

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Impact of the {omega}-3 to {omega}-6 Polyunsaturated Fatty Acid Ratio on Cytokine Release in Human Alveolar Cells.**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/115277> since 2019-04-30T09:50:33Z

*Published version:*

DOI:10.1177/0148607110372392

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Cotogni P; Muzio G; Trombetta A; Ranieri VM; Canuto RA.. Impact of the {omega}-3 to {omega}-6 Polyunsaturated Fatty Acid Ratio on Cytokine Release in Human Alveolar Cells.. JPEN. JOURNAL OF PARENTERAL AND ENTERAL NUTRITION. 35 (1) pp: 114-121.  
DOI: 10.1177/0148607110372392

The publisher's version is available at:

<http://pen.sagepub.com/cgi/doi/10.1177/0148607110372392>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/115277>

# Impact of the $\omega$ -3 to $\omega$ -6 Polyunsaturated Fatty Acid Ratio on Cytokine Release in Human Alveolar Cells

*Short title:* Fatty acids and alveolar cell cytokine release

Paolo Cotogni, MD<sup>1\*</sup>; Giuliana Muzio, PhD<sup>2</sup>; Antonella Trombetta, PhD<sup>3</sup>; V. Marco Ranieri, MD<sup>1</sup>; and Rosa Angela Canuto, MD<sup>2</sup>

<sup>1</sup>Department of Anesthesiology and Intensive Care, S. Giovanni Battista Hospital, University of Turin, Turin, Italy

<sup>2</sup>Department of Experimental Medicine and Oncology, University of Turin, Turin, Italy

<sup>3</sup>Oncology and Hematology Laboratory, Department of Medicine, S. Giovanni Battista Hospital, University of Turin, Turin, Italy

\*Present address: Clinical Nutrition, Department of Medicine, S. Giovanni Battista Hospital, University of Turin, Turin, Italy.

*Précis:* The study investigated the effects of different DHA/AA ratios on membrane composition of alveolar cells and LPS-induced balance between pro/anti-inflammatory cytokines. The supply of 1:1 and 1:2 DHA/AA ratios reversed the predominance of  $\omega$ -6 over  $\omega$ -3 in cell membranes, decrease TNF- $\alpha$ , IL-6, and IL-8 release, and increase IL-10 release.

**Keywords:** acute lung injury; acute respiratory distress syndrome; docosahexaenoic acid; inflammation; nutritional therapy; A549 cells

Address correspondence to: Paolo Cotogni, MD, Clinical Nutrition, Department of Medicine, Via Giovanni Giolitti 9, 10123, Turin, Italy; e-mail: [paolo.cotogni@unito.it](mailto:paolo.cotogni@unito.it).

**ABSTRACT.** *Background:*  $\omega$ -3 polyunsaturated fatty acids (PUFAs) and  $\omega$ -6 PUFAs have opposing influences upon inflammation. Our objective was to determine whether lipopolysaccharide (LPS)-induced cytokine release by human alveolar cells was affected by changes of the  $\omega$ -3/ $\omega$ -6 ratio in cell membranes induced by different PUFA supplies.

*Methods:* After LPS challenge, PUFAs were added to alveolar cells as docosahexaenoic acid (DHA,  $\omega$ -3) plus arachidonic acid (AA,  $\omega$ -6) in four different DHA/AA ratios (1:1, 1:2, 1:4, and 1:7) and cytokine release was measured.

*Results:* The supply of 1:1 and 1:2 DHA/AA ratios reversed the baseline predominance of  $\omega$ -6 over  $\omega$ -3 in the  $\omega$ -3/ $\omega$ -6 PUFA ratio of cell membranes. The release of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-8) was reduced by 1:1 and 1:2 DHA/AA ratios ( $p < .01$  to  $< .001$ ), but increased by 1:4 and 1:7 DHA/AA ratios ( $p < .01$  to  $< .001$ ) vs. control. The 1:1 and 1:2 ratios increased the release of anti-inflammatory IL-10 ( $p < .001$ ). The balance between pro- and anti-inflammatory cytokines showed an anti-inflammatory response with 1:1 and 1:2 ratios and a pro-inflammatory response with 1:4 and 1:7 ratios ( $p < .001$ ).

*Conclusions:* This study showed that pro-inflammatory cytokine release was dependent on the proportion of  $\omega$ -3 in  $\omega$ -3/ $\omega$ -6 ratio in alveolar cell membranes, being reduced with the supply of high proportion of DHA and increased with high proportion of AA, respectively. Our results support the biochemical basis for current recommendations to shift the PUFA supply from  $\omega$ -6 to  $\omega$ -3 in nutrition of acute lung injury patients.

Acute lung injury (ALI) is characterized by an intense inflammatory response within the alveolar spaces,<sup>1</sup> with accumulation of pro- and anti-inflammatory cytokines.<sup>2</sup> Several studies have been carried out to find strategies for reducing the severity of lung inflammatory process, e.g. by reducing the release of pro-inflammatory mediators; however, few studies have demonstrated a significant effect on mortality in patients with acute respiratory distress syndrome (ARDS).<sup>3</sup> Treatment of patients with ALI/ARDS includes nutritional support with lipids; usually, soybean oil-based lipid emulsions are used. These emulsions are rich in  $\omega$ -6 polyunsaturated fatty acids (PUFAs) (i.e. linoleic acid) and poor in  $\omega$ -3 PUFAs: thus, the ratio between  $\omega$ -3 and  $\omega$ -6 is quite low (between 1:5 and 1:7). An equally low  $\omega$ -3/ $\omega$ -6 PUFA ratio is typical of most enteral formulas. Consequently, ALI/ARDS patients are usually exposed to a relatively large amount of  $\omega$ -6 PUFAs compared with  $\omega$ -3,<sup>4</sup> although administration of high amounts of linoleic acid appears to be undesirable in ARDS patients.<sup>5</sup>

Several studies clearly demonstrated in lung cells that  $\omega$ -6 PUFAs (e.g. arachidonic acid/AA) are precursors of pro-inflammatory mediators, as well as the pivotal role of AA and its metabolites as mediators of injury.<sup>6,7</sup> Conversely, administration of  $\omega$ -3 PUFAs (e.g. eicosapentaenoic acid/EPA and docosahexaenoic acid/DHA) has been demonstrated to decrease alveolar production of pro-inflammatory mediators and to reduce organ failure in lung animal models.<sup>7-9</sup> Based on these data, several reviews suggested that  $\omega$ -3 PUFAs could modulate the pulmonary inflammatory response<sup>4,10</sup> and represent a non-ventilatory therapeutic tool for ARDS.<sup>11</sup> Three studies showed that enteral nutrition with EPA, gamma-linolenic acid, and antioxidants reduced alveolar inflammatory mediators and improved clinical outcomes in ALI/ARDS patients,<sup>12,13</sup> as well as in ventilated severe septic patients.<sup>14</sup> Parenteral nutrition (PN) with an  $\omega$ -3 enriched lipid emulsion in ARDS patients showed selective anti-inflammatory effects.<sup>15</sup> However, in contrast to  $\omega$ -3 enteral administration,<sup>16</sup> to date no randomized controlled clinical trial using  $\omega$ -3 enriched parenteral lipid emulsions has shown clear evidence of beneficial effects on clinical end-points in ALI/ARDS patients.<sup>17</sup>

Evidence is accumulating that the  $\omega$ -3/ $\omega$ -6 PUFA ratio in nutritional support may influence inflammation. Optimal  $\omega$ -3 administration is not only dose-related but is also independently affected by the  $\omega$ -3/ $\omega$ -6 ratio.<sup>18</sup> Though  $\omega$ -3/ $\omega$ -6 PUFA ratios from 1:1 to 1:4 have been proposed,<sup>4,19-25</sup> the impact of  $\omega$ -3/ $\omega$ -6 ratio on the inflammatory response is still an unresolved question. Indeed, PN with the 1:2  $\omega$ -3/ $\omega$ -6 PUFA ratio did not affect inflammation or clinical outcomes, compared to PN with a MCT/LCT emulsion in unselected critically ill medical patients.<sup>26</sup>

No previous study has investigated the effects of supply of different  $\omega$ -3/ $\omega$ -6 ratios on phospholipid composition of cell membranes and cytokine release in the presence of a pro-inflammatory stimulus in alveolar cells. The aim of our study was to determine whether changes of the  $\omega$ -3/ $\omega$ -6 PUFA ratio in cell membrane phospholipid composition induced by PUFA supply may have effects upon the release of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-8) and one anti-inflammatory cytokine (IL-10) from human alveolar cells after endotoxin challenge.

## Materials and Methods

Fatty acids (FAs) and LPS from *Escherichia coli* 055:B5 were obtained from Sigma Chemical Co. (St. Louis, MO). A human lung carcinoma cell line (A549 cells, ATCC, Rockville, MD) was used. A549 are alveolar epithelial cells with type II pneumocyte properties. The A549 cell cultures were treated as previously described.<sup>27</sup>

### *Preliminary tests*

The study was preceded by the following preliminary tests performed in triplicate to design the experimental model.

Test 1. Analysis of baseline A549 cell FA composition. The FA percentage content was determined in neutral and polar lipids as previously described.<sup>28</sup> Briefly, total lipids were isolated by the Folch<sup>29</sup> method and separated by thin-layer chromatography. FA methyl esters from phospholipids were prepared following the Metcalfe<sup>30</sup> method and separated by gas-liquid chromatography (CP 9002

Chrompack). Internal standard (methyl heptadecanoate) was added to each preparation to determine recovery.

Test 2. Effects of PUFA addition (10, 25, and 50  $\mu\text{M}$ ) without LPS stimulation on the cytokine release and the phospholipid composition of A549 cells.

Test 3. Effect of LPS challenge on A549 cells. Cells were exposed to various doses of LPS (100, 200, and 400  $\mu\text{g/ml}$ ) to determine the dose- and time-dependence of LPS on cytokine release as assessed by TNF- $\alpha$  release.

### *Experimental study*

Six different cultures of A549 cells were prepared: 1) baseline, non-stimulated cells; 2) control, cells stimulated with LPS at time 0; 3) DHA/AA1:1, cells stimulated with LPS and exposed to DHA(25  $\mu\text{M}$ )/AA(25  $\mu\text{M}$ ) ratio; 4) DHA/AA1:2, cells stimulated with LPS and exposed to DHA(17  $\mu\text{M}$ )/AA(33  $\mu\text{M}$ ) ratio; 5) DHA/AA1:4, cells stimulated with LPS and exposed to DHA(10  $\mu\text{M}$ )/AA(40  $\mu\text{M}$ ) ratio; 6) DHA/AA1:7, cells stimulated with LPS and exposed to DHA(6.5  $\mu\text{M}$ )/AA(43.5  $\mu\text{M}$ ) ratio. Three h after LPS challenge, DHA/AA ratios (50  $\mu\text{M}$ ) were added to cell cultures for 4 h. After 7 h from time 0, the release of TNF- $\alpha$ , IL-6, IL-8, and IL-10 in supernatant and the phospholipid composition of A549 cell membranes were determined (four independent experiments). All culture supernatants were harvested and stored at  $-80^{\circ}\text{C}$  for cytokine measurement via ELISA kits (Euroclone, Paignton-Devon, UK) according to the manufacturer instructions).

### *Statistical analysis*

Data were expressed as mean  $\pm$  SD. Multiple comparisons were carried out using one-way ANOVA, followed by Bonferroni post hoc test. SPSS 14 (SPSS Inc., Chicago, IL) was used for analyses. Significance was defined as  $p < .05$ .

## **Results**

### *Preliminary tests*

Test 1. The FA percentage content in phospholipids of A549 cell membranes was shown in table 1.

Test 2. Both AA and DHA addition (50  $\mu\text{M}$ ) changed respective percentage content in phospholipids and  $\omega$ -3/ $\omega$ -6 PUFA ratio of A549 cells at 7 h ( $p < .001$  vs. baseline) (Table 2). The TNF- $\alpha$  and IL-6 constitutive production was decreased by DHA (50  $\mu\text{M}$ ) ( $p < .05$  and  $< .001$ , respectively) and increased by AA (50  $\mu\text{M}$ ) ( $p < .01$  and  $< .001$ , respectively) at 7 h. Addition of 10  $\mu\text{M}$  PUFA did not induce significant modifies of the cytokine release and the phospholipid composition, whereas no difference between addition of 25 and 50  $\mu\text{M}$  PUFA was found at 7 h.

Test 3. A dose- and time-dependent effect of LPS on TNF- $\alpha$  release was observed. Concentration and exposure-time of LPS that induced the most significant TNF- $\alpha$  release were 400  $\mu\text{g}/\text{ml}$  and 7 h, respectively ( $p < .001$ ).

As previously demonstrated, A549 cell growth was decreased in a concentration- and time-dependent manner by addition of AA<sup>27</sup> and DHA,<sup>28</sup> however, at 7 h no difference on A549 cell proliferation and viability versus baseline was found after 50  $\mu\text{M}$  AA or DHA addition, as well after LPS challenge. Based on the results of these preliminary tests, we designed for the experimental study the LPS challenge of 400  $\mu\text{g}/\text{ml}$  for 7 h and the addition of DHA/AA ratios in a total final concentration of 50  $\mu\text{M}$ .

### *Experimental study*

#### *Effect of LPS and DHA/AA ratios on phospholipid composition of A549 cell membranes (Table 1)*

In control cells, we observed a remarkable decrease of AA percentage content ( $p < .001$  vs. baseline), while DHA did not change in phospholipids of cell membranes. Following addition of different DHA/AA ratios, the DHA ( $p < .001$  vs. all) and AA ( $p < .05$  to  $< .001$  vs. all, except 1:4 vs. 1:7) contents were changed. Finally, the  $\omega$ -3/ $\omega$ -6 PUFA ratio in cells exposed to 1:1 and 1:2 DHA/AA ratios was markedly changed ( $p < .001$  vs. all).

*Effect of DHA/AA ratios on LPS-induced cytokine release from A549 cells (Fig. 1 A-B-C-D)*

The release of TNF- $\alpha$ , IL-6, IL-8, and IL-10 from control was considerably increased ( $p < .001$ ) compared with baseline. The 1:1 DHA/AA ratio decreased TNF- $\alpha$ , IL-6, and IL-8 release ( $p < .001$ ) compared with control, as well as, the 1:2 DHA/AA ratio decreased TNF- $\alpha$  ( $p < .001$ ), IL-6 ( $p < .01$ ), and IL-8 ( $p < .01$ ), but less than the 1:1 ratio ( $p < .001$ ). Exposure of cell cultures to 1:4 DHA/AA and 1:7 DHA/AA ratios increased the release of TNF- $\alpha$  ( $p < .01$ ), IL-6 ( $p < .001$ ), and IL-8 ( $p < .001$ ) vs. control. Such increase in release of pro-inflammatory cytokines was also significant vs. 1:1 and 1:2 ratios ( $p < .001$ ). The release of IL-10 was increased by 1:1 and 1:2 ratios ( $p < .001$ ), while it was not affected by 1:4 and 1:7 ratios.

*Effect of DHA/AA ratios on balance between pro- and anti-inflammatory cytokines (Fig. 2 A-B-C)*

The balance between pro- and anti-inflammatory cytokines was evaluated by three cytokine ratios (TNF- $\alpha$ /IL-10, IL-6/IL-10, and IL-8/IL-10): a reduction of such ratios was considered as an anti-inflammatory response, while an increased ratio was considered a further amplification of the pro-inflammatory response. The three cytokine ratios showed the same pattern. An anti-inflammatory response was observed with 1:1 and 1:2 ratios ( $p < .001$ ), due to the net effect of a reduction of TNF- $\alpha$ , IL-6, and IL-8 plus an increase of IL-10 concentrations. On the contrary, a pro-inflammatory response was observed when 1:4 and 1:7 ratios ( $p < .001$ ).

## **Discussion**

The main finding of this study was that the supply of 1:1 and 1:2 DHA/AA ratios reversed the baseline predominance of  $\omega$ -6 over  $\omega$ -3 in the  $\omega$ -3/ $\omega$ -6 PUFA ratio of cell membranes, decreasing the release of TNF- $\alpha$ , IL-6, and IL-8 in alveolar cells. In contrast, the supply of DHA/AA ratios with an  $\omega$ -6 prevalence (i.e. 1:4 and 1:7) caused itself a further significant increase in pro-inflammatory cytokine release compared with LPS alone. Thus, the balance between the release of

pro- and anti-inflammatory cytokines showed a relevant anti-inflammatory response with 1:1 and 1:2 ratios and a pro-inflammatory response with 1:4 and 1:7 ratios.

The reason some patients with ALI develop ARDS whereas others recover remains unclear. Inflammatory cytokines are key elements in the pathogenesis of ARDS and they appear to have concentration-dependent biologic effects. Persistently alveolar elevated levels of pro-inflammatory cytokines associated with decreased production of those anti-inflammatory correlate with the severity of lung injury, and the degree of this cytokine imbalance leads to additional non-pulmonary organ dysfunction and increased mortality rates in ARDS patients.<sup>2,31,32</sup> Previous studies suggested that the lung itself can be an important cytokine-producing organ and the type II pneumocytes have a central position in the pathophysiology of the alveolar space.<sup>33</sup>

Lipids are known to have immune-modulatory properties and their administration could influence the prognosis of ALI/ARDS patients.<sup>17</sup> Indeed, after PUFA challenge many cell properties are shown to be modified, mainly the inflammatory response (e.g. eicosanoid and cytokine productions).<sup>23,25,34,35</sup> In general,  $\omega$ -3 PUFAs are regarded to be anti-inflammatory whereas  $\omega$ -6 are pro-inflammatory.<sup>34</sup> The differential impact of  $\omega$ -3 vs.  $\omega$ -6 supply on cytokine generation provoked by various stimuli was demonstrated in respiratory cells,<sup>35</sup> as well as in septic and ALI murine models.<sup>36,37</sup> In septic patients,  $\omega$ -3 PUFA parenteral administration influenced lipid mediator generation and reduced endotoxin-elicited monocyte pro-inflammatory cytokine generation, while cytokine generation was markedly amplified by  $\omega$ -6 PUFA infusion.<sup>38</sup> The alveolar cells have an intense lipid metabolism and, as demonstrated both in A549 cells and lung models, EPA/DHA are rapidly incorporated in the phospholipids of lung cell membranes, inducing rapidly (from 5 min to 4 h) changes in cell membrane FA composition<sup>9</sup> and lipid-derived inflammatory mediator generation.<sup>8,9,39</sup>

A complex network of factors regulates the relation between  $\omega$ -3 and inflammation<sup>25,34,40</sup> and the mechanisms underlining the anti-inflammatory effects of DHA are not completely clear; however, DHA seems to be more effective than EPA in alleviating LPS-induced pro-inflammatory cytokine

production in macrophages.<sup>41</sup> One of the goal of EPA/DHA supplementation is to reduce the severity of inflammatory processes by reducing the availability of AA in cell membranes.<sup>10</sup> Moreover, DHA can decrease the release of AA from membrane phospholipids by decreasing phospholipase A2 activity.<sup>42</sup> A decrease in AA leads to reduced release of pro-inflammatory mediators (prostaglandin E<sub>2</sub> and leukotriene B<sub>4</sub>).<sup>34</sup> Our findings indicate that the baseline predominance of  $\omega$ -6 over  $\omega$ -3 was reversed following the supply of 1:1 and 1:2 ratios, with the DHA exceeding the AA content nearly 4-8-fold. Thus, a remarkable change in the  $\omega$ -3/ $\omega$ -6 PUFA ratio in membranes of alveolar cells occurred, with the ratio raised from 1:5 to 6:1 and 3:1, respectively. According to numerous available data, we believe that a manipulation of PUFA supply modifies the cell membrane structure and consequently its function.<sup>9,10,20,38,43</sup> Indeed, there is a close association between the change of membrane-associated protein function linked to  $\omega$ -3 supply and the LPS-stimulated cytokine response<sup>44</sup> (e.g. DHA decreases the responsiveness of TLR-4 to LPS).<sup>42</sup> The  $\omega$ -3 fatty acid decreases the production of cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8) acting both directly, by replacing AA in cell membranes, and indirectly by decreasing activation of pro-inflammatory transcription factors (e.g. NF $\kappa$ -B) and increasing activation of anti-inflammatory transcription factors (e.g. peroxisome proliferator activated receptor  $\gamma$ ).<sup>34</sup>

An increased IL-10 (a potent anti-inflammatory cytokine) concentration was detected in supernatant of alveolar cells treated with 1:1 and 1:2 ratios. Moreover, in alveolar cells treated with the 1:1 ratio we found that the TNF- $\alpha$ /IL-10 ratio was 0.79, which is similar to that (i.e. 0.85) found in the bronchoalveolar lavage of patients who did not develop ARDS.<sup>45</sup> It was demonstrated that IL-10 suppresses LPS-induced production of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 *in vitro*, as well as reduces TNF- $\alpha$  concentrations post-LPS challenge in animals.<sup>46</sup> Thus, we hypothesized that the increased release of IL-10 could be another factor contributing to the reduction of pro-inflammatory cytokine release observed in our study.

In the alveolar spaces the balance between pro- and anti-inflammatory responses is a critical element for progression of ALI, and early activation of an anti-inflammatory response through an  $\omega$ -3 supplementation could provide a mechanism for limiting the inflammatory response. However, the  $\omega$ -3 supplementation is not completely without risk due to a potential excessive reduction in pro-inflammatory response, which could induce immunosuppression.<sup>21</sup> The  $\omega$ -3/ $\omega$ -6 ratio of 1:2.1 was considered 'neutral' in terms of 'immunosuppressive' effects.<sup>19</sup> In our study, both 1:1 and 1:2 ratios shifted the balance between pro- and anti-inflammatory cytokines towards an anti-inflammatory response; however, we believe that the 1:2  $\omega$ -3/ $\omega$ -6 ratio should be preferred because it could combine efficacy and low risk of 'immunosuppressive' effects.

The novelties of this study are the use of different PUFA ratios and the use of the alveolar cell line; the limit is that we do not investigate what transcriptional mechanisms are at work in our experimental model. In fact, the study was designed as an explorative investigation and further studies are necessary to offer more mechanistic insights.

In conclusion, this study has shown that shifting the PUFA supply from AA to DHA reduced significantly the release of pro-inflammatory cytokines in human alveolar cells undergoing inflammatory stress. Unexpectedly, we found that in the presence of a PUFA ratio with an AA predominance there was a cytokine balance more oriented to a pro-inflammatory response than with LPS alone. Our results suggest that the change of the  $\omega$ -3/ $\omega$ -6 ratio in PUFA supply could be an important factor affecting the alveolar cytokine release. Finally, these data support the biochemical basis for current recommendations<sup>47,48</sup> to shift the lipid supply from  $\omega$ -6 to  $\omega$ -3 PUFA in nutritional support of ALI/ARDS patients.

## **Acknowledgements**

We thank Dr. Fabrizio Fop (University of Turin) for the statistical analysis.

## *Funding*

This work was partially supported by the Regione Piemonte (Italy) [grant numbers 19700/27.001-2002, 1837/27.001-2003 to PC].

## References

1. Pugin J, Verghese G, Widmer MC, Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit Care Med.* 1999;27:304-312.
2. Park WY, Goodman RB, Steinberg KP, et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med.* 2001;164:1896-1903.
3. Leaver SK, Evans TW. Acute respiratory distress syndrome. *BMJ.* 2007;335:389-394.
4. Mizock BA. Nutritional support in acute lung injury and acute respiratory distress syndrome. *Nutr Clin Pract.* 2001;16:319-328.
5. Suchner U, Katz DP, Furst P, et al. Impact of sepsis, lung injury, and the role of lipid infusion on circulating prostacyclin and thromboxane A<sub>2</sub>. *Intensive Care Med.* 2002;28:122-129.
6. Grimminger F, von Kurten I, Walmrath D, Seeger W. Type II alveolar epithelial eicosanoid metabolism: predominance of cyclooxygenase pathways and transcellular lipoxygenase metabolism in co-culture with neutrophils. *Am J Respir Cell Mol Biol.* 1992;6:9-16.
7. Mancuso P, Whelan J, DeMichele SJ, et al. Effects of eicosapentaenoic and gamma-linolenic acid on lung permeability and alveolar macrophage eicosanoid synthesis in endotoxic rats. *Crit Care Med.* 1997;25:523-532.
8. Grimminger F, Wahn H, Mayer K, Kiss L, Walmrath D, Seeger W. Impact of arachidonic versus eicosapentaenoic acid on exotoxin-induced lung vascular leakage: relation to 4-series versus 5-series leukotriene generation. *Am J Respir Crit Care Med.* 1997;155:513-519.
9. Breil I, Koch T, Heller A, et al. Alteration of n-3 fatty acid composition in lung tissue after short-term infusion of fish oil emulsion attenuates inflammatory vascular reaction. *Crit Care Med.* 1996;24:1893-1902.
10. Koch T, Heller AR. Effects of intravenous fish oil on pulmonary integrity and function. *Clin Nutr.* 2002;21(Suppl 2):41-45.

11. Zadak Z, Cervinkova Z. PUFA n-3 lipid emulsion-a promising agent in ARDS treatment. *Nutrition*. 1997;13:232-233.
12. Gadek JE, DeMichele SJ, Karlstad MD, et al. Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. Enteral Nutrition in ARDS Study Group. *Crit Care Med*. 1999;27:1409-1420.
13. Singer P, Theilla M, Fisher H, Gibstein L, Grozovski E, Cohen J. Benefit of an enteral diet enriched with eicosapentaenoic acid and gamma-linolenic acid in ventilated patients with acute lung injury. *Crit Care Med*. 2006;34:1033-1038.
14. Pontes-Arruda A, Aragão AM, Albuquerque JD. Effects of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in mechanically ventilated patients with severe sepsis and septic shock. *Crit Care Med*. 2006;34:2325-2333.
15. Planas M. Effects of an  $\omega$ 3 fatty acid-enriched lipid emulsion in patients with acute respiratory distress syndrome (ARDS). *Clin Nutr Suppl*. 2007;3:7-8.
16. Pontes-Arruda A, DeMichele S, Seth A, Singer P. The use of an inflammation-modulating diet in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of outcome data. *JPEN J Parenter Enteral Nutr*. 2008;32:596-605.
17. Mayer K, Seeger W. Fish oil in critical illness. *Curr Opin Clin Nutr Metab Care*. 2008;11:121-127.
18. Heller AR, Stengel S, Stehr SN, Gama de Abreu M, Koch R, Koch T. Impact of the ratio of intravenous omega-3 vs. omega-6 polyunsaturated fatty acids in postoperative and in septic patients-A post hoc database analysis. *e-SPEN*. 2007;2:e91-e96.
19. Grimm H, Tibell A, Norrlind B, Blecher C, Wilker S, Schwemmler K. Immunoregulation by parenteral lipids: Impact of the n-3 to n-6 fatty acid ratio. *JPEN J Parenter Enteral Nutr*. 1994;18:417-421.

20. Morlion BJ, Torwesten E, Lessire H, et al. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. *Metabolism*. 1996;45:1208-1213.
21. Fürst P, Kuhn KS. Fish oil emulsions: what benefits can they bring? *Clin Nutr*. 2000;19:7-14.
22. Adolph M. Lipid emulsions in total parenteral nutrition - state of the art and future perspectives. *Clin Nutr*. 2001;20(Suppl 4):11-14.
23. Grimble R. Fatty acid profile of modern lipid emulsions: Scientific considerations for creating the ideal composition. *Clin Nutr*. 2005;1(Suppl 3):9-15.
24. Waitzberg DL, Torrinhas RS, Jacintho TM. New parenteral lipid emulsions for clinical use. *JPEN J Parenter Enteral Nutr*. 2006;30:351-367.
25. Siddiqui RA, Harvey KA, Zaloga GP, Stillwell W. Modulation of lipid rafts by Omega-3 fatty acids in inflammation and cancer: implications for use of lipids during nutrition support. *Nutr Clin Pract*. 2007;22:74-88.
26. Friesecke S, Lotze C, Köhler J, Heinrich A, Felix SB, Abel P. Fish oil supplementation in the parenteral nutrition of critically ill medical patients: a randomised controlled trial. *Intensive Care Med*. 2008;34:1411-1420.
27. Muzio G, Trombetta A, Maggiora M, et al. Arachidonic acid suppresses growth of human lung tumor A549 cells through down-regulation of ALDH3A1 expression. *Free Radic Biol Med*. 2006;40:1929-1938.
28. Trombetta A, Maggiora M, Martinasso G, Cotogni P, Canuto RA, Muzio G. Arachidonic and docosahexaenoic acids reduce the growth of A549 human lung-tumor cells increasing lipid peroxidation and PPARs. *Chem Biol Interact*. 2007;20:239-250.
29. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957;226:497-509.
30. Metcalfe LD, Pelka JR, Schmitz AA. Rapid preparation of fatty acid esters for gas-chromatographic analysis. *Anal Chem*. 1966;38:514-515.

31. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. *Chest*. 1995;107:1062-1073.
32. Ranieri VM, Suter PM, Tortorella C, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA*. 1999;282:54-61.
33. Skerrett SJ, Liggitt HD, Hajjar AM, Ernst RK, Miller SI, Wilson CB. Respiratory epithelial cells regulate lung inflammation in response to inhaled endotoxin. *Am J Physiol Lung Cell Mol Physiol*. 2004;287:L143-152.
34. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr*. 2006;83(6 Suppl):1505S-1519S.
35. Bryan DL, Forsyth KD, Hart PH, Gibson RA. Polyunsaturated fatty acids regulate cytokine and prostaglandin E2 production by respiratory cells in response to mast cell mediators. *Lipids*. 2006;41:1101-1107.
36. Sadeghi S, Wallace FA, Calder PC. Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. *Immunology*. 1999;96:404-410.
37. Schaefer MB, Ott J, Mohr A, et al. Immunomodulation by n-3- versus n-6-rich lipid emulsions in murine acute lung injury—Role of platelet-activating factor receptor. *Crit Care Med*. 2007;35:544-554.
38. Mayer K, Gokorsch S, Fegbeutel C, et al. Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. *Am J Respir Crit Care Med*. 2003;167:1321-1328.
39. Yang P, Chan D, Felix E, et al. Formation and antiproliferative effect of prostaglandin E(3) from eicosapentaenoic acid in human lung cancer cells. *J Lipid Res*. 2004;45:1030-1039.
40. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br J Pharmacol*. 2008;153(Suppl 1):S200-215.

41. Weldon SM, Mullen AC, Loscher CE, Hurley LA, Roche HM. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem.* 2007;18:250-258.
42. Lee JY, Zhao L, Youn HS, et al. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. *J Biol Chem.* 2004;279:16971-16979.
43. Senkal M, Geier B, Hannemann M, et al. Supplementation of n-3 fatty acids in parenteral nutrition beneficially alters phospholipid fatty acid pattern. *JPEN J Parenter Enteral Nutr.* 2007;31:12-17.
44. Singer P, Shapiro H, Theilla M, Anbar R, Singer J, Cohen J. Anti inflammatory properties of omega-3 fatty acids in critical illness: novel mechanisms and an integrative perspective. *Intensive Care Med.* 2008;34:1580-1589.
45. Armstrong L, Millar AB. Relative production of tumour necrosis factor alpha and interleukin 10 in adult respiratory distress syndrome. *Thorax.* 1997;52:442-446.
46. Cassatella MA, Meda L, Bonora S, Ceska M, Constantin G. Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide. *J Exp Med.* 1993;178:2207-2211.
47. McClave SA, Martindale RG, Vanek VW, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr.* 2009;33; 277-316.
48. Singer P, Berger MM, Van den Berghe G, et al. ESPEN Guidelines on Parenteral Nutrition: intensive care. *Clin Nutr.* 2009;28:387-400.

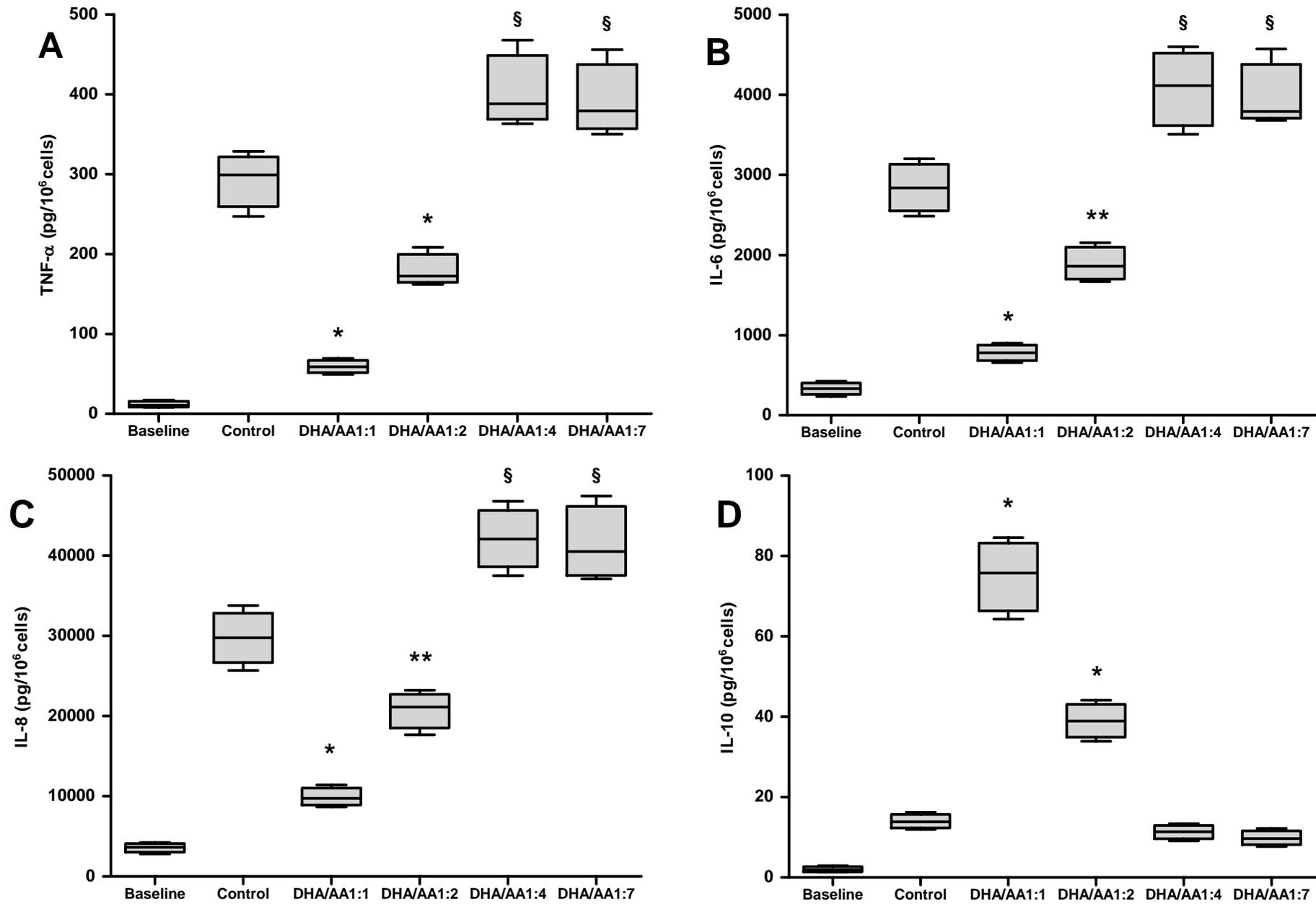
## Figure legends

### Figure 1

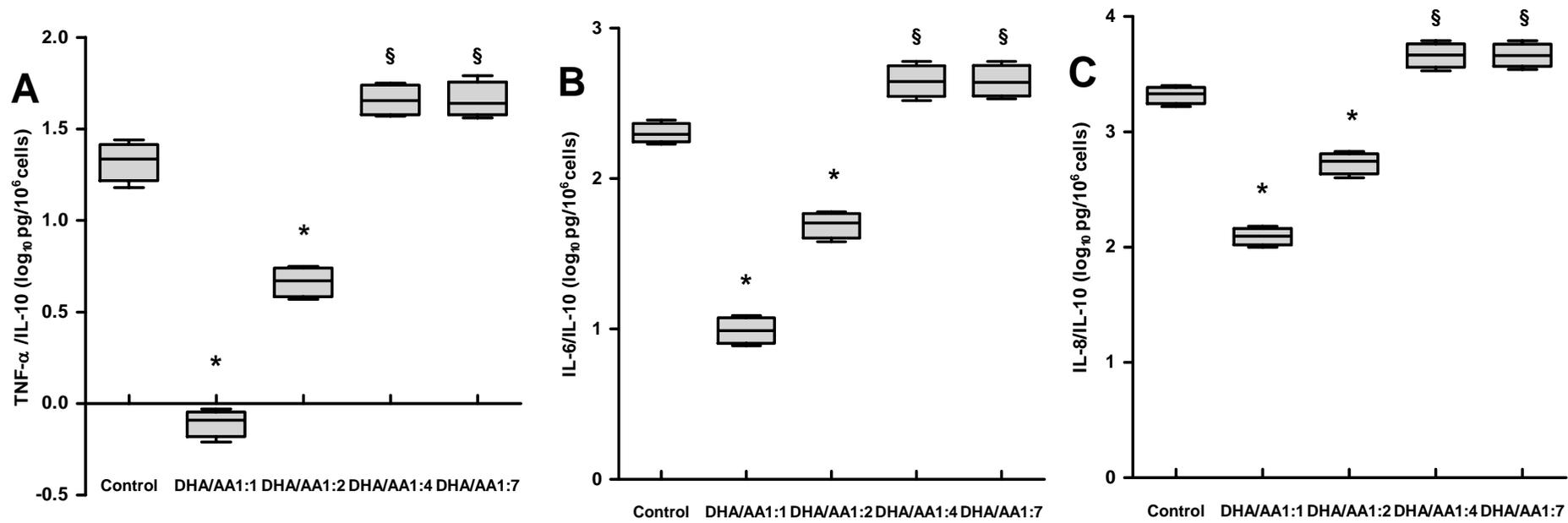
*Effect of  $\omega$ -3/ $\omega$ -6 PUFA ratios on LPS-induced cytokine release from A549 cells.* The results are expressed as picograms of released cytokines *per*  $10^6$  adherent cells (pg/ $10^6$  cells). Data are presented as median with interquartile ranges (n = 4 experiments). (A) TNF- $\alpha$ : \* $p$  < .001 vs. all; § $p$  < .01 vs. control. (B) IL-6: \* $p$  < .001 vs. all; \*\* $p$  < .01 vs. control; § $p$  < .001 vs. control and 1:2. (C) IL-8: \* $p$  < .001 vs. all; \*\* $p$  < .01 vs. control; § $p$  < .001 vs. control and 1:2. (D) IL-10: \* $p$  < .001 vs. all.

### Figure 2

*Effect of  $\omega$ -3/ $\omega$ -6 PUFA ratios on balance between pro- and anti-inflammatory cytokines.* The balance was evaluated by three cytokine ratios (TNF- $\alpha$ /IL-10, IL-6/IL-10, and IL-8/IL-10). The results are expressed as  $\log_{10}$  picograms of released cytokines *per*  $10^6$  adherent cells ( $\log_{10}$  pg/ $10^6$  cells). Data are presented as median with interquartile ranges (n = 4 experiments). \* $p$  < .001 vs. all; § $p$  < .001 vs. control.



**Figure 1**



**Figure 2**

Table 1. Percentage content of fatty acids in phospholipids of A549 cell membranes at 7 h

<b>Fatty acid</b>	<b>Baseline</b>	<b>Control</b>	<b>DHA/AA 1:1</b>	<b>DHA/AA 1:2</b>	<b>DHA/AA 1:4</b>	<b>DHA/AA 1:7</b>
<b>C14:0</b>	3.7	7.2	7	6.9	7.4	7.5
<b>C16:0</b>	31.9	56.5	51.2	52	58.2	59.7
<b>C16:1</b>	9.6	1.8	1.6	1.9	2	2.2
<b>C18:0</b>	16.8	26.6	17.2	18.1	15.2	14.2
<b>C18:1</b>	27.3	4.3	3.5	3.9	4.8	5
<b>C18:2</b>	2.8	0.5	0.6	0.8	1.6	1.8
<b>C20:4 (AA)</b>	6.2 <sup>§</sup>	0.9 <sup>§</sup>	2.2 <sup>§</sup>	3.5 <sup>§</sup>	4.5 <sup>§</sup>	4.9 <sup>§</sup>
<b>C22:6 (DHA)</b>	1.7	2.2	16.7*	12.9*	6.3*	4.7*
<b><math>\omega</math>-3/<math>\omega</math>-6 PUFA ratio</b>	1:5	1.6:1	6:1*	3:1*	1:1	1:1.4

PUFA, Polyunsaturated Fatty Acid; AA, arachidonic acid; DHA, docosahexaenoic acid.

Data are expressed as percentage of fatty acids and are means of 4 experiments. SD (not shown) was below 10% in all cases. Percentage content of fatty acids less than 0.5% were

not reported. <sup>§</sup> $p < .05$  to  $< .001$  vs. all, except 1:4 vs. 1:7. \* $p < .001$  vs. all.

Table 2. Percentage content of fatty acids in phospholipids of A549 cell membranes at 7 h

<b>Fatty acid</b>	<b>Baseline</b>	<b>AA (50 <math>\mu</math>M)</b>	<b>DHA (50 <math>\mu</math>M)</b>
<b>C20:4 (AA)</b>	6.1	13.5*	3.5 <sup>§</sup>
<b>C22:6 (DHA)</b>	1.8	1.3	9.6*
<b><math>\omega</math>-3/<math>\omega</math>-6 PUFA ratio</b>	1:5	1:12*	2:1*

PUFA, Polyunsaturated Fatty Acid; AA, arachidonic acid; DHA, docosahexaenoic acid.

Data are expressed as percentage of fatty acids and are means of 3 experiments. SD (not shown) was below 10% in all cases. <sup>§</sup> $p < .05$  vs. baseline, \* $p < .001$  vs. baseline.