Rapid modulation of lung and liver macrophage phospholipid fatty acids in endotoxicemc rats by continuous enteral feeding with n-3 and γ-linolenic fatty acids¹,²

**John D Palombo, Stephen J DeMichele, Erin E Lydon, Timothy J Gregory, Phillip LC Banks, R Armour Forse, and Bruce R Bistrian**

**ABSTRACT**  Dienic eicosanoids derived from phospholipid arachidonic acid (AA) in lung and liver macrophages promote leukosequestration, thrombosis, and tissue injury. Current enteral diets (diet A) are enriched with linoleic acid (LA), a precursor of AA. Novel diets low in LA and containing eicosapentaenoic acid (EPA) and γ-linolenic acid (GLA) foster formation of less inflammatory eicosanoids. The study objective was to assess the rapidity and extent of LA and AA displacement in vivo from alveolar macrophage (AMφ), lung, and liver Kupffer and endothelial (KE) cell phospholipids in rats fed enterally with diets enriched with 5.3% (by wt) EPA and either 1.2% or 4.6% GLA (diets B and C, respectively). After surgical placement of catheters, the rats were fed enterally and co-infused intravenously with either endotoxin or vehicle continuously for 3 or 6 d. Rats given either diet B or C had significantly lower (P < 0.01) relative percentages of AA and LA within the AMφ, lung, and KE cell phospholipids, and concomitantly higher percentages of EPA compared with rats infused with diet A after 3 d of enteral feeding irrespective of endotoxin co-infusion. Incorporation of d1homo-γ-linolenic acid (DHGLA), the metabolite of GLA, into lung and KE cell phospholipids was significant in rats given diet C. Most of the changes in fatty acid composition occurred by day 3. The polyunsaturated fatty acid composition of AMφ, lung, and KE cell phospholipids can be rapidly modified by continuous short-term enteral feeding with EPA- and GLA-enriched diets irrespective of concurrent endotoxemia.  *Am J Clin Nutr* 1996;63:208–19.

**KEY WORDS**  Polysaturated fatty acids, alveolar macrophages, lung, Kupffer cell, enteral feeding, fish oil, borage oil

**INTRODUCTION**  Given their large population of resident macrophages and extensive endothelial surface, the lung and liver play a prominent role in and, in turn, are the host immune response to infection (1–3). As immune cells, alveolar macrophages (AMφ), and hepatic sinusoidal (ie, Kupffer and endothelial [KE]) cells are capable of altering the functional properties of the proximal parenchymal cells within their respective organs. On activation, these cells release proinflammatory cytokines, eicosanoids derived from arachidonic acid (AA, 20:4n-6), and oxygen free radicals (4–6). Several eicosanoids (eg, leukotriene B₄ and thromboxane A₂) are chemotactic and promote leukosequestration to exacerbate tissue injury (7). In addition, the macrophage respiratory burst, which arises after AA activation of the NADPH oxidase complex (8), generates superoxide anions injurious to type 2 pneumocytes that synthesize surfactant (9). Persistent and uncontrolled release of these inflammatory mediators may predispose humans to the development of adult respiratory distress syndrome (ARDS) and hepatic failure (5, 10, 11).

Experimental and clinical studies have shown that the host’s immune response can be modulated by nutritional intervention with diets containing marine oils enriched with eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids. EPA, DHA, and other n-3 polyunsaturated fatty acids (PUFAs) displace AA and its dietary precursor, linoleic acid (LA, 18:2n-6) from immune cell membrane phospholipids (12, 13). Activated macrophages from animals fed n-3-enriched diets formed reduced amounts of dienoic prostaglandins (14) and leukotrienes (15), and exhibited an attenuated respiratory burst (16) relative to animals given control diets containing n-6 PUFAs (ie, LA). In addition, EPA competes with AA for binding sites on cyclooxygenase and lipoxygenase, thereby serving as the progenitor of trienoic eicosanoids, which exhibit reduced chemotactic properties relative to the dienoic series derived from AA (Figure 1). Previous investigations demonstrated that human mononuclear cells released less tumor necrosis factor α (TNFα) and interleukin 1β (17) and neutrophils were less chemotactic (18) after extended dietary supplementation with n-3 compared with n-6 PUFAs. Recent experimental studies of in vivo effects after n-3 supplementation support these findings. Guinea pigs or pigs given n-3-enriched diets exhibited less pulmonary leukosequestration (19) and/or better gas exchange after endotoxin (19, 20), or live Escherichia coli (21) challenge relative to animals receiving an LA-enriched diet.

¹ From the Departments of Surgery and Medicine, New England Deaconess Hospital, Harvard Medical School, Boston, and Ross Products Division, Abbott Laboratories, Columbus, OH.
² Address reprint requests to JD Palombo, Deaconess Hospital, CRI-116, 194 Pilgrim Road, Boston, MA 02215.

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Enteral products that are currently available have high concentrations of LA and therefore may not be appropriate for surgical or critically ill patients at risk of developing ARDS. For this reason, alternative enteral preparations containing EPA in combination with γ-linolenic acid (GLA, 18:3n-6) and low amounts of LA have been developed. Dihomo-γ-linolenic acid (DHGLA, 20:3n-6) formed from GLA competes with AA for cyclooxygenase binding sites and serves as the precursor of the monoenoic prostaglandin E₁ (PGE₁) (Figure 1). The reported biologic responses of PGE₁ include vasodilatation and suppression of leukocyte effector functions, e.g., chemotaxis and lysosomal enzyme release. Experimental studies have documented that gas exchange and oxygen delivery were improved with concurrently lower pulmonary vascular resistance, and reduced lung permeability after endotoxin challenge in pigs or rats given diets enriched with EPA and GLA.

The rapidity and extent of macrophage and lung uptake and metabolism of EPA in combination with GLA as constituents of these enteral formulations has not been determined. Modulation of cell membrane PUFAs with these dietary fatty acids to down-regulate the host’s inflammatory response in a clinically relevant time period may prove beneficial to surgical patients at risk of developing respiratory or hepatic dysfunction. In this regard, our earlier studies in rats revealed that significant replacement of LA and AA with dietary EPA occurred in liver and lung macrophage phospholipids within 72-96 h of continuous enteral feeding with a fish oil–enriched diet. We also found that displacement of LA and AA from liver sinusoidal cells was not abated by concurrent endotoxemia. The present study was undertaken to characterize dietary modulation of AMφ, lung tissue, and hepatic KE cell phospholipid PUFAs in rats enterally fed continuously for 3 or 6 d with formulations enriched with EPA in combination with either a relatively low or high percentage of GLA. Independent subsets of rats were randomly assigned to receive an intravenous co-infusion of endotoxin or vehicle during enteral feeding to assess PUFA incorporation under endotoxemic conditions.

**MATERIALS AND METHODS**

The study design was approved by the Deaconess Animal Care and Use Committee. The analytic methods were similar to those described earlier. Briefly, male Sprague-Dawley rats (Harlan, Altamont, NY) were fed a commercial stock diet in pelleted form (Prolab RMH 3000; Agway, Inc, Cornell, NY) ad libitum for 5 d before study entry. The lipid in the pellets accounted for 14.5% of total energy; total n-6 and n-3 fatty acids accounted for 35% and 2.3% of the total lipid, respectively. After anesthetization with ether (at 0830 of day −1), a medical silastic catheter (1.0 mm (0.040 in) internal diameter × 2.16 mm (0.085 in) outer diameter) was inserted saphenously through a fundal gastrostomy, advanced 2.5 cm into the duodenum, and anchored to the stomach wall with a purse string suture. A catheter (0.64 mm (0.025 in) internal diameter × 1.2 mm (0.047 in) outer diameter) was inserted in the left jugular vein for intravenous infusion of endotoxin or vehicle. The proximal ends of the catheters were tunneled subcutaneously, exteriorized at the midsacral region, and attached to a flow-through dual-channel swivel (Spalding Medical Products, Danville, PA) to permit freedom of movement. Each rat was housed individually in a wire-bottomed cage during the infusion periods and allowed water ad libitum for the study duration.

**Enteral diets**

All rats were randomly assigned before study inception to diet or endotoxin treatment. After recovery from catheterization surgery, all rats were infused with a low-fat commercial enteral diet, hereafter termed baseline diet, at one-half the energy requirement (520 kJ·kg⁻¹·d⁻¹; lipid = 9.4% of total...
TABLE 1
Composition of enteral diets

<table>
<thead>
<tr>
<th></th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td>16.7</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>63.7</td>
<td>63.3</td>
<td>64.9</td>
</tr>
<tr>
<td>Source</td>
<td>Sodium and calcium caseinates</td>
<td>Sodium and calcium caseinates</td>
<td>Sodium and calcium caseinates</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>28.1</td>
<td>28.1</td>
<td>28.1</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>102.2</td>
<td>104.2</td>
<td>103.4</td>
</tr>
<tr>
<td>Source</td>
<td>Hydrolyzed cornstarch, sucrose</td>
<td>Hydrolyzed cornstarch, sucrose</td>
<td>Hydrolyzed cornstarch, sucrose</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td>55.2</td>
<td>55.2</td>
<td>55.2</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>94.0</td>
<td>94.1</td>
<td>93.7</td>
</tr>
<tr>
<td>Source</td>
<td>Corn oil, soy lecithin</td>
<td>Canola oil, corn oil, MCT, fish oil, borage oil, soy lecithin</td>
<td>Canola oil, MCT, fish oil, borage oil, soy lecithin</td>
</tr>
<tr>
<td><strong>Energy</strong></td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>(MJ/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg TE/L)</td>
<td>36.4</td>
<td>299.5</td>
<td>303.3</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>480.9</td>
<td>915.5</td>
<td>835.4</td>
</tr>
<tr>
<td>β-Carotene (μg/L)</td>
<td>—</td>
<td>5590</td>
<td>5746</td>
</tr>
</tbody>
</table>

1 MCT, medium-chain triacylglycerols; TE, tocopherol equivalents.
2 Selectively enriched with some vitamins and minerals >100% of the US recommended dietary allowance.

energy) from 1500 of day −1 until 0900 of day 0 to facilitate rehydration and adaptation to postoperative enteral feeding. Independent subsets of rats (n = 6/group) were then infused continuously by a programmable syringe pump (Harvard Apparatus, Natick, MA) with either diet A, B, or C (Table 1) until either 0900 of day 3 or 0900 of day 6. All rats received an isovolemic, isonitrogenous, and isoenergetic (1040 kJ·kg⁻¹·d⁻¹) amount of the assigned formulation. These diets provided 16.7% of energy from protein, 28.1% of energy from carbohydrate, and 55.2% of energy from lipid. Fresh diets were provided daily to minimize the potential for bacterial growth.

The fatty acid profiles of the enteral formulations were determined by gas chromatography (Table 2). Diet A represented a standard high-fat enteral diet enriched with LA (59%), with no medium-chain triacylglycerols (MCTs). Diets B and C were enriched with deodorized sardine and borage seed oils, contained MCTs, and had reduced concentrations of LA (20.3% and 16.7%, respectively). Although diets B and C were nearly identical with respect to individual or total n−3 PUFAs (12.6% and 13%, respectively), diet B had a relatively low amount of GLA (1.25%) whereas diet C had a higher percentage of GLA (4.63%). To maintain a similar ratio of n−6 to n−3 PUFAs between diets B and C, LA was replaced by GLA in diet C. Vitamins C and E and β-carotene were increased in diets B and C relative to diet A to minimize the potential of lipid peroxide formation in vivo (Table 1).

Endotoxin infusion

Within each dietary treatment group, independent subsets of rats (n = 6) were randomly assigned to receive an intravenous co-infusion of either endotoxin (E. coli 026:B6; Difco, Detroit) or an isovolemic quantity of vehicle (0.1% albumin free of fatty acids, in saline) continuously by syringe pump starting at 1500 of day −1 and ending at the time of killing (day 0, 3, or 6). The amount of endotoxin infused (0.4 mg·kg⁻¹·d⁻¹) was nonlethal but capable of inducing physical signs of endotoxemia (conjunctival hemorrhage, lethargy, ear flattening, and piloerection), and doubling of spleen weight within 3 d. Fresh endotoxin solutions were reconstituted daily from a single lot stored at −20°C.

Isolation of hepatic KE cells

Subsets (n = 6/group) of rats were killed on day 0 (prediet baseline), or days 3 or 6 depending on the randomization schedule. Each rat was anesthetized with 50 mg pentobarbi-
ental/kg administered through the intravenous line. After abdominal laparotomy, 500 U heparin was injected into the inferior vena cava and allowed to circulate. The technique for isolation of hepatic KE cells was described previously (12). Briefly, a 16-gauge angiocatheter was inserted in the exposed hepatic veins and the liver lobes flushed in retrograde fashion with Ca\(^{2+}\)-free HEPES buffer (5 mmol/L, pH 7.2). The portal vein was severed to facilitate the flush. The liver was then flushed in situ with collagenase (0.25% each of types 2 and 4 in HEPES, pH 7.6; Sigma, St Louis). Before removal of the liver, the superior vena cava (SVC) was clamped and the SVC distal to the clamp flushed with phosphate-buffered saline (Ca\(^{2+}\)- and Mg\(^{2+}\)-free) to remove blood components from the pulmonary vasculature. The liver was then excised, incubated in a closed petri dish at 37 °C for 15 min, stripped of supporting connective tissue, and minced before further collagenase digestion for 30 min at 37 °C.

The hepatic KE cells were separated from hepatocytes by differential centrifugation (50 × g for 2 min at 4 °C, three times) followed by isolation on a 17.5% discontinuous metrizamide gradient. The enriched fraction containing KE cells was resuspended in Gey’s balanced salt solution (GBSS). Cell numbers and viability (> 90%) by trypan blue exclusion were determined by light microscopy. The KE cells were then resuspended in GBSS at a density of 2–3 × 10\(^{10}\) cells/L, capped with liquid nitrogen and stored at −20 °C for total phospholipid fatty acid analysis.

### Alveolar macrophage and lung tissue preparation

To collect AM\(\Phi\), a 14-gauge angiocatheter was inserted through a tracheostomy and the lungs were lavaged eight times with 5 mL cold phosphate-buffered saline containing 0.6 mmol EDTA/L; gentle chest massage facilitated lavage recovery (> 90%) (13). The cumulative lavage fluid was kept on ice during this procedure. After lavage, lung tissue samples (free of bronchial tissue) were excised and stored under nitrogen at −20 °C. Frozen lung tissue was homogenized in saline solution immediately before lipid extraction as described below.

The lavage fluid was spun at 400 × g for 10 min at 4 °C. The cell pellet was resuspended in phosphate-buffered saline and centrifuged at 110 × g for 10 min at 4 °C. The washed cell pellet was then resuspended in phosphate-buffered saline, layered on Ficoll Paque (Pharmacia, Piscataway, NJ), and centrifuged at 500 × g at 22 °C for 35 min to isolate the AM\(\Phi\). Purity and viability (by trypan blue exclusion) were > 95% under light microscopy. AM\(\Phi\) (5–7 × 10\(^{8}\)) were capped under nitrogen and stored in phosphate-buffered saline at −20 °C.

### Analysis of phospholipid fatty acids

Quantitation of 16 identifiable fatty acids (12–24 carbons in length) in the phospholipid fraction of AM\(\Phi\), lung tissue homogenate, and KE cells were determined by methods described previously (12). Diheptadecanoylphosphatidylcholine was used as an internal standard and was added before lipid extraction. After lipid extraction in chloroform:methanol (2:1, by vol), the phospholipid fraction was isolated on silica gel H (Analtech, Newark, DE) thin-layer plates using a mobile phase of petroleum ether, diethyl ether, and glacial acetic acid (80: 20:1, by vol), and identified relative to the migration of a phospholipid standard by using dichlorofluorescein spray. The phospholipid band was isolated, hydrolyzed, and methylated with 14% BF\(_{3}\) in methanol. The fatty acid methyl esters were separated and quantified on a Hewlett Packard (model 5890 Series II; Palo Alto, CA) gas chromatograph equipped with a 50-m fused silica capillary column containing SP-2330 as the stationary phase with a 0.20-μm film thickness (Supelco, Bellefonte, PA). The relative mole percentages of individual fatty acids were identified and quantified by using CHEM STATION software (Hewlett Packard) based on the relative responses of an external standard of pure fatty acid methyl esters (NuChek Prep, Elysian, MN).

### Statistical analyses

Orthogonal contrasts were constructed to test mean differences among the three diet groups. One comparison evaluated differences among diets A, B, and C. These between-group differences were tested by a fixed effects, analysis of variance model at day 3 and day 6. Group differences from baseline were analyzed by a two-sample t test. Separate analyses were carried out by challenge condition (ie, endotoxin or vehicle). Sensitivity analyses of model assumptions showed that the reported results were robust under most data conditions, eg, normality assumption. All tests of hypotheses were non-directional with statistical significance set at \(P < 0.05\).

### RESULTS

The control (diet A) and experimental (diets B and C) enteral diets were well tolerated by the vast majority of rats. Most rats exhibited loose stools that resolved after the first 24 h of diet infusion irrespective of endotoxin or vehicle co-infusion. There were no significant differences in initial or final body weight (overall ± SD: 254 ± 12 and 257 ± 14 g, respectively) or change in body weight among the dietary and endotoxin treatment groups.

The between-diet group comparisons of relative mole percentages of the principal PUFAs of interest were similar across the endotoxin or vehicle stratifications (ie, statistically significant interaction effects) for the AM\(\Phi\), lung tissue, or KE cell phospholipids. Therefore, for purposes of simplifying this report, only the rats treated with endotoxin are discussed below.

### Alveolar macrophage fatty acids

Significant changes in the mean relative percentage of the majority of unsaturated fatty acids in AM\(\Phi\) phospholipids occurred within the first 3 d of enteral feeding without further change between days 3 and 6, irrespective of diet (Table 3, Figures 2 and 3). This fatty acid modulation largely reflected the differences between the amount and composition of dietary fatty acids present in the stock diet pellets (13) and the low-fat baseline diet (day-0 rats) with that present in the three enteral diets tested. For example, AM\(\Phi\) from rats randomly assigned to receive the LA-enriched diet (diet A) had significantly higher percentages of LA, total n−6 PUFAs, and ratios of n−6 to n−3 PUFAs by day 3 relative to rats at baseline (day 0, Table 3). Similarly, AM\(\Phi\) from rats randomly assigned to receive the diets enriched with GLA and EPA (diets B and C) had significantly higher (\(P < 0.001\)) concentrations of EPA and other n−3 PUFAs (Figure 2a and Table 3), and a trend toward
increased concentrations of DHGLA by day 3 than those from either the rats receiving the baseline diet or diet A.

With regard to the principal PUFAs that compete for cyclooxygenase binding sites, the relative percentage of AMΦ AA (20:4n−6) in rats given diet A increased over time and was significantly greater than that at baseline by day 6 (Figure 3a). In contrast, the relative percentage of AMΦ AA in rats given diets B and C for 3 or 6 d remained similar to that on day 0. In comparison with the rats given diet A, the mean relative percentage of EPA (Figure 2), docosapentaenoic acid (DPA; 22:5n−3), and DHA (Table 3) in AMΦ from rats receiving diets B and C were significantly higher, and AA (Figure 3) and LA (Table 3) were concurrently lower on both days 3 and 6. In accordance with these modifications, total n−3 PUFAs were significantly higher (P < 0.001) whereas total n−6 PUFAs and ratios of n−6 to n−3 PUFAs were lower in AMΦ from rats infused with diets B or C relative to those given diet A (Table 3).

![FIGURE 2](https://academic.oup.com/ajcn/article-abstract/63/2/208/4650536)
Although DHGLA, the metabolite of dietary GLA, is the precursor of AA, there was no evidence to suggest that the presence of GLA in either diet B or C resulted in an increased appearance of AA in the AMϕ phospholipids on either day 3 or day 6 (Figure 3). Although there was a trend for higher concentrations of DHGLA in AMϕ from rats provided diets containing either low (diet B) or high (diet C) amounts of GLA than from rats provided diet A (Figure 2), the variation within the DHGLA data confounded the identification of statistical differences among the diet groups on either day 3 or day 6.

Of interest was the finding that the rats receiving the higher-GLA diet (diet C) had significantly lower amounts of LA on day 6 than did the rats receiving the low-GLA diet (diet B) (Table 3). This may have been, in part, because diet C had a slightly lower amount of LA than diet B (Table 2; 16.7% compared with 20.3%, respectively). It may also have reflected further displacement of LA by GLA. However, neither of the GLA-supplemented groups exhibited consistently detectable concentrations of GLA, or concentrations of DHGLA higher than those at baseline. The variability in the AMϕ data may have obscured any real dietary effect, because it will be shown below that the relative percentage of DHGLA measured in lung and KE cells from rats treated with diet C for 6 d was significantly higher than that in rats treated with either diet A or B.

The eicosanoid precursor ratio (EPA + DHGLA/AA) is a relative index of 20-carbon PUFAs that compete directly for cyclooxygenase binding sites. A higher ratio indicates a milieu more favorable toward formation of the trienoic and monoenoic series of eicosanoids from EPA and DHGLA over the dienoic series derived from AA. In this regard, AMϕ from the rats given either diet B or C had a significantly higher mean ratio ($P < 0.01$) compared with either the rats receiving the baseline diet or diet A on day 3, without further change occurring on day 6 (Figure 3). AMϕ from rats on diets B and C had similar eicosanoid precursor ratios on both days 3 and 6.

The unsaturation index (UI), which is an indicator of the number of double bonds within phospholipid PUFAs (27), revealed that AMϕ from rats given either diet B or diet C for 3 d had a higher UI than that calculated for the rats at baseline ($P < 0.05$), or those given diet A for 3 d ($P < 0.06$); this latter comparison was statistically significant by day 6. Total PUFAs (n−3 + n−6, data not shown) in AMϕ and the ratios of polyunsaturated to saturated fatty acids (P:S) for all diet groups were significantly higher ($P < 0.05$) than baseline values by day 6. No differences in total polyunsaturates, monounsaturated fatty acids, or P-S ratios existed among the three diet groups on either day 3 or 6. Interestingly, the sum of the relative percentages of all saturated fatty acids for each diet group was lower ($P < 0.05$) after 3 d of enteral feeding, relative to baseline values. This relative decrease in saturated fatty acids appeared to be attributable to the concomitant increase in total PUFAs as stated above.

**Lung tissue fatty acids**

As observed for AMϕ, modulation of lung tissue phospholipid fatty acids by enteral feeding reflected the fatty acid composition of the enteral diets. The largest changes in the mean relative percentage of lung PUFAs occurred within the first 3 d of enteral feeding (Table 4, Figures 4 and 5). However, further modulation of lung PUFAs between days 3 and 6 was selective, eg: 1) LA and the sum of n−6 PUFAs decreased further ($P < 0.05$) in rats given diet C (Table 4), as observed for the AMϕ; 2) rats given diet B (EPA + low GLA) experienced a further increase ($P < 0.06$) in lung EPA (Figure 4) with a concurrent decrease in total n−6 PUFAs; and 3) the ratio of eicosanoid precursors (ie, EPA + DHGLA/AA) increased further between days 3 and 6 for rats infused with either diet B or C (Figure 5).

The relative percentage of AA in lung of rats given diet A increased 15% between days 0 and 3 (Figure 5) without further
change on day 6. In contrast, lungs from rats given diets B and C had significantly lower (P < 0.01) percentages of AA by day 6 than did rats at baseline or rats given diet A. LA as well as ratios of n-6 to n-3 were also lower in rats given diets B and C relative to those given diet A (Table 4). Rats given diet B (EPA + low GLA) experienced a greater (P < 0.05) reduction (29%) in lung AA between days 0 and 6 than did rats given diet C (14%). This reduction occurred in concert with the rise in the relative percentages of EPA and/or DHGLA.

The mean relative percentages of phospholipid EPA (Figure 4), DPA, DHA, and total n-3 PUFAs (Table 4) in lung tissue from rats given diets B and C were significantly higher (P < 0.001) than the corresponding means at baseline, as well as in lung tissue from rats given diet A on both day 3 and day 6. Diet groups B and C had similar percentages of individual and total n-3 PUFAs on day 3. The mean relative percentage of lung phospholipid DHGLA (1.3%) was significantly higher by day 3 in rats given diet C (i.e., the EPA + high-GLA diet) in comparison with rats given either diet B (0.9%, P < 0.05) or diet A (0.8%, P < 0.01) (Figure 4). Although lung DHGLA in the rats given diet C increased further to 1.5% by day 6, there were no changes in lung DHGLA in the rats given diet B. GLA was not detected in any lung homogenates.

The resultant differences in the eicosanoid precursor ratio (Figure 5) between the group of rats provided with diet A and those given either diet B or C for 3 d further exemplified the rapidity and extent of dietary modulation of these lung phospholipid PUFAs. Rats receiving diet B or C had a significantly higher eicosanoid precursor ratio than did rats at baseline or those given diet A. The ratio increased further between days 3 and 6 for rats given diet B or C. The UIs of lung phospholipid fatty acids from rats given diet B or C were also significantly higher (P < 0.001) on day 3 than those at baseline or those of rats given diet A (Table 4). The P.S of lung phospholipid fatty acids increased significantly (P < 0.05) above baseline for rats given either diet A or diet C by day 3. However, there were no differences among the mean P-S ratios of the three enteral groups on day 6, or between baseline values and those on day 6.

### Liver Kupffer and endothelial cell fatty acids

The effects of enteral feeding on liver KE cell phospholipid PUFAs paralleled those described above for AMΦ and lung tissue. Comparisons of each of the major PUFAs of interest revealed significant differences between rats given the high-LA diet (diet A) and those given the EPA + GLA diets (diets B and C) on days 3 and 6 (Table 5). With few exceptions, the majority of significant changes in relative percentages of individual unsaturated fatty acids and the P-S and n-6 to n-3 PUFA ratios occurred within the first 3 d without further modification by day 6. One notable exception was that further displacement of LA occurred between days 3 and 6 for rats given diet C, which was similar to the reductions observed above for AMΦ (Table 3) and lung tissue (Table 4).

Given the larger pool size of phospholipid PUFA in liver KE cells compared with the lung (40% and 30%, respectively) (12, 13), the capacity of dietary lipids to influence the PUFA composition of these liver sinusoidal cells tended to be greater than that observed in either the AMΦ or lung tissue. Modulation of KE cell AA occurred in a manner similar to that observed for AMΦ, i.e., rats given diet A showed a significant increase (P < 0.01) in the relative percentage of AA from a baseline value of 15.8% to 21.7% on day 3, whereas the concentration of AA in rats given diets B or C remained at baseline values throughout the study (Table 5). The increased percentages of individual n-3 PUFAs in the KE phospholipids from rats provided with diets B and C were accompanied by significant decreases in LA. Of interest was the finding that rats given diet B (EPA + low GLA) exhibited further incorporation of EPA between days 3 and 6, to the extent that EPA accounted

### Table 4

Principal fatty acids of lung tissue phospholipids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Day 0 Baseline</th>
<th>Day 3 Diet A</th>
<th>Day 3 Diet B</th>
<th>Day 3 Diet C</th>
<th>% by wt of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>1.5 ± 0.6</td>
<td>0.8 ± 0.1*</td>
<td>1.7 ± 0.3*</td>
<td>1.4 ± 0.3*</td>
<td>0.9 ± 0.1*</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>39.0 ± 3.0</td>
<td>35.2 ± 2.4</td>
<td>35.1 ± 3.3</td>
<td>34.3 ± 3.4</td>
<td>36.1 ± 2.1</td>
</tr>
<tr>
<td>Palmitoleic (16:1n-7)</td>
<td>1.8 ± 0.5</td>
<td>1.0 ± 1.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.5</td>
<td>0.6 ± 0.2*</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>14.4 ± 1.3</td>
<td>16.5 ± 0.6*</td>
<td>15.3 ± 1.0*</td>
<td>15.2 ± 1.4*</td>
<td>16.8 ± 1.6</td>
</tr>
<tr>
<td>Oleic (18:1n-9)</td>
<td>11.2 ± 0.8</td>
<td>11.1 ± 0.6*</td>
<td>12.7 ± 1.1*</td>
<td>13.3 ± 0.3*</td>
<td>11.2 ± 0.8*</td>
</tr>
<tr>
<td>Linoleic (18:2n-6)</td>
<td>9.2 ± 1.0</td>
<td>11.4 ± 0.9*</td>
<td>7.9 ± 0.5*</td>
<td>7.1 ± 0.6*</td>
<td>11.6 ± 0.4*</td>
</tr>
<tr>
<td>Docosapentaenoic (22:5n-3)</td>
<td>2.4 ± 0.6</td>
<td>1.3 ± 0.3*</td>
<td>3.6 ± 0.2*</td>
<td>3.5 ± 0.6*</td>
<td>1.0 ± 0.2*</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6n-3)</td>
<td>2.1 ± 0.6</td>
<td>1.6 ± 0.2*</td>
<td>2.4 ± 0.2*</td>
<td>2.5 ± 0.5*</td>
<td>1.6 ± 0.3*</td>
</tr>
<tr>
<td>Total saturation</td>
<td>56.6 ± 2.2</td>
<td>54.4 ± 2.1</td>
<td>54.0 ± 1.5</td>
<td>52.4 ± 2.1</td>
<td>55.9 ± 2.4*</td>
</tr>
<tr>
<td>Total monounsaturates</td>
<td>14.1 ± 0.9</td>
<td>13.6 ± 1.5*</td>
<td>15.3 ± 0.7*</td>
<td>16.6 ± 0.7*</td>
<td>13.1 ± 1.0*</td>
</tr>
<tr>
<td>Total n-3 polyunsaturates</td>
<td>5.6 ± 1.8</td>
<td>3.3 ± 0.6*</td>
<td>10.4 ± 0.9*</td>
<td>10.2 ± 1.3*</td>
<td>2.8 ± 0.6*</td>
</tr>
<tr>
<td>Total n-6 polyunsaturates</td>
<td>23.7 ± 1.6</td>
<td>28.6 ± 0.9*</td>
<td>20.3 ± 0.6*</td>
<td>20.8 ± 1.0*</td>
<td>28.2 ± 2.8*</td>
</tr>
<tr>
<td>n-6:n-3</td>
<td>4.7 ± 1.5</td>
<td>8.8 ± 1.4*</td>
<td>2.0 ± 0.2*</td>
<td>2.1 ± 0.3*</td>
<td>10.4 ± 2.3*</td>
</tr>
<tr>
<td>P:S²</td>
<td>0.52 ± 0.05</td>
<td>0.59 ± 0.04</td>
<td>0.57 ± 0.03</td>
<td>0.59 ± 0.06</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td>Unsaturation index</td>
<td>1.18 ± 0.10</td>
<td>1.19 ± 0.06*</td>
<td>1.32 ± 0.04*</td>
<td>1.36 ± 0.10*</td>
<td>1.14 ± 0.11*</td>
</tr>
</tbody>
</table>

1 ± SD; n = 6/group. Values with different superscript letters within each time interval are significantly different, P < 0.05 (ANOVA). Comparisons over time are discussed in the Results section.

2 Ratio of polyunsaturates to saturates.

3 Σ (% unsaturated fatty acids × number of double bonds)/100.
for 8.5% of total fatty acids by day 6. For this reason, both EPA and total n−3 PUFAs in KE cells from rats given diet B exceeded \( P < 0.01 \) that measured in KE cells of rats given diet C (EPA + high GLA) by day 6.

Similarly, liver KE cells showed a greater propensity to incorporate DHGLA into the phospholipid pools than either AM\( \Phi \) or lung tissue when GLA was present in the diet. The mean relative percentage of KE cell DHGLA was 2.6% in the rats given diet C on day 3, which was significantly higher \( P < 0.001 \) than the proportion of DHGLA measured in the rats given diet B (1.4%) or diet A (0.8%) (Table 5). However, the relative percentage of DHGLA in the KE cell phospholipids from rats given the low-GLA diet (ie, diet B) was also greater \( P < 0.05 \) than that measured in the rats given diet A. GLA was not detected in these liver sinusoidal cells.

The eicosanoid precursor ratios followed a pattern similar to that observed in lung tissue (Figure 5b), ie, the ratio increased significantly in rats on diets B and C relative to diet A by day

**FIGURE 4.** Mole percentage of eicosapentaenoic acid (EPA) and dihomo-\( \gamma \)-linolenic acid (DHGLA) in rat lung tissue phospholipids. See Figure 2 for a description of the diet groups. \( \bar{x} \pm SD; n = 6 / \text{group.} \) For a given fatty acid, values with different superscript letters are significantly different, \( P < 0.05. \)

**FIGURE 5.** Mole percentage of arachidonic acid (AA) and the ratio of eicosapentaenoic acid (EPA) + dihomo-\( \gamma \)-linolenic acid (DHGLA) to AA in rat lung tissue phospholipids. See Figure 2 for a description of the diet groups. \( \bar{x} \pm SD; n = 6 / \text{group.} \) For a given fatty acid or ratio, values with different superscript letters are significantly different, \( P < 0.05. \)
TABLE 5
Principal fatty acids of liver Kupffer and endothelial cell phospholipids<sup>1</sup>

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Diet A</td>
<td>Diet B</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>0.6 ± 0.3</td>
<td>0.2 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>31.1 ± 4.0</td>
<td>24.4 ± 1.9</td>
<td>24.0 ± 1.3</td>
</tr>
<tr>
<td>Palmitoleic (16:1n-7)</td>
<td>0.9 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>22.9 ± 1.7</td>
<td>23.5 ± 1.0</td>
<td>21.4 ± 1.1</td>
</tr>
<tr>
<td>Oleic (18:1n-9)</td>
<td>7.2 ± 1.6</td>
<td>7.8 ± 0.7</td>
<td>9.2 ± 3.2</td>
</tr>
<tr>
<td>Linoleic (18:2n-6)</td>
<td>11.7 ± 1.5</td>
<td>13.9 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic (20:3n-6)</td>
<td>0.98 ± 0.39</td>
<td>0.80 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arachidonic (20:4n-6)</td>
<td>15.8 ± 0.42</td>
<td>21.7 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.9 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5n-3)</td>
<td>1.5 ± 1.1</td>
<td>0.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Docosapentaenoic (22:5n-3)</td>
<td>1.7 ± 0.6</td>
<td>0.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6n-3)</td>
<td>2.5 ± 0.7</td>
<td>2.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total saturates</td>
<td>55.5 ± 5.1</td>
<td>49.3 ± 2.9</td>
<td>46.7 ± 1.8</td>
</tr>
<tr>
<td>Total monounsaturates</td>
<td>9.3 ± 2.3</td>
<td>9.9 ± 0.9</td>
<td>11.4 ± 2.7</td>
</tr>
<tr>
<td>Total n-3 polyunsaturates</td>
<td>5.8 ± 1.8</td>
<td>2.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total n-6 polyunsaturates</td>
<td>29.1 ± 5.2</td>
<td>37.6 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.1 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-6:n-3</td>
<td>5.6 ± 1.9</td>
<td>14.5 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P/S&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.64 ± 0.23</td>
<td>0.82 ± 0.10</td>
<td>0.89 ± 0.09</td>
</tr>
<tr>
<td>Unsaturation index&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.32 ± 0.23</td>
<td>1.46 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>x ± SD; n = 6/group. Values with different superscript letters within each time interval are significantly different, P < 0.05 (ANOVA). Comparisons over time are discussed in the Results section.

<sup>2</sup>Ratio of polyunsaturates to saturates.

<sup>1</sup>Σ (% unsaturated fatty acids × number of double bonds)/100.

3, and by day 6 rats on diet B had a significantly higher mean ratio than rats given either diet A or diet C. The mean UIs of the KE cell phospholipids from the rats given diets B and C for 3 d were also significantly higher than those at baseline (P < 0.01) or those of rats given diet A (P < 0.05)(Table 5). This increased degree of unsaturation was sustained on day 6; however, differences between the UI of rats given diet A and those of rats given either diet B or C were not statistically significant (P < 0.08).

**DISCUSSION**

Lung and liver macrophages and endothelium play a central role in the inflammatory response to injury or sepsis. As discussed earlier, functional alterations in lung and liver have been associated with the release of lipid mediators derived from AA. Recent treatment modalities for ARDS have therefore been directed toward down-regulation of these lipid metabolites by pharmacologic means (28–30). However, few clinical studies have investigated the efficacy of nutritional intervention to modulate the synthesis of lipid mediators derived from immune cell phospholipid PUFAs.

The capacity of select dietary PUFAs to reduce the relative quantity of AA and LA present in macrophage membranes and thereby attenuate inflammatory processes driven by dienoic eicosanoids may prove clinically relevant for critically ill patients at risk of developing pulmonary or hepatic dysfunction. Incorporation of dietary EPA into mononuclear cell phospholipids has been associated with decreased formation of several vasoactive eicosanoids derived from AA and reduced synthesis of TNFα and interleukin 1β (14, 17). Similarly, provision of GLA as a dietary supplement has been associated with reduced inflammation secondary to formation of monoenoic eicosanoids derived from DHGLA (22). Because the majority of commercially available enteral diets contain high amounts of LA, the precursor of AA, novel enteral formulations containing small amounts of LA and supplemented with EPA and/or GLA have been assessed for potential application in the critical care setting. The outcome of preliminary experiments with pigs fed novel enteral preparations lends further support to the concept that pulmonary and systemic inflammation as sequelae of endotoxemia can be attenuated by short-term (<8 d) nutritional pretreatment with EPA alone (20) or EPA in combination with GLA (24, 25). However, although oral nutrient pretreatment has proven advantageous in these models, whether or not provision of these formulations enterally or during septic complications would confer the same functional benefits remains unresolved. A second concern has been that such intervention therapies would be practical only if their application can impart a beneficial effect within a clinically relevant time span. In this regard, the rapidity and extent of incorporation of EPA and metabolism of GLA to DHGLA within lung and liver macrophages in vivo during endotoxemia has not been well characterized.

For this reason, the present study was conducted to determine the capacity of short-term, continuous feeding with two enteral preparations enriched with EPA from fish oil and GLA from borage oil to modulate phospholipid fatty acid composition in rat lung and liver macrophages under endotoxemic conditions. This study has revealed several important findings. The first was the rapidity and extent to which dietary fatty acids were incorporated or metabolized (ie, DHGLA from GLA) by the macrophages and lung tissue through continuous enteral feeding. This feeding modality optimizes the exposure of lung and liver cell membranes to dietary PUFAs that are packaged as components of lipoproteins released either from the liver.
into the venous circulation or from the intestine into the lymphatic circulation. An alternate but less prominent route for distribution of dietary PUFAs would be as fatty acids within the portal circulation (31). As reported earlier, plasma phospholipid fatty acids are also significantly affected by the dietary lipid profile as well as by the feeding pattern (12, 32–34). Furthermore, remodeling of phospholipid PUFAs is facilitated by the normally extensive turnover of cell membranes in vivo (35). There were only a few instances in the present study wherein further changes in the relative percentage of macrophage or lung tissue phospholipid PUFA occurred after feeding for an additional 3 d. Given the similarities between humans and rats with regard to tissue esterification and incorporation of dietary PUFAs (36), provision of select dietary PUFAs by continuous enteral feeding could prove to be a practical and effective treatment modality for down-regulating inflammatory processes within the lung and liver. It is not yet known whether application of nutritional intervention in a discontinuous manner, ie, cyclic enteral feeding, would promote release of endogenous LA from adipose stores into the plasma postabsorptively (32, 37), thereby retarding modification of phospholipid PUFAs by exogenous lipids. Additional studies are necessary to address these concerns.

A second outcome of potential importance was that endogenous LA and AA within the resident lung and liver macrophage and lung tissue phospholipids were significantly displaced by highly unsaturated n-3 (ie, EPA and DHA) and n-6 (ie, DHGLA) PUFAs in rats given the EPA- and GLA-enriched diets. Rats given diets B or C for 3 d had significantly higher U1s of macrophage and lung tissue phospholipids in comparison with rats at baseline or those given diet A (Tables 3–5). The elevated U1 coupled with unchanged P-S ratios indicate that provision of diet B or C facilitated an increase in the number of double bonds within the phospholipid PUFAs without altering in the relative mole percentage of total PUFAs. Thus, these two diets promoted a qualitative but not quantitative change in macrophage and lung phospholipid PUFAs relative to the control diet. The physiologic significance of this increased unsaturation is unknown. The fatty acid composition of membrane phospholipids appears to influence membrane fluidity, ie, the mobility of proteins (eg, receptors, G proteins) and other lipids within the phospholipid bilayer. In this regard, receptor exposure, receptor affinity for their ligands, and subsequent signal transduction may be modified by the type of PUFA present within the membrane lipid bilayer (38). In general, increased activities or processes, such as macrophage adhesion and phagocytosis, are directly correlated with increased membrane fluidity and the U1 of PUFA (27, 35).

A third finding was that endotoxemia did not significantly alter the dietary modification of lung macrophage and liver sinusoidal phospholipid PUFAs (unpublished comparisons with vehicle-infused control rats). This confirmed the results of an earlier study that revealed that the extent of n-3 PUFA incorporation into liver sinusoidal cells from endotoxemic rats enterally infused for 4 d with an elemental diet enriched with fish-oil PUFAs was similar to that observed in nonendotoxemic control rats (12). These results suggest that application of enteral nutritional regimens for remodeling of phospholipid PUFAs may not be limited to nonstressed patients or preemptive situations only, but may also be used postoperatively or during septic complications (39, 40).

The results for the lung (Table 4) and KE cell (Table 5) phospholipids also suggested that diet C, which contained similar proportions of GLA and EPA (4.6% and 5.3%, respectively, Table 2), more effectively displaced LA between days 3 and 6 than did diet B, which had a relatively lower proportion of GLA to EPA (1.2% and 5.4%, respectively). Although the possibility exists that these effects were due to the slight differences in LA content between diets B and C (Table 2), the fact that DHGLA increased concomitantly in rats treated with diet C supports the concept that a specific class or classes of unidentified phospholipids may have preferentially incorporated either GLA or DHGLA at the expense of LA. The significant rise of the relative percentage of DHGLA in lung (Figure 4) and KE cells (Table 5) coupled with the inability to detect GLA in these samples suggests that these cells may possess the capacity to elongate GLA to DHGLA similar to peritoneal macrophages (41). However, analysis of plasma samples taken from the non-endotoxemic rats given diet C for 3 d revealed that the mean relative percentage of phospholipid DHGLA was 1.6%, compared with 0.7% in plasma from diet A rats (unpublished observations). Thus, the lung and its constituent cells may have derived some portion (or all) of the DHGLA from the plasma pool. It also appears that the KE cells were better able to metabolize low amounts of GLA present in diet B than was lung tissue, given the significant increase of phospholipid DHGLA observed only in the KE cells after 3 d (Table 5). This may be due, in part, to a “first pass” exposure to dietary GLA absorbed from the portal circulation by KE cells (31). However, the possibility remains that hepatocytes proximal to the KE cells supplied DHGLA by elongating GLA.

Interestingly, providing GLA enterally at 4.6% of the total lipid energy (diet C) did not foster increased formation of AA in either the AMφ or KE cells (Figure 3 and Table 5). Although DHGLA is the immediate precursor of AA, this conversion by Δ5 desaturase appears to be tightly regulated (42). Given the imposition of factors that potentially reduce its activity—ie, surgical stress, endotoxemia, and the presence of EPA (41, 42)—it is not known whether hepatic Δ5 desaturase activity was altered in the present study. Both clinical (32) and experimental (33) studies have demonstrated that provision of GLA without EPA does not promote increased concentrations of plasma AA during stress. Similar findings were reported for peritoneal macrophage phospholipids from nonstressed mice fed orally with a diet enriched with GLA (25.6% of total fatty acids) (22). However, AA concentrations in those phospholipids may already have been maximal, rendering further incorporation unnecessary. The lack of an effect of GLA on macrophage AA concentrations in the present study does not appear to be due to the cells’ incapacity to further increase phospholipid AA content; the relative percentage of AA increased above baseline values in the lung and liver macrophages (Figure 3 and Table 5, respectively) and lung tissue (Figure 5) from rats given the LA-enriched formulation (diet A). Note that n-3 PUFAs present at baseline were concomitantly displaced after infusion of diet A (Tables 3–5). Although the lung samples from rats given diet C tended to have higher concentrations of AA than those from rats given diet B (Figure 5), this difference was relatively small in comparison with AA concentrations in lungs from rats given diet A (Figure 5). The physiologic significance of this difference in relative percent-
age of lung phospholipid AA observed between rats given diet B or diet C is not evident at this time.

As mentioned above, rats infused with diet A had significantly higher relative percentages of AMΦ (Figure 3), lung (Figure 5), and KE cell (Table 5) phospholipid AA by day 3 than those in the rats at baseline (day 0). These notable increases appear to be due in part to the displacement of endogenous EPA (1–1.5%) and other n-3 PUFAs that had been incorporated before surgery from the commercial food pellets, which contained fish meal (13). These results emphasize that the membrane phospholipids of these cells rapidly respond to changes in plasma PUFA patterns during continuous feeding with novel or, in this instance, conventional lipids (12). In contrast, lung (Figure 3) and liver macrophages (Table 5) from rats given diets B and C (which had identical total lipid energy but low concentrations of LA) for 6 d maintained relative concentrations of AA similar to those observed in the rats at baseline. Displacement of phospholipid AA to concentrations significantly lower than baseline was only achieved in lung tissue (Figure 5) from rats given either diet B or C for 6 d. Within these dietary groups, the mean relative percentage of lung phospholipid AA continued to decrease between days 3 and 6 in conjunction with increased incorporation of EPA (P < 0.06) in rats given diet B (Figure 4) or increased incorporation of DHGLA (P < 0.05) in rats given diet C (Figure 4) during that interval.

The biologic response elicited after eicosanoid release is dependent on the net balance of eicosanoids derived from AA, EPA, and GLA. At this time, it is not known whether concomitant alterations in eicosanoid metabolism occur in conjunction with the rapid modification of the macrophage phospholipid PUFAs after short-term enteral feeding. Various ratios of n-6 and n-3 families of PUFAs have been used to predict potential formation of the respective dienoic rather than trienoic series of eicosanoids. In this regard, the commonly used ratio of n-6 to n-3 PUFAs was significantly lower in the lung and liver samples analyzed from rats infused with diets B or C relative to rats given diet A. Note, however, that DHGLA, the precursor of the monoenoic series, is included in the n-6 family despite the fact that under pathophysiologic conditions it may not be desaturated to AA. For this reason, a more relevant ratio (ie, EPA + DHGLA/AA) was used to combine those PUFAs, ie, EPA and DHGLA, that compete with AA for cyclooxygenase binding. Elevating values of this ratio of precursor PUFAs signify that potential formation of the trienoic and monoenoic eicosanoids relative to dienoic eicosanoids is increased. However, this ratio is only useful from the standpoint of availability of each precursor PUFA based on relative mole percentages. It cannot account for any actual differences that may exist in selective PUFA distribution among the inner and outer membrane phospholipids, or relative binding affinities, or spatial proximity of the individual PUFA to membrane cyclooxygenase. With that caveat in mind, ratios of EPA + DHGLA to AA calculated for the macrophage (Figure 3 and Table 5) and lung tissue (Figure 5) phospholipids in rats receiving the EPA + GLA diets (ie, diets B and C) were 4- to 10-fold higher than those in rats infused with the LA-enriched diet (diet A) at either day 3 or day 6.

In summary, this study reveals that the PUFA composition of resident lung and liver macrophage phospholipids is sensitive to the PUFA content of enteral diets, and that modulation of immune cell membrane PUFAs occurs rapidly during short-term continuous enteral feeding. Modulation of immune cell membrane PUFAs with select dietary fatty acids such as EPA and GLA to down-regulate the host’s inflammatory response within a clinically relevant time frame may prove beneficial to surgical patients at risk of developing pulmonary and hepatic dysfunction.

We are grateful for the technical assistance of Pei-Linn Chen and Sean Angerman.

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