly higher

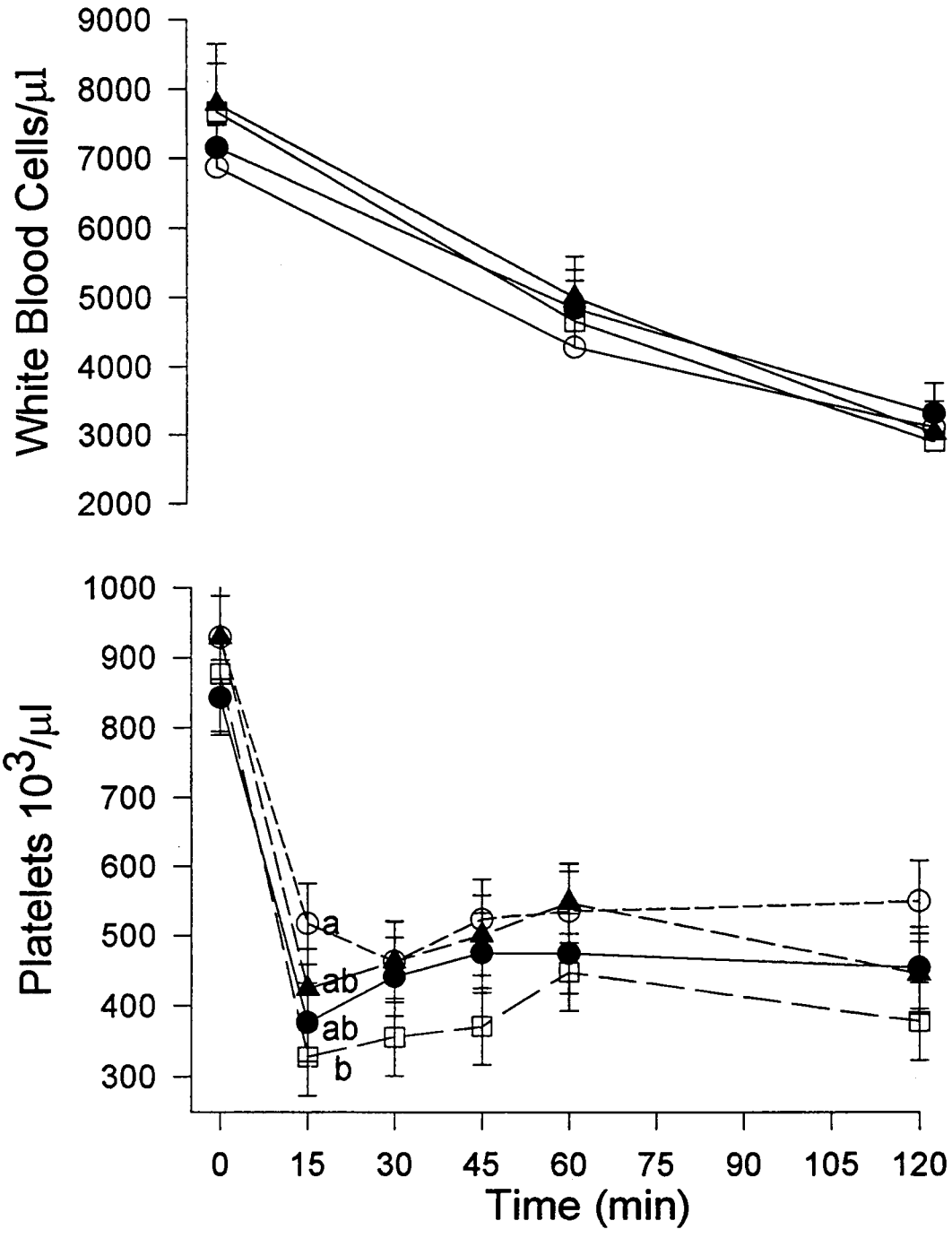


Figure 3. Arterial white blood cells (a) and platelets (b) following an iv bolus injection of *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) in rats fed diets supplemented with corn oil (●), 20% fish oil (□), 20% fish and 5% borage oil (▲), 20% fish and 20% borage oil (○) for 21 days. Values are means ± SEM for 9-11 rats. Error bars are shown in one direction for clarity.

Table 2. Effect of feeding diets supplemented with corn oil, 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil for 21 days on liver phospholipid fatty acid composition (mol %).

Fatty Acid	Corn Oil	20% Fish Oil	20% Fish and 5% Borage Oil	20% Fish and 20% Borage Oil
16:0	16.5 ± 0.38	16.1 ± 0.36	17.4 ± 0.34	16.4 ± 0.36
16:1	0.08 ± .02^{a}	0.40 ± 0.02^{b}	0.58 ± 0.14^{b}	0.34 ± 0.05^{b}
18:0	27.4 ± 0.41^{a}	26.4 ± 0.41^{a}	23.0 ± 0.49^{b}	25.3 ± 0.55^{b}
18:1	3.10 ± 0.20^{a}	4.34 ± 0.50^{a}	8.05 ± 0.83^{b}	5.72 ± 0.29^{b}
18:2n-6	15.8 ± 0.89^{a}	16.1 ± 0.76^{a}	14.03 ± 0.55^{b}	10.4 ± 0.30^{c}
18:3n-6	0.09 ± 0.02^{a}	0.01 ± 0.02^{b}	0.15 ± 0.05^{a}	0.46 ± 0.03^{c}
18:3n-3	0.05 ± 0.06	0.07 ± 0.05	0.04 ± 0.04	0.10 ± 0.04
20:3n-6	0.50 ± 0.04^{a}	1.88 ± 0.12^{b}	2.18 ± 0.05^{c}	3.71 ± 0.30^{d}
20:4n-6	31.8 ± 0.59^{a}	18.7 ± 0.51^{b}	20.7 ± 0.53^{c}	25.4 ± 0.29^{d}
20:5n-3	ND	4.78 ± 0.35^{a}	3.15 ± 0.37^{ab}	2.53 ± 0.19^{b}
22:5n-3	0.26 ± 0.03^{a}	1.62 ± 0.10^{b}	1.56 ± 0.11^{b}	1.46 ± 0.10^{b}
22:6n-3	4.29 ± 0.21^{a}	9.53 ± 0.29^{b}	8.76 ± 0.40^{c}	7.96 ± 0.28^{c}
(n-6)/(n-3) PUFA	22.23 ± 0.98^{a}	2.31 ± 0.11^{b}	2.75 ± 0.05^{c}	3.35 ± 0.12^{d}
DGLA/AA	0.02 ± 0.001^{a}	0.10 ± 0.006^{b}	0.11 ± 0.003^{b}	0.15 ± 0.007^{c}

ND, Not Detected; Values (n = 6-7) are means ± SEM. Values in the same row with different letters are significantly different ($P \leq 0.05$).

with 20% fish and 20% borage oil as compared with the other groups. Arachidonic acid levels were significantly lower with 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil as compared with corn oil, but progressively increased with increasing levels of borage oil. The levels of eicosapentaenoic acid and its elongation products, docosapentaenoic acid (22:5n-3) and docosapentaenoic acid, were significantly higher with 20% fish oil, 20% fish and 5% borage oil, 20% fish and 20% borage oil as compared with corn oil, and progressively decreased with increasing levels of borage oil. The n-6/n-3 ratio of polyunsaturated fatty acids was significantly higher with corn oil as compared with the other groups. There were significant differences between 20% fish oil, 20% fish and 5% borage oil and 20% fish and 20% borage oil with a trend of increasing n-6/n-3 ratios with increasing levels of borage oil in the diet. The ratio of dihomo-γ-linolenic acid:arachidonic acid was highest with 20% fish and 20% borage oil and lowest with corn oil as compared with the other groups.

Alveolar Macrophages Phospholipid Fatty Acid Composition. The fatty acid compositions of alveolar macrophages acquired from the bronchoalveolar lavage fluid of rats fed corn oil, 20% fish oil, and 20% fish and 20% borage oil for 21 days in experiment 2 are shown in Table 3. The level of linoleic acid with 20% fish oil was approximately 50% lower than with corn oil. As the level of borage oil in the diet increased from 0 to 20%, the level of linoleic acid decreased significantly. The level of dihomo-γ-linolenic acid was significantly

Table 3. Effect of feeding diets supplemented with corn oil, 20% fish oil, and 20% fish and 20% borage oil for 21 days on alveolar macrophage phospholipid fatty acid composition (mol %).

Fatty Acid	Corn Oil	20% Fish Oil	20% Fish and 20% Borage Oil
16:0	48.7 ± 1.87	52.6 ± 2.47	52.53 ± 2.13
16:1	4.11 ± 0.29	4.93 ± 0.44	5.04 ± 0.40
18:0	11.25 ± 0.54	9.48 ± 0.79	9.94 ± 0.57
18:1	7.05 ± 0.41[a]	8.48 ± 0.32[b]	8.19 ± 0.45[b]
18:2n-6	14.49 ± 0.45[a]	7.00 ± 0.51[b]	5.16 ± 0.28[c]
18:3n-6	0.05 ± 0.03[a]	ND	0.58 ± 0.02[b]
18:3n-3	0.03 ± 0.02[a]	0.23 ± 0.01[b]	ND
20:3n-6	0.22 ± 0.04[a]	0.24 ± 0.04[a]	0.62 ± 0.06[b]
20:4n-6	11.99 ± 0.77[a]	5.47 ± 0.24[b]	8.21 ± 0.94[c]
20:5n-3	ND	2.68 ± 0.23[a]	1.93 ± 0.13[b]
22:5n-3	0.38 ± 0.07[a]	2.06 ± 0.08[b]	1.82 ± 0.13[b]
22:6n-3	0.96 ± 0.12[a]	4.14 ± 0.13[b]	3.97 ± 0.20[b]
(n-6)/(n-3) PUFA	20.72 ± 1.91[a]	1.48 ± 0.11[b]	1.88 ± 0.09[c]
DGLA/AA	0.02 ± 0.002[a]	0.05 ± 0.018[b]	0.07 ± 0.002[b]

ND, Not Detected; Values (n = 6-7) are means ± SEM. Values in the same row with different letters are significantly different ($p \leq 0.05$).

higher with 20% fish and 20% borage oil as compared with 20% fish oil and corn oil, which were not significantly different from each other. Arachidonic acid levels were significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. Arachidonic acid levels, however, were significantly higher with 20% fish and 20% borage oil as compared with fish oil. Eicosapentaenoic acid was not detected in the corn oil group but was significantly higher with 20% fish oil as compared with 20% fish and 20% borage oil. The levels of both docosapentaenoic acid and docosahexaenoic acid were significantly higher with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. The n-6/n-3 ratio with 20% fish oil and 20% fish and 20% borage oil was substantially lower as compared with corn oil. The dihomo-γ-linolenic acid:arachidonic acid ratio was the highest with 20% fish oil and 20% borage oil as compared with 20% fish oil and corn oil and 20% fish oil was significantly higher than corn oil.

In Vitro Eicosanoid Production after Maximal Stimulation with A23187. The effects of stimulating alveolar macrophages *in vitro* with the calcium ionophore A23187 on the generation of leukotriene B_4 and other eicosanoids (experiment 2) are shown in Figure 4 and Table 4, respectively. The release of leukotriene B_4 from stimulated alveolar macrophages was significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. In addition, the amount of the 5-lipoxygenase product of eicosapentaenic acid, leukotriene B_5, was significantly higher with 20% fish oil and 20% fish and 20%

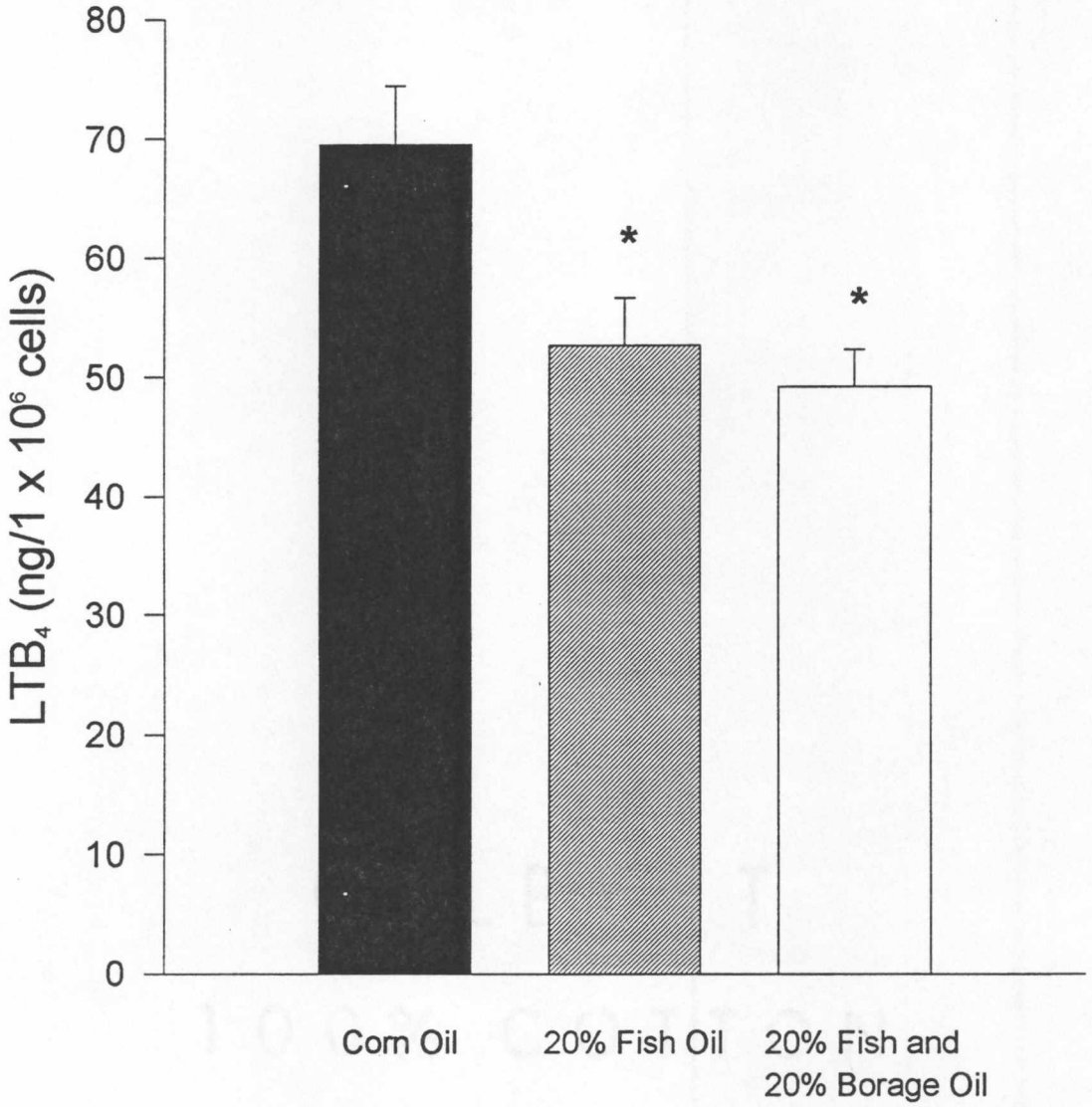


Figure 4. LTB_4 levels (ng/1 $x10^6$ cells) released from alveolar macrophages following stimulation in vitro with 10 µM A23187 for 15 min from rats fed diets supplemented with corn oil, 20% fish oil, or 20% fish and 20% borage oil for 21 days. Values are means ± SEM for 9 rats. Bars with an asterisk are significantly different from corn oil ($P \leq 0.05$).

Table 4. Effect of feeding diets supplemented with corn oil, 20% fish oil, or 20% fish and 20% borage oil on eicosanoid release from alveolar macrophages following stimulation in vitro with A23187 (ng/1x10^6 cells).

Eicosanoids	Corn Oil	Fish Oil	20% Fish and 20% Borage Oil
LTB_5	ND	7.32 ± 0.62[a]	4.69 ± 0.30[b]
TXB_2	6.06 ± 0.16[a]	4.58 ± 0.55[b]	4.40 ± 0.44[b]
PGE_2	1.83 ± 0.24[a]	1.03 ± 0.26[b]	0.95 ± 0.14[b]
6-keto-$PGF_{1\alpha}$	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.02

ND, Not Detected; Values are means ± SEM. Values in the same row with different letters are significantly different ($P \leq 0.05$). TXB_2, thromboxane B_2; LTB_5, leukotriene B_5; PGE_2, prostaglandin E_2; 6-keto-prostaglandin $F_{1\alpha}$, (n = 3 pooled samples).

borage oil as compared with corn oil. There were no significant differences in the level of 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin) between any of the dietary treatment groups. However, the levels of thromboxane B_2 (stable metabolite of thromboxane A_2) and prostaglandin E_2 were significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil.

E. Discussion

Gram-negative sepsis and endotoxemia initiate inflammatory responses that have been implicated in the pathogenesis of ARDS (25). The clinical treatment of critically ill patients with ARDS is usually supportive and consists primarily of fluid resuscitation for circulatory support and mechanical ventilation to improve arterial oxygenation. However, current treatment modalities are associated with a mortality rate greater than 50% (26) and have not addressed the role of proinflammatory mediators in the pathogenesis of ARDS. High fat low carbohydrate enteral diets have been designed to reduce minute ventilation and ventilatory demand by lowering CO_2 production in ventilator-dependent patients (27,28). However, the lipids found in these formulations are typically rich in linoleic acid. Linoleic acid is converted by desaturation and elongation to arachidonic acid for incorporation into tissue phospholipids. However, with injury there is an activation of phospholipase A_2 which releases arachidonic acid from tissue phospholipids. The enzymes, cyclooxygenase (prostaglandin H

synthase) and 5-lipoxygenase, act to convert arachidonic acid to either the 2-series prostaglandins or 4-series leukotrienes, respectively, which have various proinflammatory effects. These eicosanoids are important mediators of inflammation of the lung (4).

This study showed that prefeeding high fat enteral formulations containing dietary fatty acids derived from fish and borage oil modulated many of the pathophysiologic responses to an iv injection of endotoxin in rats. Computerized gamma-scintigraphy has been shown to be a sensitive, noninvasive, dynamic technique for the detection and quantitation of pulmonary microvascular leakage of protein (29). We assessed changes in protein conductance across the pulmonary microvascular endothelium by calculating the lung:heart radioactivity ratio of radiolabelled albumin before and after the iv administration of endotoxin. The lung:heart radioactivity ratio was constant for two hours in rats given an iv injection of saline (data not shown). Previous studies have shown that an increase in the lung:heart radioactivity ratio is due to an increase in pulmonary microvascular permeability resulting from microvascular injury (29). We observed that the endotoxin-induced increase in pulmonary microvascular protein permeability was markedly reduced with diets containing 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil as compared with corn oil. Such a reduction in permeability may have coordinated beneficial effects by decreasing transcapillary fluid flux and pulmonary edema; thereby facilitating gas exchange leading to an improvement

in arterial oxygenation (29).

The severity of thrombocytopenia typically observed within 15 min following endotoxin administration was attenuated with 20% fish and 20% borage oil as compared with 20% fish oil; however, while this trend continued for the duration of the experiment these differences were only significant at the early time point (15 min). The early and late hypotensive effects of endotoxin were attenuated with 20% fish oil, 20% fish and 5% borage oil, and 20% fish and 20% borage oil as compared with corn oil with no statistical differences at the intermediate time points. Although 20% fish oil has been widely shown to depress vascular reactivity to agonists and lower blood pressure in hypertension (30), the results of this and the studies by Murray et al. (13,31) indicate that fish oil does not exacerbate the hypotensive effects of endotoxin but rather diminishes the fall in mean arterial blood pressure as compared with corn oil. Murray et al (31) showed sustained improvements in pulmonary vascular resistance, cardiac index, and oxygen delivery following an iv infusion of *Escherichia coli* endotoxin in pigs prefed fish and borage oil as compared with fish oil or corn oil. It is possible that the reduction in endotoxin-induced lung microvascular permeability with fish oil or fish and borage oil may be related to reduced synthesis of arachidonic acid derived proinflammatory eicosanoids. Fish oils are effective in suppressing eicosanoid biosynthesis and our previous study demonstrated that dietary supplementation with borage oil modulated arachidonic acid derived 2-series prostaglandins after burn and

endotoxin injury (19).

We observed a decrease in the level of arachidonic acid that was accompanied by higher levels of n-3 fatty acids (eicosapentaenoic acid, docosapentaenoic acid, and docosapentaenoic acid) in the phospholipids of alveolar macrophages and liver with fish oil or fish and borage oil. These findings are in agreement with other studies showing that dietary fish oil supplementation displaces arachidonic acid from tissue phospholipids (14,15,33). The incorporation of these n-3 fatty acids into tissue phospholipids is significant for many reasons. First, an increase in tissue eicosapentaenoic acid suppresses the formation of arachidonic acid derived prostaglandins by inhibiting cyclooxygenase. Second, eicosapentaenoic acid is the precursor of the 3-series prostaglandins which are less biologically active than 2-series prostaglandins. Lastly, eicosapentaenoic acid suppresses 4-series leukotriene formation (derived from arachidonic acid) (15). The significant increases in dihomo-γ-linolenic acid and the ratio of dihomo-γ-linolenic acid to arachidonic acid in the phospholipids of alveolar macrophages and liver with 20% fish and 20% borage oil suggest that dihomo-γ-linolenic acid was not completely converted to arachidonic acid. Previous studies have reported similar increases in dihomo-γ-linolenic acid in tissue phospholipids (32) and plasma (19) following dietary γ-linolenic acid supplementation. The release of leukotriene B_4 from alveolar macrophages following stimulation with A23187 was significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil.

This may have been due to a combined effect of a decrease in arachidonic acid (the metabolic precursor of leukotriene B_4) in the phospholipids of alveolar macrophages and to competitive inhibition of 5-lipoxygenase by eicosapentaenoic acid (33,34). Although there were significantly higher arachidonic acid levels in the alveolar macrophages with 20% fish and 20% borage oil as compared with 20% fish oil, this did not result in increases in eicosanoid synthesis. In addition, 15-OH-20:3n-6 (15-HETrE), a 15-lipoxygenase metabolite of dihomo-γ-linolenic acid, has been shown to attenuate leukotriene B_4 formation by inhibiting 5-lipoxygenase (32). Importantly, dihomo-γ-linolenic acid is not a substrate for 5-lipoxygenase and cannot be converted to leukotrienes through this pathway (32). Leukotriene B_4 is elevated in the bronchoalveolar lavage fluid of patients with ARDS (2,7) and has been shown to increase pulmonary microvascular permeability in the rabbit (35). Leukotriene B_4 is a potent chemoattractant of polymorphonuclear neutrophils. Neutrophil-mediated endothelial injury has been implicated in animal models of acute lung injury (35,36). An inhibition of leukotriene B_4 formation has been shown to significantly reduce lung microvascular permeability and edema in acute lung injury in the rat (37) and the dog (36). In addition, alveolar macrophages stimulated with A23187 produced elevated levels of leukotriene B_5, a 5-lipoxygenase metabolite of eicosapentaenoic acid, with 20% fish oil and 20% fish and 20% borage oil. Leukotriene B_5 is much less potent as a chemoattractant for polymorphonuclear neutrophils as

compared with leukotriene B_4 (18). Taken together, these results indicate that there was an overall decrease in the potential for neutrophil chemotaxis with 20% fish oil and 20% fish and 20% borage oil.

Thromboxane A_2 is an important mediator in the development of pulmonary hypertension in ARDS and in endotoxin-induced lung injury (7,38). Thromboxane synthase inhibitors have been shown to significantly attenuate pulmonary hypertension in a porcine model of bacteremia (39) and increase survival after endotoxin infusion in the rat (40). Although thromboxane A_2 may not have a direct effect on microvascular permeability, thromboxane receptor inhibition may indirectly inhibit neutrophil adherence thus reducing endothelial cell damage (7). The protection against endotoxin-induced increases in lung permeability with fish oil or fish and borage oil may be due in part to reduced formation of thromboxane A_2 in vivo. The release of this eicosanoid, as inferred by measurement of its stable metabolite, thromboxane B_2, was reduced from alveolar macrophages stimulated with A23187 in vitro in 20% fish oil and 20% fish and 20% borage oil compared with corn oil.

Prostaglandin E_2 is required for normal immune function and T-cell differentiation at very low concentrations ($<10^{-9}$ M). Following injury, elevated concentrations of prostaglandin E_2 ($>10^{-8}$ M) are immunosuppressive (41). In this study, prostaglandin E_2 from stimulated alveolar macrophages was significantly lower with 20% fish oil and 20% fish and 20% borage oil as compared with corn oil. Thus, modulation of prostaglandin E_2 formation by

dietary fish oil or fish and borage oil may normalize immune function and T-cell differentiation.

In summary, prefeeding high fat enteral formulas enriched with fish oil or fish and borage oil as compared with corn oil for 21 days was protective against endotoxin-induced acute lung injury in the rat; however, their order of effectiveness was not always consistent. The 20% fish oil and 20% fish and 20% borage oil diets had very similar effects on eicosanoid production, while 20% fish oil and 20% fish and 5% borage oil diets appeared to be somewhat more protective than 20% fish and 20% borage oil diets against increases in pulmonary microvascular protein permeability and hypotension. Thrombocytopenia was muted the most with 20% fish and 20% borage oil and the least with 20% fish oil. It is premature at this time to speculate about the effectiveness of dietary interventions in patients with ARDS. However, the use of diets containing anti-inflammatory fatty acids which modulate eicosanoid metabolism may attenuate lung microvascular protein permeability and hemodynamic insufficiency in critically ill patients at risk of developing ARDS.

REFERENCES

1. Brigham KL, Meyrick B: Endotoxin and lung injury. *Am Rev Respir Dis* 1986; 133:913-927

2. Stephenson AH, Lonigro AJ, Hyers TM, et al: Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* 1988; 138:714-719

3. Hoover RL, Karnovsky KF, Austen EJ, et al: Leukotriene B_4 action on endothelium mediates augmented neutrophil/endothelial adhesion. *Proc Natl Acad Sci USA* 1984; 81:2191-2193

4. Sharar SR, Winn RK, Harlan JM: Endotoxin-induced interactions of inflammatory cells with the lungs. In: Brigham KL., ed. Endotoxin and the lungs. New York: Marcel Dekker, Inc.; 1994: 229-265

5. Abela A, Daniel EE: Neural and myogenic effects of leukotrienes C_4, D_4, and E_4, on canine bronchial smooth muscle. *Am J Physiol.* 1994; 266:L414-L425

6. Cohn SM, Kruithoff KL, Rothchild HR, et al: Beneficial effects of LY203647, a novel leukotriene C_4/D_4 antagonist, on pulmonary function and mesenteric perfusion in a porcine model of endotoxic shock and ARDS. *Circ Shock* 1991; 33:7-16

7. Makhlouf M, Durando M, Cook JA, et al: Role of eicosanoids in endotoxin-induced lung dysfunction. In: Brigham KL., ed. Endotoxin and the Lungs. New York City: Marcel Dekkar, Inc.; 1994; 153-169

8. Burton JL: Dietary fatty acids and inflammatory skin disease. *Lancet* 989; 7:27-31

9. Tate G, Mandell BF, LaPosata M, et al: Suppression of acute and chronic inflammation by dietary gamma linolenic acid. *J Rheumatol* 1989; 16:729-734

10. Teo TC, DeMichele SJ, Selleck KM, et al: Administration of structured lipid composed of MCT and fish oil reduces net protein catabolism in enterally fed burned rats. *Ann Surg* 1989; 210:100-107

11. Simopoulos AP: Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991; 54:438-463

12. Tate GA, Mandell BF, Karmali RA, et al: Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. *Arthrit Rheumat* 1988; 31:1543-1551

13. Murray MJ, Svingen BA, Holman RT, et al: Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia. *J Parenter Enter Nutr.* 1991; 15:152-158

14. Whelan J, Broughton KS, Kinsella JE: The comparative effects of dietary alpha-linolenic acid and fish oil on 4- and 5-series leukotriene formation in vivo. *Lipids* 1991; 26:119-126

15. Lands WE, LeTellier PR, Rome LH: Vanderhoek JY. Inhibition of prostaglandin biosynthesis. *Adv Biosci* 1972; 9:15-27

16. Lands WEM: Biochemistry and physiology of n-3 fatty acids. *FASEB J.* 1992; 6:2530-2536

17. Fischer S, Weber PC: Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature* 1984; 307:165-168

18. Lee TH, Mencia-Huerta J, Shih C, et al: Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J.Biol.Chem* 1984; 259:2383-2389

19. Karlstad MD, DeMichele SJ, Leathem WD, et al: Effect of intravenous lipid emulsions enriched with gamma-linolenic acid on plasma n-6 fatty acids and prostaglandin biosynthesis after burn and endotoxin injury in rats. *Crit Care Med* 1993;21:1740-1749

20. Fan YY, Chapkin RS: Mouse peritoneal macrophage prostaglandin E_1 synthesis is altered by dietary gamma-linolenic acid. *J Nutr* 1992; 122:1600-1606

21. Chapkin RS, Miller CC, Somers SD, et al: Ability of 15-hydroxyeicosatrienoic acid (15-OH-20:3) to modulate macrophage arachidonic acid metabolism. *Biochem and Biophys Res Commun* 1988; 153:799-804

22. Abraham E, Park YC, Covington P, et al: Liposomal prostaglandin E_1 in acute respiratory distress syndrome: A placebo-controlled, randomized, double-blind, multicenter clinical trial. *Crit Care Med* 1996; 24:10-15

23. Orning L, Bernstrom K, Hammarstrom S: Formation of leukotrienes E_3, E_4, and E_5 in rat basophilic leukemia cells. *European Journal of Biochemistry.* 981; 120:41-45

24. Whelan J, Broughton KS, Surette ME, Kinsella JE: Dietary arachidonic and linoleic acids: Comparative effects on tissue lipids. *Lipids* 1992; 27:85-88

25. Read MA, Meyrick BO: Effects of Endotoxin on Lung Endothelium. In: Brigham KL., ed. Endotoxin and the Lung. New York, NY: Marcel Dekker, Inc.; 1994: 83-110

26. Kollef MH, Schuster DP: The acute respiratory distress syndrome. *N Eng J Med* 1995; 332:27-37

27. Askanazi J, Rosenbaum SH, Hyman AI, et al: Respiratory changes induced by the large glucose loads of total parenteral nutrition. *JAMA* 1980; 243:1444-1447

28. Skeie B, Askanazi J, Rothkopf MM, Rosenbaum SH, et al: Intravenous fat emulsions an dlung function: a review. *Crit Care Med* 1988; 16:183-194

29. Sugerman HJ, Strash AM, Hirsch JI, et al: Sensitivity of scintigraphy for detection of pulmonary capillary albumin leak in canine oleic acid ARDS. *J Trauma* 1991; 21:520-527

30. Chin JPF, Dart AM: How do fish oils effect vascular function. *Clin Exper Pharm* 1995; 22:71-81

31. Murray MJ, Kumar M, Gregory TJ, et al: Enteral diets enriched in eicosapentaenoic acid and gamma-linolenic acid attenuate cardiopulmonary dysfunction in a porcine model of acute lung injury. *Am J Physiol* 1995; 269:H2090-H2099

32. Ziboh VA, Fletcher MP: Dose-response effects of dietary τ-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B_4. *Am J Clin Nutr* 1992; 55:39-45

33. Billiar TR, Bankey PE, Svingen BA, et al: Fatty acid intake and kuppfer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* 1988; 104:343-349

34. Kinsella JE, Broughton KS, Whelan JW: Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis. *J Nutr Biochem.* 1990; 1:123-141

35. Yoshimura K, Nakagawa S, Koyama S, et al: Leukotriene B_4 induces lung injury in the rabbitt: role of neutrophils and effect of indomethacin. *J Appl Physiol* 1993; 74:2174-2179

36. Sprague RS, Stephenson AH, Lonigro AJ: OKY-046 prevents increases in LTB_4 and pulmonary edema in phorbol ester-induced lung injury in dogs. *J Appl Physiol* 1992; 73:2493-2498

37. Ball HA, Cook JA, Spicer KM, et al: Essential fatty acid-deficient rats are resistant to oleic acid-induced pulmonary injury. *J Appl Physiol* 1989; 57:811-816

38. Wisner D, Sturm J, Sutter G, Ellendorf B, Nerlich M: Thromboxane receptor blockade in an animal model of ARDS. *Surgery* 1988; 104:91-97

39. Svartholm E, Bergqvist D, Hedner U, Ljungberg J, Haglund U: Arachidonic acid cascade metabolites in porcine *E. coli* shock. *Acta Chir Scand Suppl* 1988; 154:133-139

40. Cook JA, Wise WC, Halushka PV: Elevated thromboxane levels in the rat during endotoxic shock: protective effects of imidazole, 13-azaprostanoic acid, or essential fatty acid deficiency. *J Clin Invest* 1980; 65:227-230

41. Santoli D, Phillips PD, Colt TL, Zurier RB: Suppression of interleukin 2-dependent human T cell growth in vitro by prostaglandin E and their precursor fatty acids. *J Clin Invest* 1990; 85:424-432

II. DIETARY FISH AND FISH AND BORAGE OIL SUPPRESS INTRAPULMONARY PROINFLAMMATORY EICOSANOID BIOSYNTHESIS AND ATTENUATE PULMONARY NEUTROPHIL ACCUMULATION IN ENDOTOXIC RATS[1]

A. Abstract

Proinflammatory eicosanoids and cytokines are important mediators of local inflammation in acute lung injury. We determined if enteral nutrition with anti-inflammatory fatty acids, eicosapentaenoic acid and γ-linolenic acid would reduce the intrapulmonary synthesis of proinflammatory eicosanoids and cytokines and pulmonary neutrophil accumulation in a rat model of acute lung injury. Rats were randomly assigned to three dietary treatment groups and fed nutritionally complete diets (300 kcal•kg•day^{-1}) containing 55.2% of the total calories from fat with either 97% corn oil, 20% fish oil, or 20% fish and 20% borage oil for 21 days. On day 22, bronchoalveolar lavage was performed 2 hours following an iv injection of *Salmonella Enteritidis* endotoxin (10 mg/kg) or saline. Bronchoalveolar lavage fluid was analyzed for leukotriene B_4, leukotriene C_4/D_4, thromboxane B_2, prostaglandin E_2, 6 keto-prostaglandin $F_{1\alpha}$, TNF-α, and MIP-2 (macrophage inflammatory protein-2). Lung myeloperoxidase activity (a marker for neutrophil accumulation) and phospholipid fatty acid composition were also determined.

Measurements and Main Results: Lung phospholipid levels of arachidonic acid

were lower and the levels of eicosapentaenoic acid and docosahexaenoic acid were higher with fish oil and fish and borage oil as compared with corn oil. Dihomo-γ-linolenic acid, the desaturated and elongated intermediate of γ-linolenic acid, was elevated with fish and borage oil as compared with fish oil and corn oil. The levels of leukotriene B_4, leukotriene C_4/D_4, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 with corn oil were significantly elevated with endotoxin as compared with saline. In contrast to the corn oil group, endotoxin did not significantly increase bronchoalveolar lavage levels of leukotriene B_4, leukotriene C_4/D_4, and thromboxane B_2 above those of saline treated rats with fish oil and fish and borage oil. Lung myeloperoxidase activity was significantly elevated with endotoxin treated rats compared with those given saline in all dietary treatment groups. However, lung myeloperoxidase activity was significantly lower with either fish oil or fish and borage oil as compared with corn oil after endotoxin. Although endotoxin increased the levels of TNF-α and MIP-2 with all dietary treatment groups as compared with saline treated rats, there were no significant differences in the levels of either cytokine between the dietary treatment groups. These results indicate that dietary fish oil and fish and borage oil as compared with corn oil may provide protection against endotoxin induced acute lung injury by suppressing the levels of proinflammatory eicosanoids (but not TNF-α or MIP-2) in bronchoalveolar lavage fluid and pulmonary neutrophil accumulation.

[1]This manuscript has been submitted for publication in similar form with co-authors Jay Whelan, Stephen J. DeMichele, Carolyn C. Snider, Julie A. Guszcza, and Michael D. Karlstad.

B. Introduction

The acute respiratory distress syndrome (ARDS) is characterized by pulmonary edema, increased microvascular permeability and pulmonary vascular resistance, decreased lung compliance, and progressive hypoxemia (1,2). These functional alterations have been associated with the excessive release of arachidonic acid-derived mediators from inflammatory cells. The systemic injection of endotoxin in the rat has been used as a model for sepsis-induced ARDS since it increases the levels of intrapulmonary proinflammatory mediators and produces the functional and morphological pulmonary pathophysiology associated with ARDS in humans. Alveolar macrophages have the potential to release a wide variety of proinflammatory mediators (cytokines and eicosanoids) and have long been considered to be central cells in orchestrating local inflammatory events in ARDS and experimental models of pulmonary inflammation (3). The release of chemotactic agents (leukotriene B_4 and macrophage-inflammatory protein-2) from alveolar macrophages initiates neutrophil recruitment and activation in the pulmonary vasculature (4,5). Neutrophils damage vascular endothelium by the release of proteolytic enzymes and reactive oxygen species during adherence and diapedesis through vascular

tissue (6).

Recent treatment modalities for ARDS have been focused on down-regulation of proinflammatory mediators by pharmacologic intervention (7,8). However, the efficacy of nutritional support to modulate the local inflammatory response to pulmonary injury requires further investigation. Optimal nutritional support of the patient at risk of developing ARDS remains a complex issue. A primary goal of nutritional support has been to lessen the demands on the respiratory system in these patients. For example, high fat, low carbohydrate enteral diets have been shown to reduce minute ventilation and ventilatory demand by lowering the respiratory quotient and CO_2 production in ventilator-dependent patients (9). Moreover, the use of the anti-inflammatory fatty acids, eicosapentaenoic acid and γ-linolenic acid, would provide added benefits. Animal and clinical studies have shown that nutritional intervention with diets containing fish oils containing eicosapentaenoic acid can favorably modulate proinflammatory eicosanoid production (10-12). Similarly, there has been recent interest in the potential benefits of dietary supplementation with γ-linolenic acid found in borage oil (13). γ-Linolenic acid is rapidly elongated to dihomo-γ-linolenic acid and subsequently incorporated in tissue phospholipids. Dihomo-γ-linolenic acid is an inhibitor of leukotriene biosynthesis (14) and is further metabolized to prostaglandin E_1. Prostaglandin E_1 has been shown to increase cardiac output and systemic oxygen delivery while reducing pulmonary arterial pressure and vascular resistance in patients with ARDS (15).

Previously, we have shown that alveolar macrophages from rats fed enteral formulas containing eicosapentaenoic acid and γ-linolenic acid versus a control diet produced less proinflammatory mediators (leukotriene B_4, thromboxane B_2 and prostaglandin E_2) when stimulated *in vitro* (16). Along with these effects on eicosanoid metabolism, eicosapentaenoic acid and γ-linolenic acid provided protection against increases in lung microvascular permeability and hemodynamic insufficiency in endotoxic rats (16). However, we have yet to demonstrate that enteral formulas containing eicosapentaenoic acid and γ-linolenic acid affect local intrapulmonary production of proinflammatory eicosanoids *in vivo*. Therefore, to further characterize the anti-inflammatory properties of diets containing eicosapentaenoic acid and γ-linolenic acid, intrapulmonary proinflammatory mediators were assessed in the bronchoalveolar lavage fluid of endotoxin-challenged rats.

C. Materials and Methods

Approval of this study was granted by the Animal Care Committee of the University of Tennessee Graduate School of Medicine in accordance with guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Experimental design. Male Long-Evans rats weighing 150 ± 10 g were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate to the animal facility prior to the start of Experiments 1 and 2. Three

high fat, low carbohydrate enteral diets were fed to rats for 21 days in both experiments. The fatty acid composition of lung phospholipids was assessed in control rats in Experiment 1. Eicosanoid and cytokine levels recovered in bronchoalveolar lavage fluid and lung neutrophil accumulation (myeloperoxidase activity) in endotoxic and control rats were assessed in Experiment 2.

Enteral nutrition. Rats were randomly assigned to one of three dietary groups. Rats within the same dietary group were housed two per cage in a temperature (21-23°C) and light controlled facility (12 h light/12 h dark). Animals were fed isovolemic, isocaloric (300 $kcal \cdot kg^{-1} \cdot day^{-1}$), and isonitrogenous enteral diets containing corn oil, fish oil, or fish and borage oil for 21 days. Enteral diets were provided in sterilized feeding bottles and a separate bottle provided water ad libitum. Diets were changed daily to minimize the potential for bacterial growth. The diets provided 16.7% of calories from protein, 28.1% of calories from carbohydrate, and 55.2% of calories from lipid. The corn oil diet represents a standard high fat, low carbohydrate enteral formula enriched with linoleic acid devoid of medium chain triglycerides. The fish oil and fish and borage oil diets had similar levels of fish oil and medium-chain triglycerides. To maintain a similar n-6 to n-3 fatty acid ratio between these three diets, linoleic acid was replaced with γ-linolenic acid in the fish and borage oil diet. The fatty acid composition of the enteral diets was determined by gas chromatography (Table 5). The diets were enriched with vitamins and minerals at or above the recommended daily requirement for humans. Diets were formulated and

Table 5. Fatty acid content of enteral diets.

FATTY ACID	% OF TOTAL,		
	Corn Oil	Fish Oil	Fish and Borage Oil
Caproic (6:0)	ND[1]	0.09	0.09
Caprylic (8:0)	ND	12.56	13.05
Capric (10:0)	ND	9.54	9.93
Lauric (12:0)	0.09	0.20	0.20
Myristic (14:0)	0.15	1.22	1.24
Palmitic (16:0)	11.3	6.26	5.95
Palmitoleic (16:1n-6)	0.04	1.70	1.69
Stearic (18:0)	2.27	1.48	1.86
Oleic (18:1n-9)	24.59	28.0	25.96
Linoleic (18:2n-6)	59.35	21.67	16.68
γ-linolenic (18:3n-6)	ND	0.08	4.63
α-linolenic (18:3n-3)	1.01	3.68	3.92
Stearidonic (18:4n-3)	ND	0.70	0.71
Arachidic (20:0)	0.41	0.31	0.27
Eicosenoic (20:1n-9)	0.30	1.08	1.92
Arachidonic (20:4n-6)	ND	0.38	0.38
Eicosapentaenoic (20:5n3)	ND	5.52	5.31
Erucic (22:1n-9)	0.04	0.57	1.12
Docosapentaenoic (22:5n3)	ND	0.55	0.54
Docosahexaenoic (22:6n3)	ND	2.30	2.52
Others	0.45	2.11	2.03
Total n-6/Total n-3	58.76	1.74	1.67

1. ND-Not Detectable.

supplied by Ross Products Division, Abbott Laboratories, Columbus, OH.

In Experiment 1 (n=19), rats were randomly assigned to three dietary groups; corn oil (n=6), fish oil (n=6), or fish and borage oil (n=7). After 21 days of feeding, rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL) and their lungs were removed for phospholipid fatty acid analysis.

Lung Phospholipid Fatty Acid Analysis. Lungs were perfused with cold phosphate buffered saline and the lipids were extracted as described previously (17). Briefly, lungs (100mg) were homogenized in cold saline (0.9%) and the homogenates were resuspended in 3 ml methanol-chloroform (2:1, v/v), extracted once with chloroform-saline (1:1, v/v), and twice with chloroform. The pooled chloroform fractions were evaporated to dryness under nitrogen, redissolved in chloroform, and the phospholipids were separated by thin layer chromatography using a chloroform:methanol (8:1 v/v) solvent system. The phospholipids were recovered by scraping the appropriate band from the thin layer chromatography plate and suspending the scrapings in toluene. The phospholipids were then saponified with 0.5N potassium hydroxide in methanol for eight min at 86°C. Free fatty acids were acidified in 0.7N hydrochloric acid in methanol, extracted twice with hexane, and evaporated to dryness under nitrogen. After methylating with ethereal diazomethane, the fatty acid methyl esters were resuspended in hexane and analyzed by gas chromatography (Hewlett-Packard Model #5890 Series II) using a DB 23 capillary column

(0.25mm x 30m) (J & W Chromatography Folsum, CA) with hydrogen as the carrier gas. Free fatty acids were quantified using pentadecanoic acid as an internal standard and expressed as mole percent.

In experiment 2, (n=48) rats were randomly assigned to one of three dietary groups corn oil (n=16), fish oil (n=16), or fish and borage oil (n=16), and fed for 21 days. On the morning of day 22, rats were anesthetized with isoflurane and given an iv (penile vein) injection of (1.0 ml/kg body wt.) either *Salmonella enteritidis* endotoxin (10 mg/kg) (n=8) or saline (n=8) (control). The rats were then returned to their cages and given free access to water until the bronchoalveolar lavage procedure was performed.

Bronchoalveolar Lavage and Tissue Collection. Two hours following the iv injection of either endotoxin or saline, rats were anesthetized with isoflurane prior to performing the bronchoalveolar lavage procedure. The trachea was cannulated with a Jelco™ 14 gauge teflon IV catheter placement unit (Critikon, Tampa, FL) and secured in placed with ligatures. The lungs were lavaged with six separate 6 ml aliquots of ice cold phosphate buffered saline using a 6 ml syringe. The lavage fluid was pooled for each animal. The pulmonary artery was isolated and perfused with 12 ml cold phosphate buffered saline to flush the blood from the lung. The lungs were then excised and the left lung was quick frozen in liquid nitrogen for assay of myeloperoxidase activity.

Eicosanoid Extraction of Bronchoalveolar Lavage Fluid. The total recovered bronchoalveolar lavage fluid from each rat was centrifuged at 400 x g

for 10 min at 4°C to pellet the cells. The supernatant was diluted 1:1 (v/v) with 20% methanol containing 3 mM formic acid. Eicosanoids were isolated by solid phase extraction using a C-18 cartridge (Burdick and Jackson, Muskegon, MI), washed with distilled water and eluted off the cartridge with methanol. The remaining bronchoalveolar lavage fluid was aliquoted into 1.5 ml polypropylene tubes and stored at -80°C until use for cytokine analysis.

Prostaglandin and Leukotriene Analysis. Eicosanoids were analyzed as described previously (18) with the following modifications: The eluents were evaporated to dryness under nitrogen and resuspended in phosphate buffered saline containing 0.1% (w/v) gelatin. Each sample was analyzed for leukotriene B_4, leukotriene C_4/D_4, thromboxane B_2, prostaglandin E_2, and 6-keto-prostaglandin $F_{1\alpha}$ by RIA according to the manufacturer's instructions (Advanced Magnetics, Cambridge, MA) and tritiated eicosanoids were obtained from New England Nuclear (Dupont Boston, MA). Cross reactivities at half maximum binding of various leukotrienes with leukotriene C_4/D_4 antiserum are as follows: leukotriene C_4(100%), leukotriene D_4 (100%), leukotriene E_4, (20%), leukotriene B_4 (<1%). Cross reactivities at half maximum binding of various leukotrienes with leukotriene B_4 antiserum are as follows: leukotriene B_4 (100%), leukotriene C_4 (<1%), and leukotriene D_4 (<1%). Cross reactivities at half maximum binding of various prostanoids with 6-keto-prostaglandin $F_{1\alpha}$ antiserum are as follows: 6-keto-prostaglandin $F_{1\alpha}$ (100%), prostaglandin $F_{1\alpha}$ (7.8%), 6-keto-prostaglandin E_1 (6.8%), prostaglandin $F_{2\alpha}$ (2.2%), prostaglandin

E_2 (<1%), and thromboxane B_2 (<1%). Cross reactivities at half maximum binding of various prostanoids with prostaglandin E_2 are as follows: prostaglandin E_2 (100%), prostaglandin E_1 (50%), and 6-keto-prostaglandin $F_{1\alpha}$ (<1%) and thromboxane B_2 (<1%). Cross reactivities at half maximum binding of various prostanoids with thromboxane B_2 antiserum are as follows: thromboxane B_2 (100%), prostaglandin E_2 (<1%), and 6-keto-prostaglandin $F_{1\alpha}$ (<1%).

TNF-α and MIP-2 Assays. TNF-α and MIP-2 levels were determined on bronchoalveolar lavage fluid using enzyme-linked immuno-sorbent assay kits (Biosource Cytoscreen Immunoassay™, Camarillo, CA) according to the manufacturer's instructions. Standards and samples were done in triplicate and the absorbance was read at 450 nm on a Minireader II (Dynatech Laboratories, Chantilly, VA) 96 well plate reader. The minimum detectable amount of TNF-α and MIP-2 was 30 and 10 pg/ml, respectively.

Myeloperoxidase Tissue Extraction. A 100 mg sample of the lower lobe of each left lung was homogenized for 30 sec with 5 ml of 80 mM potassium phosphate buffer (KH_2PO_4), pH 7.4, and centrifuged at 40,000 x g (18,000 rpm) for 20 min at 4°C. The supernatants were discarded and the pellets were resuspended in 5 ml of 50 mM potassium phosphate buffer with 0.5% hexadecyltrimethylammonium (HTAB), pH 6.0 and sonicated for 1 min. The sample tubes were frozen in liquid nitrogen for 20 min and then incubated in a 60°C water bath for 2 hours and centrifuged. The tubes were centrifuged and

the supernatants were aliquoted and frozen at -80°C.

Myeloperoxidase Activity Assay . Myeloperoxidase activity was measured by measuring the H_2O_2-dependent oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) (39). Samples were thawed at room temperature and centrifuged at 250 g for 10 min to remove any particulate matter. An aliquot (50 µL) of each sample and standard were added to a flat bottom 96 well plate in triplicate. Following the addition of 100% (w/v) TMB (100µL) to each well, the plates were agitated for 30 min on a plate shaker. The reaction was terminated by the addition of 20 µl of 10M sulfuric acid and shaken for an additional 2 min. Plates were read at 450 nm using a Minireader II 96 well plate reader (Dynatech Laboratories, Chantilly, VA).

Statistical Analysis. Values were analyzed using a one way analysis of variance (ANOVA) and the Student-Newman-Kuels' test for differences between groups. Data are expressed as mean ± SEM. $P \leq 0.05$ was considered significant. Non-parametric data was analyzed using Kruskal-Wallis ANOVA on ranks.

D. Results

Caloric Intake and Body Weight Gain. There were no differences in total caloric intake or the percentage of initial body weight gained between the dietary groups over the 21 day feeding period in experiments 1 and 2. There were no visible signs of intolerance to the diets in either experiment.

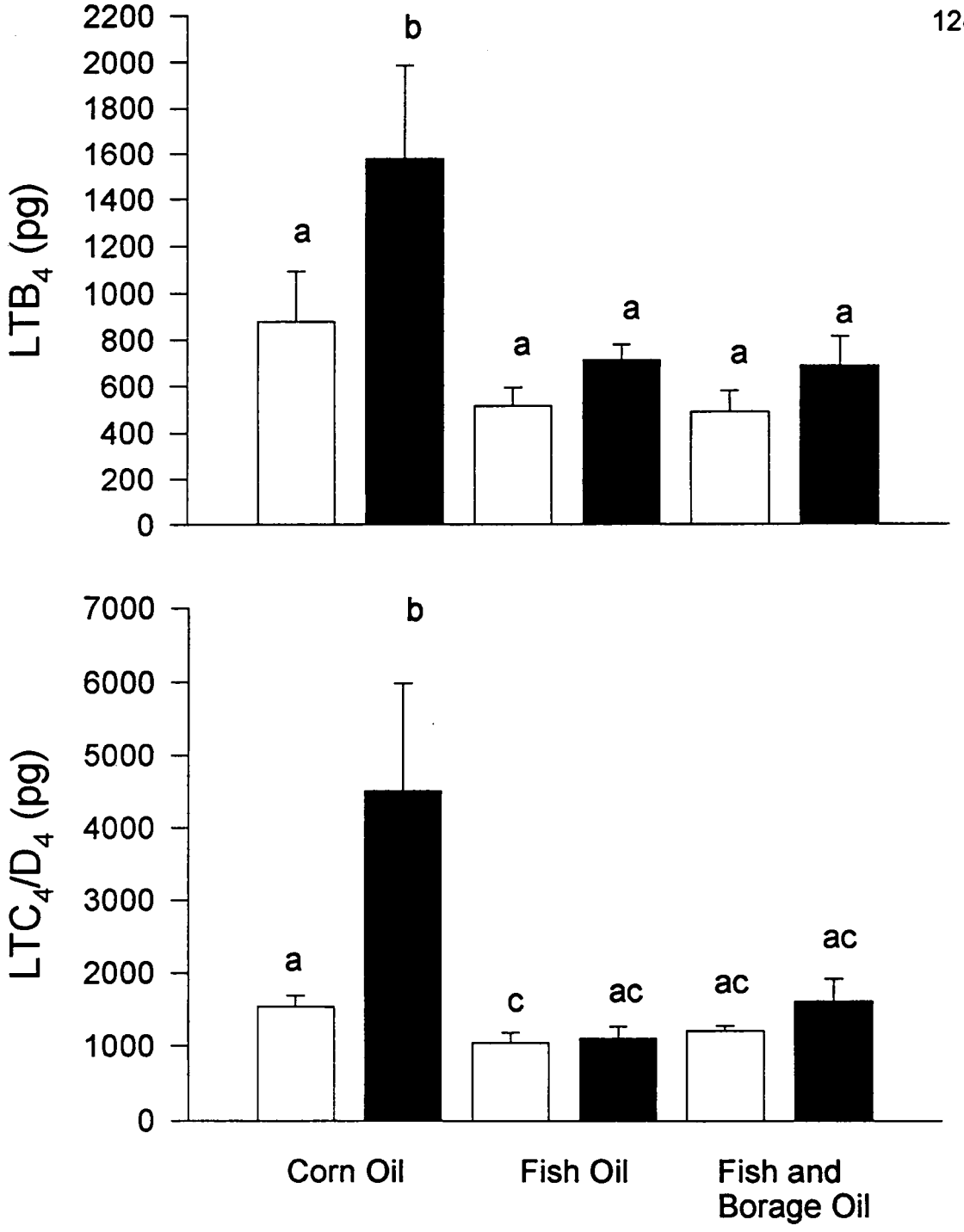
Lung Phospholipid Fatty Acid Composition. Lung phospholipid fatty acid composition in control rats is presented in Table 6. γ-Linolenic acid was present at a low level with fish and borage oil but was not detected with corn oil or fish oil. Dihomo-γ-linolenic acid was significantly higher with fish and borage oil as compared with the other groups. Arachidonic acid was substantially lower with fish oil and fish and borage oil as compared with corn oil. In addition, there was a significantly higher level of arachidonic acid with fish and borage oil as compared with fish oil. Eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids were significantly higher with fish oil and fish and borage oil as compared with corn oil. Eicosapentaenoic acid was not detected in the corn oil group. The n-6/n-3 fatty acid ratio was significantly higher with corn oil as compared with fish oil and fish and borage oil. The ratio of dihomo-γ-linolenic acid to arachidonic acid was significantly higher with fish and borage oil as compared with the other groups.

Bronchoalveolar Lavage Fluid Leukotriene Levels. Leukotriene B_4 and leukotrienes C_4/D_4 in recovered bronchoalveolar lavage fluid of control and endotoxic rats are presented in Figures 5a and 5b, respectively. Leukotriene $B_4$was not significantly affected by diet in the saline control groups (Figure 5a). Following endotoxin administration, leukotriene B_4 was significantly increased with corn oil; however, leukotriene B_4 synthesis with fish and fish and borage oil

Table 6. Effect of feeding diets containing corn oil, fish oil, and fish and borage oil for 21 days on lung phospholipid fatty acid composition (mol%).

Fatty Acid	Corn Oil	Fish Oil	Fish and Borage Oil
16:0	35.1 ± 0.68	34.1 ± 0.62	34.3 ± 0.36
16:1	0.55 ± 0.06[a]	1.99 ± 0.37[b]	2.11 ± 0.44[b]
18:0	17.8 ± 0.37[a]	16.1 ± 0.32[b]	17.05 ± 0.32[c]
18:1	13.6 ± 0.25[a]	17.2 ± 0.60[b]	16.5 ± 0.29[b]
18:2n-6	14.8 ± 0.42[a]	10.1 ± 0.43[b]	7.33 ± 0.14[c]
18:3n-6	ND	ND	0.08 ± 0.08
18:3n-3	0.05 ± 0.05	0.32 ± 0.35	ND
20:3n-6	0.72 ± 0.03[a]	0.70 ± 0.02[a]	1.81 ± 0.03[b]
20:4n-6	15.35 ± 0.30[a]	7.73 ± 0.47[b]	9.96 ± 0.70[c]
20:5n-3	ND	3.28 ± 0.14[a]	2.43 ± 0.12[b]
22:5n-3	0.50 ± 0.11[a]	3.76 ± 0.15[b]	3.51 ± 0.11[c]
22:6n-3	0.87 ± 0.05[a]	3.55 ± 0.06[b]	3.56 ± 0.11[b]
(n-6)/(n-3) PUFA	23.5 ± 3.85[a]	1.70 ± 0.09[b]	2.03 ± 0.13[b]
DGLA/AA	0.05 ± 0.00[a]	0.08 ± 0.012[b]	0.19 ± 0.02[c]

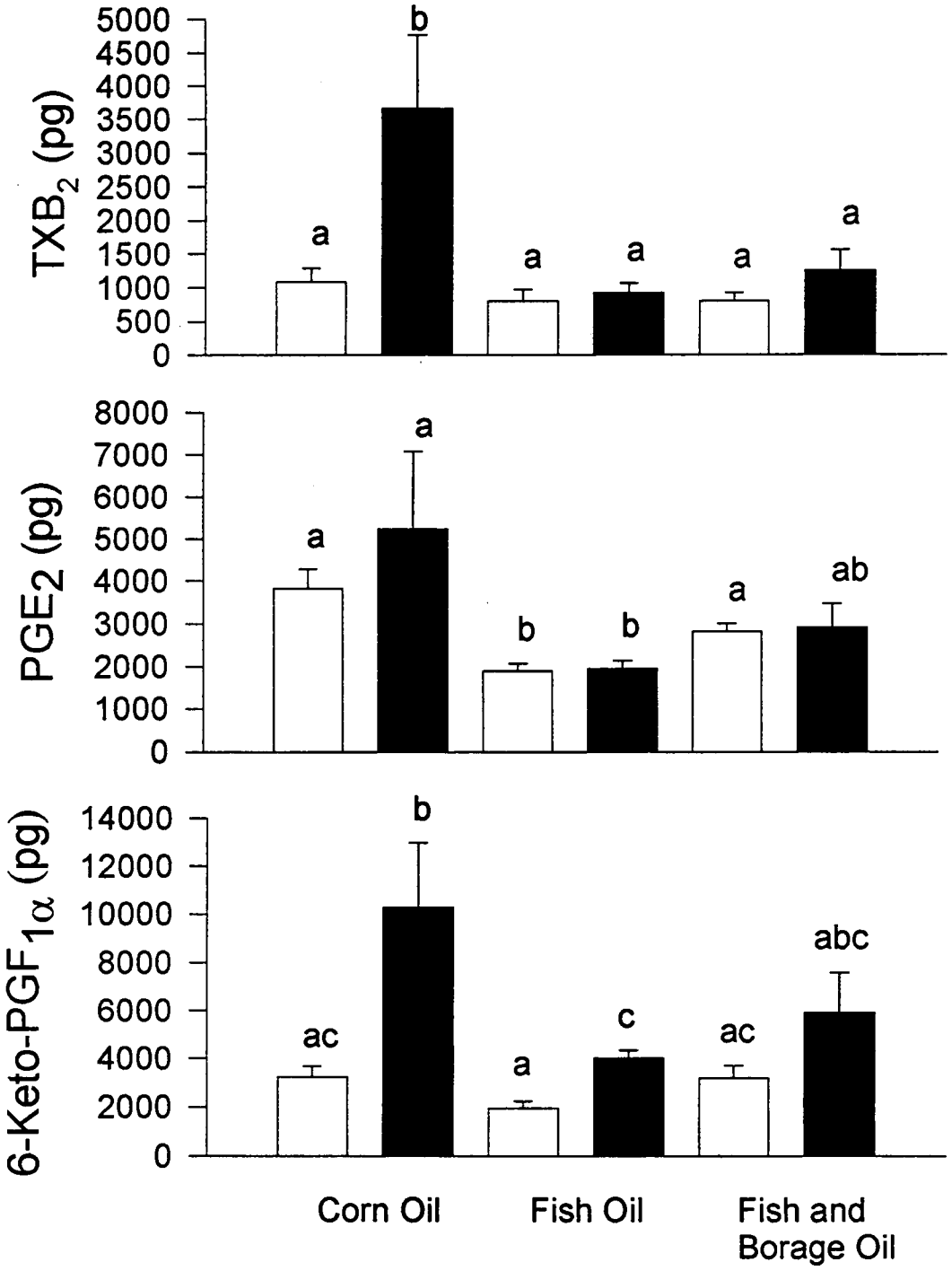
ND- Not detectable; Values are means ± SEM. Values in the same row with different letters are significantly different ($P \leq 0.05$); (n=6-7).



Figures 5. Total leukotriene B_4 (a) and leukotriene C_4/D_4 (b) levels in bronchoalveolar lavage fluid 2 hours after iv injection of either *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) (solid bars) or saline (control) (open bars) from rats fed corn oil, fish oil, or fish and borage oil diets for 21 days. Values are means ± SEM for 8 rats. Bars with different letters are significantly different ($P \leq 0.05$).

failed to respond to endotoxin challenge as leukotriene B_4 did not significantly increase in either of these groups. Moreover, leukotriene B_4 was significantly lower with fish oil and fish and borage oil as compared with corn oil following endotoxin administration. The basal level (saline control) of leukotriene C_4/D_4 was significantly higher with corn oil as compared with fish oil but was not different between corn oil and fish and borage oil. Following endotoxin administration, leukotriene C_4/D_4 increased significantly (~3-fold) over the basal level (saline control) with corn oil, but leukotriene C_4/D_4 synthesis with fish oil or fish and borage oil failed to respond to endotoxin challenge as leukotriene C_4/D_4 did not significantly increase in either of these groups. Moreover, leukotriene C_4/D_4 was significantly lower with fish oil and fish and borage oil as compared with corn oil following endotoxin administration.

Bronchoalveolar Lavage Fluid Prostaglandin Levels. Thromboxane B_2 (stable metabolite of thromboxane A_2), prostaglandin E_2 and 6-Keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin) in recovered bronchoalveolar lavage fluid of control and endotoxic rats are presented in Figure 6a, 6b, and 6c, respectively. Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$were not significantly affected by diet in the saline control groups (Figures 6a and 6c). However, prostaglandin E_2 was significantly affected by diet in the

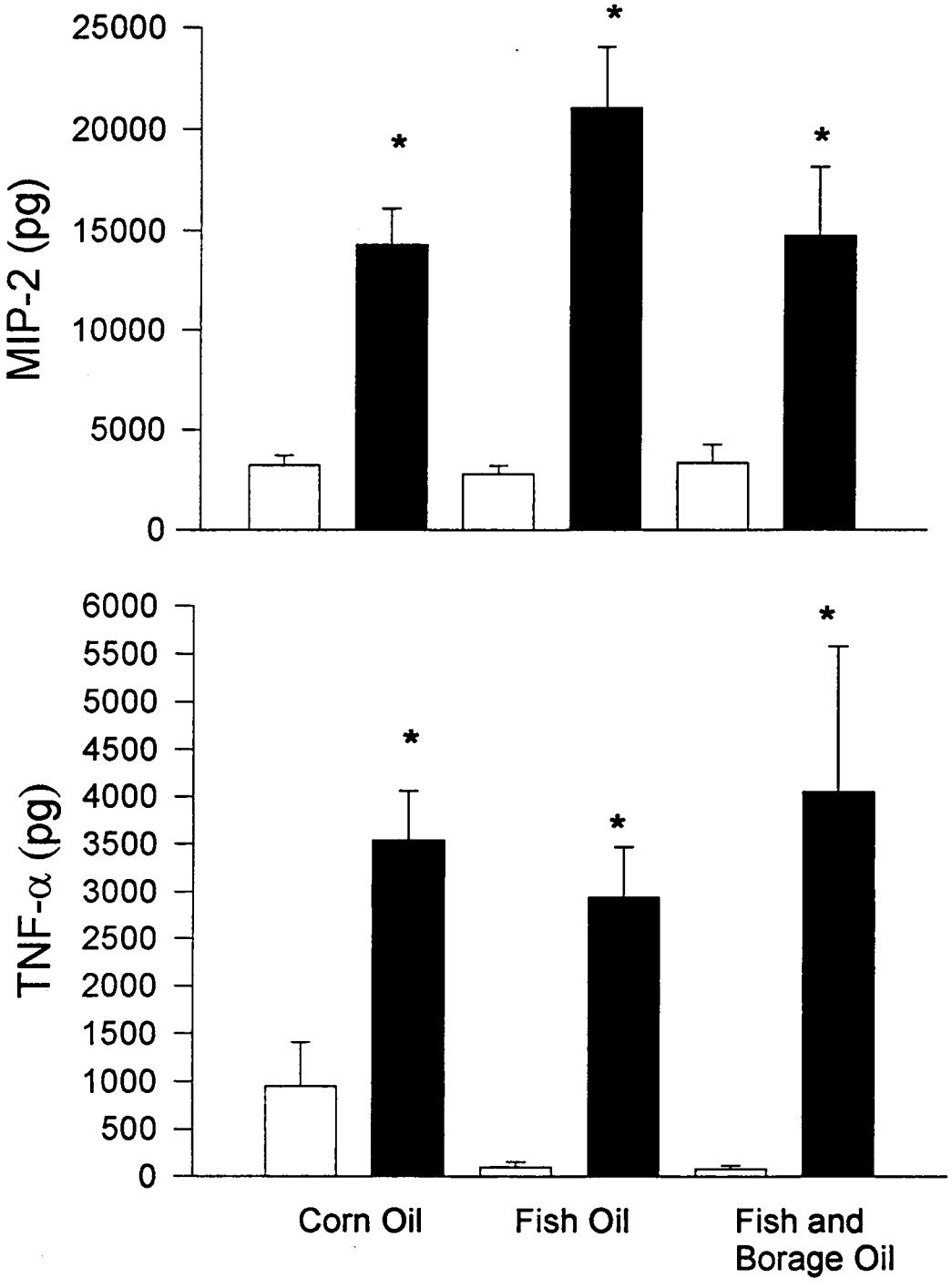


Figures 6. Total thromboxane B_2 (a) , prostaglandin E_2 (b), and 6-keto-prostaglandin $F_{1\alpha}$ (c) levels in bronchoalveolar lavage fluid 2 hours after iv injection of either *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) (solid bars) or saline (control) (open bars) from rats fed corn oil, fish oil, or fish and borage oil diets for 21 days. Values are means ± SEM for 8 rats. Bars with different letters are significantly different ($P \leq 0.05$).

saline control groups. It was significantly lower with fish oil as compared with corn oil and fish and borage oil (Figure 6b). Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ increased significantly, whereas the increase in PGE_2 was not significantly different with corn oil following endotoxin administration as compared with their respective saline controls (Figures 6a-c). Thromboxane B_2 and prostaglandin E_2 failed to respond to endotoxin challenge with fish oil or fish and borage oil as compared with their respective saline controls. In addition, thromboxane B_2 was significantly lower with fish oil and fish and borage oil and prostaglandin E_2 was significantly lower with fish oil as compared with corn oil following endotoxin administration. 6-keto-prostaglandin $F_{1\alpha}$ was significantly lower with fish oil as compared with corn oil, and fish and borage oil was not significantly different from either corn oil or fish oil following endotoxin administration.

Bronchoalveolar Lavage Fluid Cytokine Levels. MIP-2 and TNF-α in recovered bronchoalveolar lavage fluid of control and endotoxic rats are shown in Figures 7a and 7b, respectively. MIP-2 and TNF-α were not significantly affected by diet in the saline control groups. MIP-2 and TNF-α significantly increased to approximately the same level in all three dietary groups following endotoxin administration as compared with their saline control groups.

Lung Myeloperoxidase Activity. Lung myeloperoxidase activity of control and endotoxic rats is presented in Figure 8. Lung myeloperoxidase activity was not significantly affected by diet in the saline control groups. Lung



Figures 7. Total MIP-2 (a) and TNF-α (b) levels in bronchoalveolar lavage fluid 2 hours after iv injection of either *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) (solid bars) or saline (control) (open bars) from rats fed corn oil, fish oil, or fish and borage oil diets for 21 days. Values are means ± SEM for 8 rats. Bars with different letters are significantly different ($P \leq 0.05$).

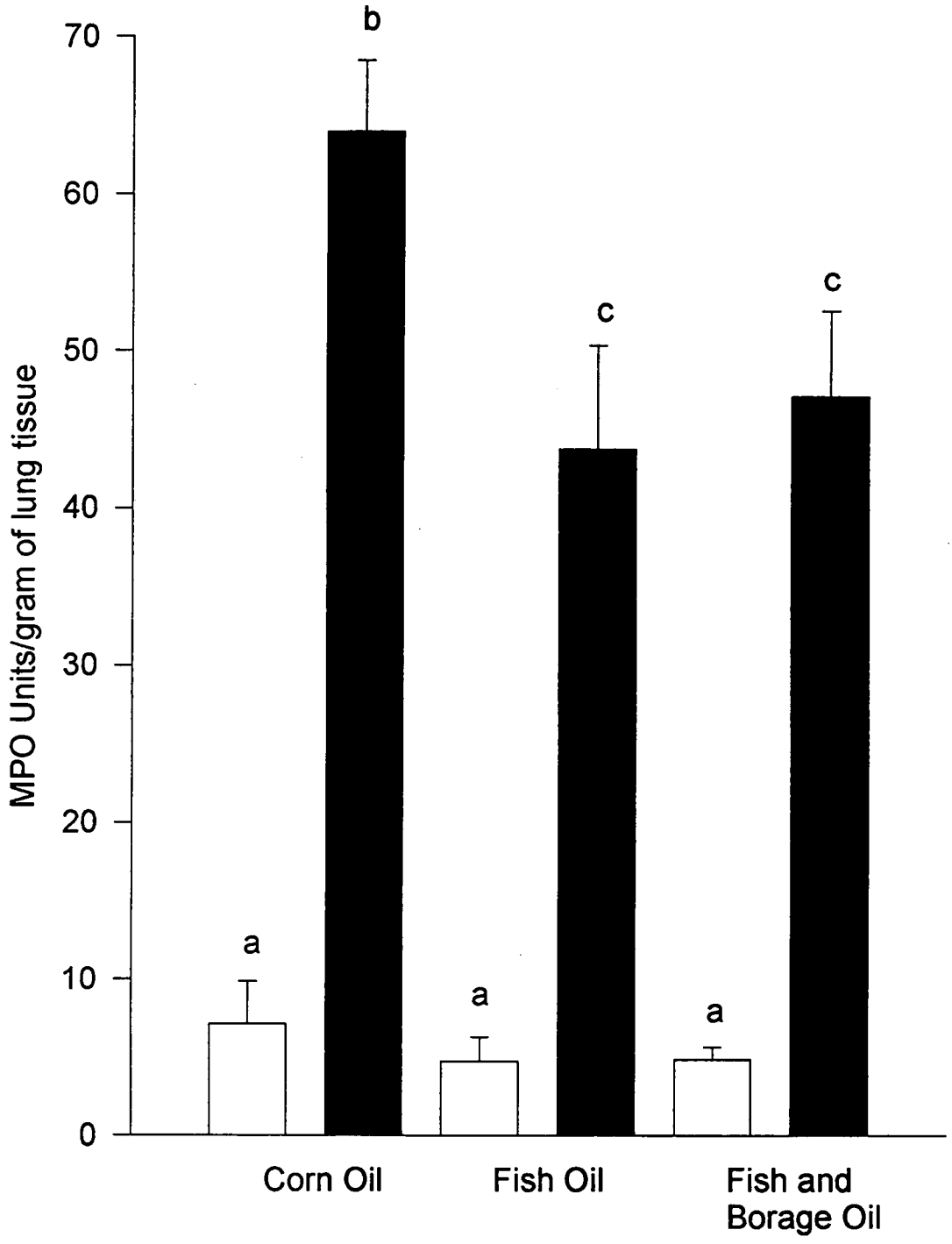


Figure 8. Lung myeloperoxidase activity 2 hours after iv injection of either *Salmonella enteritidis* endotoxin (10 mg/kg of body weight) (solid bars) or saline (control) (open bars) in rats fed corn oil, fish oil, or fish and borage oil diets for 21 days. Values are means ± SEM for 8 rats. Bars with different letters are significantly different ($P \leq 0.05$).

myeloperoxidase activity was significantly increased in all three diets groups following endotoxin administration as compared with their respective saline control groups. Lung myeloperoxidase activity was significantly lower in the fish oil and fish and borage oil groups as compared with corn oil following endotoxin administration. Lung myeloperoxidase activity was not statistically different between the fish oil and fish and borage oil groups following endotoxin administration.

E. Discussion

The rationale for nutritional support with a high fat, low carbohydrate enteral formula containing fish oil alone or in combination with borage oil instead of corn oil is based upon previous studies in endotoxin-induced acute lung injury (16,19). Fish oil and fish and borage oil attenuated proinflammatory eicosanoid production by alveolar macrophages *in vitro* and protected against increases in lung microvascular permeability in rats (16) and improved gas exchange and oxygen delivery in pigs (19). In the current study, we examined the effects of feeding enteral diets containing corn oil, fish oil, or a mixture of fish and borage oil for 21 days on the incorporation of fatty acids into lung phospholipids and on lung myeloperoxidase content and levels of proinflammatory eicosanoids and cytokines in bronchoalveolar lavage fluid two hours after the intravenous administration of endotoxin or saline in rats. We demonstrated that fish oil alone or in combination with borage oil, as compared

with corn oil, reduced lung myeloperoxidase content and suppressed the levels of thromboxane B_2, leukotriene B_4, and leukotriene C_4/D_4 but not TNF-α and MIP-2 in bronchoalveolar lavage fluid of endotoxic rats. These results, in conjunction with our previous study using enteral nutrition with fish oil alone or in combination with borage oil (16), indicate that the decrease in lung neutrophil accumulation and protection against elevated lung microvascular permeability may be due to the suppression of proinflammatory eicosanoid synthesis but not the proinflammatory cytokines, TNF-α and MIP-2, in endotoxin-induced acute lung injury.

This study demonstrates that both dietary fish oil and borage oil can significantly alter the fatty acid composition of lung phospholipids by decreasing arachidonic acid and increasing eicosapentaenoic acid with fish oil and increasing dihomo-γ-linolenic acid when combined with borage oil. These changes in fatty acid composition are consistent with the overall suppression of eicosanoids associated with systemic inflammatory response syndrome, viz. 2-series prostaglandins and 4-series leukotrienes. Furthermore, it has been shown that the incorporation of eicosapentaenoic acid into phospholipids shifts eicosanoid metabolism, to the less biologically active 3-series prostaglandins and 5-series leukotrienes (20-22). The fact that fish and borage oil increased the ratio of dihomo-γ-linolenic acid to arachidonic acid in lung phospholipids suggests that γ-linolenic acid is desaturated and elongated to dihomo-γ-linolenic acid and is not entirely converted to arachidonic acid. These changes

in fatty acid composition are directly associated with the overall suppression of important eicosanoid mediators of the systemic inflammatory response syndrome, the 2-series prostaglandins and 4-series leukotrienes. Increasing phospholipid content of dihomo-γ-linolenic acid has been associated with an expected increase in the biosynthesis of prostaglandin E_1 and an increase in the 15-lipoxygenase metabolite 15-hydroxyeicosatrienoate (15-HETrE). 15-HETrE has been shown to inhibit 5-lipoxygenase and attenuate leukotriene biosynthesis (14).

Leukotrienes have been found in high concentrations in the bronchoalveolar lavage fluid of patients with ARDS (23) and are considered to be key mediators of pulmonary inflammation in ARDS. Leukotrienes are released from a number of inflammatory cells including alveolar macrophages, neutrophils, and mast cells in the lung (24). It is likely that these sources contributed to the elevated levels of leukotrienes in bronchoalveolar lavage fluid of endotoxic rats. The cysteinyl-leukotrienes, C_4 and D_4, have been shown to increase both lung microvascular permeability (25,26) and pulmonary arterial pressure (25) when administered into the pulmonary circulation of animals. Cohn et al (25) showed that a leukotriene C_4/D_4 antagonist significantly decreased lung extravascular water accumulation in endotoxic pigs. In addition, pulmonary edema formation can be attenuated by a leukotriene D_4 receptor antagonist in endotoxic rats (27). Thus suppression of leukotriene C_4/D_4 biosynthesis may limit the severity of pulmonary microvascular protein leakage

in endotoxin-induced acute lung injury (16).

Similarly, inhibition of leukotriene B_4 formation has been shown to significantly reduce lung microvascular permeability and edema formation in the rat (28) and dog (29). Leukotriene B_4 is a potent chemoattractant of neutrophils and activated neutrophils are thought to mediate vascular endothelial cell damage by the release of proteases, reactive oxygen species, hypochlorous acid, leukotrienes, cytokines, and platelet activating factor (30). We have shown previously, using the same enteral diets as in this study, that the synthesis of leukotriene B_4 from A23187-stimulated alveolar macrophages was significantly less with diets containing fish oil and fish and borage oil as compared with corn oil (16). This study suggests that suppressed synthesis of leukotriene B_4 in bronchoalveolar lavage fluid may be due to a reduced capacity of alveolar macrophages to synthesize leukotriene B_4 *in vivo*. This would reduce the chemotactic signal for neutrophil recruitment and is consistent with our current findings of reduced myeloperoxidase activity and neutrophil accumulation in the lung and our previous findings of protection against increased lung microvascular protein permeability in endotoxic rats (16).

In general, eicosanoid formation in bronchoalveolar lavage fluid was significantly lower with fish oil and fish and borage oil as compared with corn oil following endotoxin challenge. Moreover, a striking feature of this dietary study is the lack of response in eicosanoid production by endotoxin-challenged rats fed fish oil and fish and borage oil. As observed with leukotrienes,

thromboxane B_2 (stable metabolite of thromboxane A_2) and prostaglandin E_2 failed to increase above their saline controls. Thromboxane A_2 has been shown to increase lung microvascular permeability and pulmonary arterial pressure in acute lung injury (31). Murray et al (11,19) demonstrated that dietary fish oil or fish and borage oil significantly attenuated plasma thromboxane B_2 levels and alleviated pulmonary arterial hypertension in endotoxic pigs. Prostaglandin E_2 is immunosuppressive at concentrations ($>10^{-8}$) and at low concentration ($<10^{-9}$) is required for normal T-cell function (32). Thus, modulation of prostaglandin E_2 synthesis may normalize immune function. The results of this study paralleled our previous study where we showed that alveolar macrophages from rats fed fish oil alone or in combination with borage oil synthesized less prostaglandin E_2 as compared with corn oil (16). In contrast to thromboxane B_2 and prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin) was not completely suppressed following endotoxin challenge with fish oil and fish and borage oil as compared with their saline controls. Prostacyclin opposes the vasoactive and platelet-aggregating activities of thromboxane A_2 and therefore may represent a beneficial response in the endotoxic lung.

TNF-α is another important mediator of endotoxin-induced inflammation (33) and acute lung injury (34). Infusion of recombinant human TNF-α induces acute lung injury (34) and symptoms that mimic the acute respiratory distress syndrome in rats. TNF-α is elevated in the bronchoalveolar lavage fluid and serum in patients with the acute respiratory distress syndrome (32). Diet had

no effect on attenuating TNF-α levels in bronchoalveolar lavage fluid following endotoxin challenge in this study. These results are in contrast to studies reporting that dietary fish oil enhances the *in vitro* production of TNF-α from peritoneal macrophages in mice (35) and rats (36).

The macrophage inflammatory protein-2 (MIP-2) is a member of the α branch of the supergene family of chemokines (37). It is rapidly produced by alveolar macrophages and pulmonary epithelial cells in response to endotoxin and is a potent chemoattractant of neutrophils (38). We show that endotoxin-induced increase in MIP-2 levels in bronchoalveolar lavage fluid was not affected by diet. However, it is likely that MIP-2 played a major role in attracting neutrophils to the pulmonary microvasculature following endotoxin in this study. It appears that the reduction in pulmonary neutrophil accumulation, as shown by a decrease in myeloperoxidase activity, with fish oil alone or in combination with borage oil as compared with corn oil, was due to the suppression of leukotriene B_4 synthesis but not MIP-2 in the bronchoalveolar lavage fluid of endotoxic rats.

In summary, changes in lung fatty acid phospholipid composition with dietary fish oil and fish and borage oil were associated with suppressed synthesis of proinflammatory 2-series prostaglandins and 4-series leukotrienes in bronchoalveolar lavage fluid of endotoxic rats. These results suggest that the reduction in pulmonary neutrophil accumulation and the previously reported attenuation of lung microvascular protein permeability (16) may be related to

the reduced synthesis of proinflammatory eicosanoids but not TNF-α or MIP-2 in bronchoalveolar lavage fluid of endotoxic rats. Enteral nutrition with high fat, low carbohydrate formulas containing anti-inflammatory fatty acid lipid blends may provide protection against increases in lung microvascular permeability and pulmonary neutrophil accumulation in critically ill patients at risk of developing the ARDS.

REFERENCES

1. Kollef MH, Schuster DP: The acute respiratory distress syndrome. *N Eng J Med* 1995; 332:27-37

2. Hudson LD: New therapies for ARDS. *Chest* 1995; 108:79S-91S

3. Fels AOS, Cohn ZA: The alveolar macrophage. *J Appl Physiol* 1986; 60:353-369

4. Yoshimura K, Nakagawa S, Koyama S, et al: Roles of neutrophil elastase and superoxide anion in leukotriene B_4-induced lung injury in rabbit. *J Appl Physiol* 1994; 76:91-96

5. Driscoll KE, Hassenbein DG, Howard BW, et al: Cloning, expression, and functional characterization of rat MIP-2: a neutrophil chemoattractant and epithelial cell mitogen. *J Leuk Biol* 1995; 58:359-364

6. Sharar SR, Winn RK, Harlan JM: Endotoxin-induced interactions of inflammatory cells with the lungs. In: Brigham KL, ed. Endotoxin and the Lungs. New York: Marcel Dekker, Inc; 1994: 229-265

7. Bernard GR, Reines HD, Halushka PV, et al: Prostacyclin and thromboxane A_2 formation is increased in human sepsis syndrome: Effects of cyclooxygenase inhibition. *Am Rev Respir Dis* 1991; 144:1095-1101

8. Haupt MT, Jastremski MS, Clemmer TP, et al: Effect of ibuprofen in patients with severe sepsis: a randomized, double-blind, multicenter study. *Crit Care Med* 1991; 19:1339-1347

9. Al-Saady NM, Blackmore CM, Bennett ED: High fat, low carbohydrate, enteral feeding lowers $PaCO_2$ and reduces the period of ventilation in artificially ventilated patients. *Int Care Med* 1989; 15:290-295

10. Kenler AS, Swails WS, Driscoll DF, et al: Early enteral feeding in postsurgical cancer patients: Fish oil structed lipid-based polymeric formula versus a standard polymeric formula. *Ann Surg* 1996; 223:316-333

11. Murray MJ, Svingen BA, Yaksh TL, et al: Effects of endotoxin on pigs prefed omega-3 vs. omega-6 fatty acid-enriched diets. *Am J Physiol* 1993; 265:E920-E927

12. Lee TH, Hoover RL, Williams JD, et al: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 1985; 312:1217-1224

13. Ziboh VA, Fletcher MP: Dose-response effects of dietary τ-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B_4. *Am J Clin Nutr* 1992; 55:39-45

14. Chapkin RS, Miller CC, Somers SD, et al: Ability of 15-hydroxyeicosatrienoic acid (15-OH-20:3) to modulate macrophage arachidonic acid metabolism. *Biochem Biophys Res Commun* 1988; 153:799-804

15. Abraham E, Park YC, Covington P, et al: Liposomal prostaglandin E_1 in acute respiratory distress syndrome: A placebo-controlled, randomized, double-blind, multicenter clinical trial. *Crit Care Med* 1996; 24:10-15

16. Mancuso P, Whelan J, DeMichele SJ, et al: Effects of eicosapentaenoic acid and gamma-linolenic acid on lung permeability and alveolar macrophage eicosanoid synthesis in endotoxic rats. *Crit Care Med* 1996; In Press

17. Surette ME, Whelan J, Lu G, et al: Dependence on dietary cholesterol for n-3 polyunsaturated fatty acid-induced changes in plasma cholesterol in the Syrian hamster. *J Lipid Res* 1992; 33:263-271

18. Whelan J, Broughton KS, Kinsella JE: The comparative effects of dietary alpha-linolenic acid and fish oil on 4- and 5-series leukotriene formation *in vivo*. *Lipids* 1991; 26:119-126

19. Murray MJ, Kumar M, Gregory TJ, et al: Enteral diets enriched in eicosapentaenoic acid and gamma-linolenic acid attenuate cardiopulmonary dysfunction in a porcine model of acute lung injury. *Am J Physiol* 1995; 269:H2090-H2099

20. Lee TH, Mencia-Huerta J, Shih C et al: Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J Biol Chem* 1984; 259:2383-2389

21. Fischer S, Weber PC: Prostaglandin I_3 is formed *in vivo* in man after dietary eicosapentaenoic acid. *Nature* 1984; 307:165-168

22. Needleman P, Raz A, Minkes MS, et al: Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci USA* 1979; 76:944-948

23. Stephenson AH, Lonigro AJ, Hyers TM, et al: Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* 1988; 138:714-719

24. Holtzman MJ: Arachidonic acid metabolism. Implications of biological chemistry for lung function and disease. *Am Rev Respir Dis* 1991; 143:188-203

25. Cohn SM, Kruithoff KL, Rothchild HR, et al: Beneficial effects of LY203647, a novel leukotriene C_4/D_4 antagonist, on pulmonary function and mesenteric perfusion in a porcine model of endotoxic shock and ARDS. *Circ Shock* 1991; 33:7-16

26. Cook JA, Li EJ, Spicer KM, et al: Effect of leukotriene receptor antagonists on vascular permeability during endotoxic shock. *Circ Shock* 1990; 32:209-218

27. Turner CR, Lackey MN, Quinlan MF, et al: Therapeutic intervention in a rat model of adult respiratory distress syndrome: II. Lipooxygenase pathway inhibition. *Circ Shock* 1991; 34:263-269

28. Ball HA, Cook JA, Spicer KM, et al: Essential fatty acid-deficient rats are resistant to oleic acid-induced pulmonary injury. *J Appl Physiol* 1989; 57:811-816

29. Sprague RS, Stephenson AH, Lonigro AJ: OKY-046 prevents increases in LTB_4 and pulmonary edema in phorbol ester-induced lung injury in dogs. *J Appl Physiol* 1992; 73:2493-2498

30. Gee MH, Albertine KH: Neutrophil-endothelial cell interactions in the lung. *Ann Rev Physiol* 1993; 55:227-248

31. Turnage RH, Guice KS, Oldham KT: Pulmonary microvascular injury following intestinal reperfusion. *New Horizons* 1994; 2:463-475

32. Hyers TM, Tricomi SM, Dettenmeier PA, et al: Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991; 144:268-271

33. Parsons PE, Moore FA, Moore EE et al: Studies on the role of tumor necrosis factor in adult respiratory distress syndrome. *Am Rev Respir Dis* 1992; 146:694-700

34. Ferrari-Balliviera E, Mealy K, Smith RJ, et al: Tumor necrosis factor induces adult respiratory distress syndrome in rats. *Arch Surg* 1989; 124:1400-1405

35. Hardardottir I, Kinsella JE: Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochim Biophys Acta* 1991; 1095:187-195

36. Carrick JB, Schnellmann RG, Moore JN: Dietary source of omega-3 fatty acids affects endotoxin-induced peritoneal macrophage tumor necrosis factor and eicosanoid synthesis. *Shock* 1994; 2:421-426

37. Driscoll KE, Hassenbein DG, Carter J, et al: Macrophage inflammatory proteins 1 and 2: Expression by rat alveolar macrophages, fibroblasts, and epithelial cells and in rat lung after mineral dust exposure. *Am J Resp Cell Mol Biol* 1993; 8:311-318

38. Huang S, Paulauskis JD, Godleski JJ, et al: Expression of macrophage inflammatory protein-2 and KC mRNA in pulmonary inflammation. *Am J Pathol* 1992; 141:981-988

39. Schierwagen C, Bylund-Fellenius A-C, Lundberg C: Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. *J Pharmacol Meth* 1990; 179-186.

PART 4

CONCLUSIONS

I. CONCLUSIONS

In the experiments presented in this dissertation, we have demonstrated that commercially prepared enteral formulas containing anti-inflammatory fatty acids from fish oil alone or in combination with borage oil as compared with corn oil provided protection against endotoxin-induced lung microvascular protein permeability, neutrophil accumulation, and hemodynamic insufficiency. Although the addition of borage oil to the diets did not provide additional protection against endotoxin-induced lung microvascular protein permeability, hemodynamic insufficiency, or lung neutrophil accumulation, it (20% fish and 20% borage oil) significantly muted thrombocytopenia as compared with fish oil alone. The beneficial effects of the diets containing fish oil and borage oil as compared with corn oil may have been due to the modulation of tissue phospholipid fatty acids and attenuation of intrapulmonary proinflammatory eicosanoid biosynthesis but not TNF-α or MIP-2 in endotoxic rats. It is premature at this time to predict the effectiveness of the diets containing anti-inflammatory lipid blends in a clinical setting. However, these diets may provide protection against eicosanoid and neutrophil mediated increases in lung microvascular permeability and hemodynamic insufficiency in critically ill patients at risk of developing ARDS by attenuating proinflammatory eicosanoid formation.

VITA

Peter Mancuso, the 4th of 5 children, was born in Somerville, NJ on July 15, 1962 and grew up in Mt. Freedom, NJ. He attended Center Grove and Shongum Elementary Schools and Randolph Intermediate School in Randolph, NJ. He graduated from Randolph High School in May, 1981. In August of the same year, he attended Purdue University in West Lafayette, IN and graduated with a Bachelors degree in Agriculture with a major in Food Science in May, 1985. In the summer of 1985, he worked for Thomas J. Lipton, Co. in beverage product development as a summer intern. In September of that year, he attended the University of Tennessee, Knoxville, and graduated with a Masters' degree in Agriculture, with a major in Food Technology and Science and a minor in Nutrition, in December, 1987. On September, 1987, Peter married Alison Hightower in Knoxville, TN and began working for Ragu Foods, Co. in Shelton, CT. in October, 1987 where he was employed as a Food Scientist in Product Development. He was promoted to Research Food Scientist in April, 1990. In August, 1990, Peter enrolled in the Ph.D. program in Exercise Physiology at the University of Tennessee, Knoxville. In January, 1992, he transferred to the Life Sciences Program in Physiology. He started his dissertation project in January 1993 in Dr. Michael Karlstad's laboratory and defended his dissertation in November, 1996. In January, 1997, Peter will begin working as a postdoctoral fellow at the University of Michigan Medical Center in the Division of Pulmonary and Critical Care Medicine.