

# Effects of dietary fish oil supplementation on cellular adhesion molecule expression and tissue myeloperoxidase activity in hypercholesterolemic mice with sepsis

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## Abstract

This study investigated the effects of fish oil on adhesion molecule expression and tissue myeloperoxidase (MPO) activity in hypercholesterolemic mice with sepsis. There were one control and two experimental groups in this study. The control group (C) was fed a regular chow diet for 7 weeks, while hypercholesterolemia in the experimental group was induced by feeding a high-fat diet (20%, w/w) with cholesterol (2%, w/w) for 4 weeks. Then the experimental group was divided into two subgroups with identical nutrient distributions except that one subgroup was fed soybean oil (SO), while part of the soybean oil was replaced by fish oil (FO) in the other one for 3 weeks. After feeding the diets for 7 weeks, sepsis was induced in all three groups by cecal ligation and puncture (CLP), and mice were sacrificed at 0, 6 or 24 h after CLP, respectively. The results showed that the FO group had a higher intracellular interferon- $\gamma$ /interleukin-4 ratio and lower tumor necrosis factor- $\alpha$  and monocyte chemoattractant protein-1 concentrations in peritoneal lavage fluid at 6 h after CLP than those in the C and SO groups. Lymphocyte CD11a/CD18 expressions were higher at 0 and 6 h and neutrophil CD11b/CD18 were higher at 6 h in the SO group than in the FO and C groups. The SO group had higher plasma intercellular adhesion molecule (ICAM)-1 levels than C group at 0 and 6 h, whereas no difference in ICAM-1 concentrations were observed between the C and FO groups at 0 h after CLP. Hypercholesterolemia resulted in higher tissue MPO activities. There were no differences in MPO activities in various organs between the two experimental groups. These results suggest that hypercholesterolemic mice fed FO did not exhibit immunosuppression when complicated with sepsis. FO administration reduced adhesion molecule expressions and inflammatory-related mediators at the site of injury at an early but not a late stage of sepsis. However, compared with the SO group, the influences of FO on MPO activities in various organs were not obvious in hypercholesterolemic mice with sepsis.

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**Keywords:** Fish oil; Hypercholesterolemia; Sepsis; Adhesion molecule; Myeloperoxidase

## 1. Introduction

Cardiovascular diseases (CVDs) constitute the most common causes of death in Western societies and in Taiwan. Hypercholesterolemia resulting from long-term consumption of a high-fat, high-cholesterol diet increases the incidence of

CVDs. CVDs evoke a specific form of a chronic inflammatory process that may result in endothelial dysfunction [1]. Endothelial dysfunction accompanied by up-regulated inflammatory mediators is a major contributing factor to the pathogenesis of vascular diseases [2]. Hypercholesterolemia usually occurs prior to the onset of atherosclerosis and renders tissue more susceptible to inflammation [3].

Sepsis is a common clinical problem with extremely high mortality rates. Several components of the immune system are implicated in the process of sepsis, including the release

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of proinflammatory mediators and activation of endothelial cells (ECs) and polymorphonuclear leukocytes (PMNs) [4,5]. On activated endothelium, members of immunoglobulin family of adhesion molecule — intercellular adhesion molecule (ICAM) and vascular cell adhesion molecules (VCAMs) — are expressed. CD11a/CD18 and CD11b/CD18 are members of the leukocyte adhesion molecule  $\beta 2$  integrin. Cellular adhesion molecules (CAMs) and integrins play central roles in mediating the firm adhesion of leukocytes to ECs [6]. Overexpressions of these adhesion molecules facilitate leukocyte-endothelial interactions, which result in endothelial dysfunction and tissue damage [7,8]. The macrophage-derived chemokine, monocyte chemoattractant protein-1 (MCP-1), plays a significant role in the recruitment of leukocytes during endotoxemia and atherosclerosis [9,10]. Elevated levels of MCP-1 have been detected in the plasma of patients with sepsis [11].

Fish oil is rich in the n-3 fatty acids (FAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A number of clinical trials have shown that fish oil has immune modulatory effects [12,13]. The major advantages of n-3 FAs are related to their postulated reductions in proinflammatory effects. The anti-inflammatory effect of fish oil has been shown by its ability to suppress proinflammatory cytokines and modulate the gene expression of adhesion molecules [14]. A study by Zhang et al. [15] showed that the anti-inflammatory effect of fish oil was due to the suppression of Th1 cytokine production. Several studies have shown that dietary fish oil has beneficial clinical effects on Th1-mediated diseases including rheumatic arthritis, multiple sclerosis, insulin-dependent diabetic mellitus, etc. [16]. However, some studies revealed that fish oil supplementation suppresses lymphocyte proliferation and interleukin (IL)-2 production and has immunosuppressive properties [17,18]. In accordance with such observations, laboratory animals fed fish oil showed lower survival to challenges with bacteria than those fed other types of fat [19,20]. Conflicting results were also observed in septic conditions when n-3 FAs were administered [20,21]. We designed this study to investigate the effect of fish oil on the inflammatory response in hypercholesterolemic mice complicated with sepsis because animal models sometimes fail to mimic human sepsis. Most animal studies are performed acutely in young healthy animals, whereas a significant percentage of the clinical population is elderly with many secondary complications such as diabetes, systemic vascular disease, hypertension, etc. [22]. Previous studies concerned with the influence of fish oil focused exclusively on the condition of hyperlipidemia or sepsis. Studies investigating the effects of dietary fish oil on comorbidities are rare. Therefore, we induced polymicrobial sepsis after treating hypercholesterolemic mice with fish oil to investigate the effect of n-3 FAs on adhesion molecules and inflammatory cytokines. Because oxyradicals released from leukocytes that accumulate in organs may damage organ cells and induce organ dysfunction [23], we analyzed the myeloperoxidase (MPO) activities in organs as an indicator for identifying the extent of tissue injury resulting from hypercholesterolemia with sepsis.

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## 2. Materials and methods

### 2.1. Animals

Male ICR mice weighing 25–30 g were purchased from the Animal Center of National Taiwan University, College of Medicine. Mice were maintained in a temperature- $(23\pm 2^{\circ}\text{C})$  and humidity-controlled  $(55\%\pm 15\%)$  room with a 12-h light/dark cycle. All mice were allowed free access to a standard chow diet and water for 1 week before the study. All experimental procedures of the laboratory animals were approved by the Animal Care Committee of Taipei Medical University.

### 2.2. Experimental design and procedures

There were one control and two experimental groups in this study. The control group was fed a standard chow diet (C,  $n=30$ ) for 7 weeks. The experimental groups were fed a high-fat (20%, w/w) with cholesterol (2%, w/w) semipurified diet for 4 weeks to induce hypercholesterolemia. Then, mice in the experimental groups were divided into an fish oil group (FO,  $n=30$ ) and soybean oil group (SO,  $n=30$ ). The SO group was exclusively fed soybean oil (Taiwan Sugar, Taipei, Taiwan), while the FO group had 23% fish oil (Denofa, Fredrikstad, Norway) and 77% soybean oil, for 3 weeks (Table 1). The fish oil contained 34% EPA, 27% DHA and 72% total n-3 FAs, while the mixed tocopherols were 2.4 mg/g. The n-3/n-6 ratio in the FO group was 1:2 in this study. The soybean oil contained 6.5% n-3 FAs and 55% n-6 FAs according to the

Table 1  
Composition of the experimental diets (g/kg)

Component	Soybean oil (SO)	Fish oil (FO)
Soybean oil	200	154
Fish oil	0	46
Casein	200	200
Maize starch	498	498
Cholesterol	20	20
Salt mixture <sup>a</sup>	35	35
Vitamin mixture <sup>b</sup>	10	10
Methyl cellulose	31	31
Choline bitartrate	3	3
Methionine	3	3

<sup>a</sup> The salt mixture contained the following (mg/g): calcium phosphate diatomic, 500; sodium chloride, 74; potassium sulfate, 52; potassium citrate monohydrate, 20; magnesium oxide, 24; manganese carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01 and chromium potassium sulfate, 0.55.

<sup>b</sup> The vitamin mixture contained the following (mg/g): thiamin hydrochloride, 0.6; riboflavin, 0.6; pyridoxine hydrochloride, 0.7; nicotinic acid, 3; calcium pantothenate, 1.6; D-biotin, 0.05; cyanocobalamin, 0.001; retinyl palmitate, 1.6; DL- $\alpha$ -tocopherol acetate, 20; cholecalciferol, 0.25 and menaquinone, 0.005.



manufacturer. The n-3 FAs content in the FO and SO diets were 8.6% and 2.6% of total energy. After feeding the respective diets for 7 weeks, sepsis was induced in all three groups of mice by cecal and ligation and puncture (CLP) according to the method of Ayala et al. [24]. Briefly, mice were lightly anesthetized with ether, and their abdomens were opened through a midline incision. The cecum was punctured twice with a 22-gauge needle, and fecal residue was allowed to exclude. After CLP, the cecum was replaced into the abdominal cavity, and the abdominal wound was closed in layers. Mice in each group were sacrificed at 0, 6 and 24 h after CLP, respectively. Blood was collected by heart puncture and transferred into tubes containing EDTA- $\text{Na}_2$  for the CD11a/CD18, CD11b/CD18, interferon (IFN)- $\gamma$ , IL-4, cholesterol and ICAM-1 analyses. The abdomen was opened, and the peritoneal cavity was lavaged with 2 ml phosphate-buffered saline (PBS). The peritoneal lavage fluid (PLF) was collected for the tumor necrosis factor (TNF)- $\alpha$ , monocyte chemoattractant protein (MCP)-1 and prostaglandin (PG)  $\text{E}_2$  analyses. Tissues including the liver, heart, kidneys and lungs were rapidly harvested and stored at  $-80^\circ\text{C}$  for the measurement of MPO activities.

### 2.3. Measurements of plasma triglyceride, cholesterol and ICAM-1 concentrations

Plasma triglyceride (TG), cholesterol levels were determined by a colorimetric method after an enzymatic reaction with peroxidase (Randox, Antrim, Ireland). Concentrations of ICAM-1 were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). Antibodies specific for mouse ICAM-1 were coated onto the wells of the microtiter strips provided. Procedures followed the manufacturer's instructions. The detection limit for ICAM-1 was 0.03 ng/ml.

### 2.4. Measurement of TNF- $\alpha$ , MCP-1 and PG $\text{E}_2$ levels in PLF

Concentrations of TNF- $\alpha$  and MCP-1 were measured using commercially available ELISA kits. Antibodies specific for mouse TNF- $\alpha$  and MCP-1 were coated onto the wells of the microtiter strips provided (R&D Systems). PG  $\text{E}_2$  concentrations were also measured by ELISA. The surfaces of the microtiter plates were precoated with a mouse monoclonal antibody. Acetylcholinesterase covalently coupled to PG  $\text{E}_2$  was used as the enzymatic tracer (R&D Systems). The detection limits for TNF- $\alpha$ , MCP-1 and PG  $\text{E}_2$  were 5, 2 and 10 pg/ml, respectively.

### 2.5. Analysis of CD11a/CD18 distributions in lymphocytes and PMN expressions of CD11b/CD18

One hundred microliters of fresh blood was incubated with 10  $\mu\text{l}$  fluorescein isothiocyanate (FITC)-conjugated rat monoclonal anti-mouse CD11a and phycoerythrin (PE)-conjugated rat monoclonal anti-mouse CD18 (Serotec, Oxford, UK) for 15 min at  $4^\circ\text{C}$ . FITC-conjugated rat

immunoglobulin G (IgG) 2a (IgG2a) and PE-conjugated rat IgG2a were used for the isotope control (Serotec). Subsequently, erythrocytes were lysed with lysis buffer. The distribution of CD11a/CD18 expressed on lymphocytes was analyzed by flow cytometry (Beckman Coulter, Miami, FL, USA). The data are presented as a percentage of CD11a-presenting cells in  $1 \times 10^5$  leukocytes. To measure CD11b/CD18 expression on PMN, FITC-conjugated rat monoclonal anti-mouse CD11b and PE-conjugated rat monoclonal anti-mouse CD18 (Serotec) were added to 100  $\mu\text{l}$  of fresh blood. The isotope control was the same as that mentioned above. Fluorescence data were collected on  $1 \times 10^5$  leukocytes, which were also analyzed by flow cytometry. The results are presented as a percentage of CD11b/CD18 expression in  $1 \times 10^5$  leukocytes. Lymphocytes and PMNs were gated on the basis of the forward- and side-scatter profiles and were analyzed for the expressions of CD11a/CD18 and CD11b/CD18, respectively.

### 2.6. Analysis of intracellular IFN- $\gamma$ and IL-4 percentage

Percentages of intralymphocyte IFN- $\gamma$  and IL-4 expressions in fresh blood were analyzed by flow cytometry. Fifty microliters of fresh blood was immediately incubated with 100  $\mu\text{l}$  Leucoperm (Serotec) reagent A for 15 min at room temperature to fix the leukocytes, then 5 ml PBS was added and centrifuged for 5 min at 300g. After discarding the supernatants, 100  $\mu\text{l}$  Leucoperm reagent B was added to the cell pellets to penetrate the leukocytes. After that, 10  $\mu\text{l}$  FITC-conjugated rat monoclonal anti-mouse IFN- $\gamma$  (XGM1.2, Serotec) and 5  $\mu\text{l}$  PE-conjugated IL-4 (BVD 6-24G2, Serotec) were incubated for 30 min at room temperature. FITC-conjugated rat IgG1 and PE-conjugated rat IgG1 were used for the isotope control (Serotec). Penetrated and incubated leukocytes were washed with PBS. After removing the supernatant and resuspending cells in the sheath fluid, lymphocytes were gated on the basis of their forward- and side-scatter profiles. The results are presented as a percentage of cytokine-producing cells in  $1 \times 10^5$  lymphocytes.

### 2.7. Measurement of MPO activity in organs

The method of measuring MPO activity was modified from that previously described [25]. Tissue samples were homogenized with 50 mM potassium phosphate buffer (pH 6.0), and centrifuged at 2000g at  $4^\circ\text{C}$  for 15 min. After discarding the supernatant, the pellets were suspended in a solution containing 0.5% hexadecyl-trimethyl-ammonium bromide dissolved in potassium phosphate buffer (pH 7.0) and centrifuged for 30 min at 15,000g at  $4^\circ\text{C}$ . An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM  $\text{H}_2\text{O}_2$ . The rate of change in the absorbance was measured by a spectrophotometer at O.D. (optical density) 650 nm. The MPO activity was defined as the quantity of enzyme degrading



1  $\mu\text{mol}$  of peroxide per min at 37°C and the data are expressed in units per gram weight of wet tissue.

### 2.8. Statistical analysis

All data are expressed as the mean $\pm$ S.D. Differences among groups were analyzed by two-way ANOVA using Duncan's multiple-range test as the post hoc analysis. A  $P$  value of  $<.05$  was regarded as statistically significant.

## 3. Results

### 3.1. Body weights and plasma lipid levels

There were no differences in the initial body weights. After feeding the various diets for 7 weeks, the body weights of the mice in the experimental groups were significantly higher than those in the control group ( $47.4\pm 4.3$  vs.  $41.3\pm 2.8$  g,  $P<.05$ ). Plasma cholesterol levels were higher in the hypercholesterolemic groups than in the control group ( $173.8\pm 11.8$  vs.  $103.3\pm 7.8$  mg/dl,  $P<.05$ ), whereas TG levels were lower in the hypercholesterolemic groups than in the control group ( $25.7\pm 3.8$  vs.  $38.3\pm 7.1$  mg/dl,  $P<.05$ ). There were no differences in plasma cholesterol concentrations or body weights between the FO and SO groups. Also, no differences were observed before and after sepsis in both experimental groups (data not shown).

### 3.2. PG E<sub>2</sub>, TNF- $\alpha$ and MCP-1 concentrations in PLF

PG E<sub>2</sub> concentrations were significantly higher in the experimental groups than the control group at 6 and 24 h after CLP. No significant differences were observed between the FO and SO groups at various time points (Fig. 1). Concentrations of TNF- $\alpha$  (Fig. 2) and MCP-1 (Fig. 3) were significantly lower in the FO group than the control and SO groups at 6 h after CLP.

### 3.3. Intralymphocyte IFN- $\gamma$ /IL-4 ratio

The intralymphocyte IFN- $\gamma$ /IL-4 ratio in the FO group was significantly higher than those in the control and SO groups 6 h after CLP (Fig. 4).

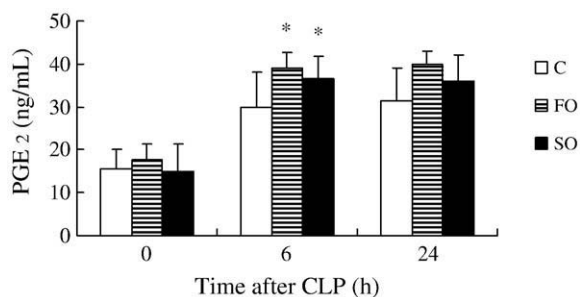


Fig. 1. Concentrations of PG E<sub>2</sub> in peritoneal lavage fluid at different time points after CLP in the C, FO and SO groups. Values are expressed as the mean $\pm$ SD. \*Significantly different from the C group at 6 h ( $P<.05$ ).

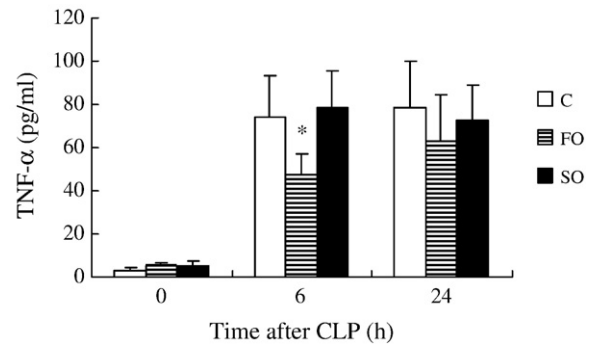


Fig. 2. TNF- $\alpha$  concentrations in peritoneal lavage fluid at different time points after CLP among the C, FO and SO groups. \*Significantly different from the C and SO groups at the same time point ( $P<.05$ ).

### 3.4. Expressions of CD11a/CD18 on lymphocytes and CD11b/CD18 on PMNs

Lymphocyte CD11a/CD18 expressions were higher at 0 and 6 h in the SO group than in the FO and control group. The percentages of CD11a/CD18 were higher at 24 h in the FO and SO groups than in the control group (Fig. 5A). Neutrophil CD11b/CD18 expressions were higher in the SO group than in the FO and control group at 6 h. CD11b/CD18 expressions were significantly higher in the FO group than in the control group, whereas there were no differences between the FO and SO groups at 24 h after CLP (Fig. 5B).

### 3.5. Plasma ICAM-1 levels

The SO group had higher ICAM-1 levels than the control and FO groups at 0 h, and the concentrations of ICAM-1 in the FO and SO groups were higher than that of the control group 6 h after CLP. No significant differences in ICAM-1 concentrations were observed between the 2 experimental groups at 6 and 24 h after CLP (Fig. 6).

### 3.6. MPO activities in the liver, lungs, kidneys and intestines

Tissue MPO activities increased with the progression of sepsis. Both the FO and SO groups had higher MPO

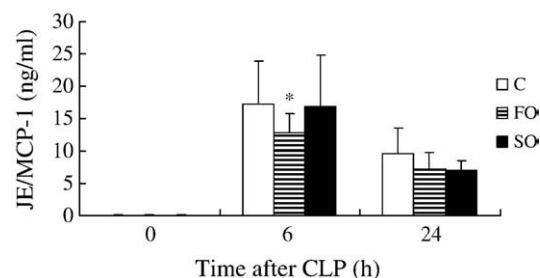


Fig. 3. Macrophage chemoattractant protein-1 (MCP-1) concentrations in peritoneal lavage fluid at different time points after cecal ligation and puncture (CLP) among the control (C), fish oil (FO) and soybean oil (SO) groups. MCP-1 concentrations were not detectable at 0 h. \*Significantly different from the C and SO groups at the same time point ( $P<.05$ ).



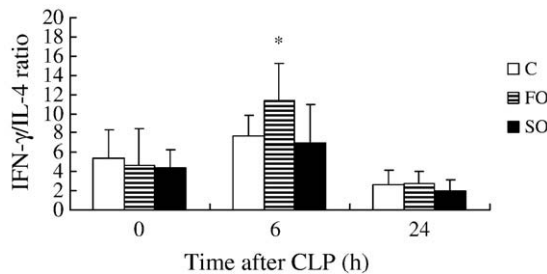


Fig. 4. The ratio of intra-lymphocyte interferon (IFN)- $\gamma$  to interleukin (IL)-4 at different time points after cecal ligation and puncture (CLP) in the control (C), fish oil (FO) and soybean oil (SO) groups. \*Significantly different from the C and SO groups at 6 h ( $P < .05$ ).

activities than those in the control group in the liver at 0 and 6 h and in the kidney at 6 h after CLP. The FO group had higher MPO activities in the kidneys, whereas the SO group had higher activities in the lungs than in the control group at 24 h after CLP. There were no differences in MPO activities between the SO and FO groups in various organs at any time point (Table 2).

#### 4. Discussion

In this study, the n-3/n-6 ratio was adjusted to 1: 2 in the FO group. This ratio is considered to exert the most favorable modulation of lipid mediator synthesis [26]. We used CLP as a sepsis model because CLP is clinically

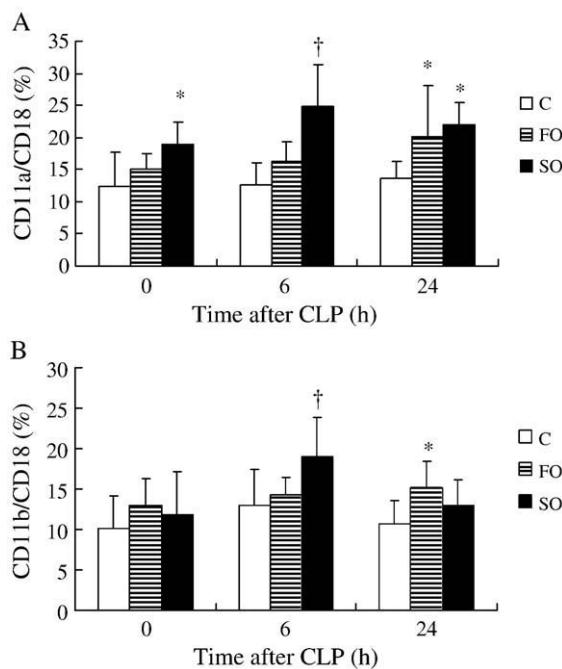


Fig. 5. Expressions of lymphocyte CD11a/CD18 (A) and neutrophil CD11b/CD18 (B) during sepsis in the control (C), fish oil (FO) and soybean oil (SO) groups. Values are expressed as the mean  $\pm$  SD depicted by vertical bar. \*Significantly different from the C group at the same time point ( $P < .05$ ). †Significantly different from the C and FO groups at 6 h after CLP ( $P < .05$ ).

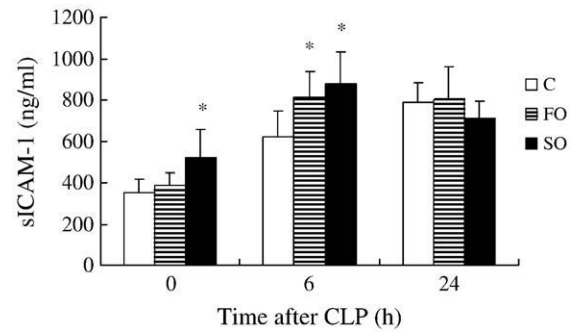


Fig. 6. Concentrations of plasma intercellular adhesion molecule-1 (ICAM-1) during sepsis in the control (C), fish oil (FO) and soybean oil (SO) groups. \*Significantly different from the C group at the same time point ( $P < .05$ ).

relevant and is considered a simple and reproducible model of gut-derived sepsis in rodents [27]. We observed that the experimental groups had lower plasma TG levels than the control group after feeding high fat diet for 7 weeks. This result was similar with the study by Petit et al. [28], they reported that chronic high fat diet influenced the absorption of fat and thus reduced blood TG levels. Besides, control group fed with chow diet had higher carbohydrate content than those in the test diets that may enhance endogenous TG synthesis.

PG  $E_2$  is a product of arachidonic acid metabolism. Numerous studies have shown that an increased dietary intake of n-3 FAs suppresses PG  $E_2$  synthesis [13,29]. However, a study reported that high fat intake reduced PG  $E_2$  production, and no further reduction was observed when n-3 polyunsaturated fatty acids intake increased [30]. This result was in agreement with our finding that compared with the SO group, the PG  $E_2$  level was not lowered in the FO group when 20% (w/w) fat was provided as in this study. TNF- $\alpha$  is an important mediator involved in the onset and regulation of

Table 2  
MPO activities in organ homogenates

	Liver	Kidneys	Intestines	Lungs
	(U/g tissue)			
0 h				
C	0.45 $\pm$ 0.24	4.57 $\pm$ 1.28	0.16 $\pm$ 0.09	4.47 $\pm$ 2.31
FO	0.98 $\pm$ 0.28 *	5.77 $\pm$ 2.43	0.19 $\pm$ 0.11	4.20 $\pm$ 0.75
SO	0.94 $\pm$ 0.65 *	7.66 $\pm$ 2.71 *	0.24 $\pm$ 0.11	5.06 $\pm$ 2.41
6 h				
C	0.59 $\pm$ 0.37	4.34 $\pm$ 1.64	0.20 $\pm$ 0.09	5.52 $\pm$ 1.82
FO	0.86 $\pm$ 0.32 *	6.58 $\pm$ 2.48 *	0.22 $\pm$ 0.11	4.51 $\pm$ 1.61
SO	0.98 $\pm$ 0.50 *	8.19 $\pm$ 2.18 *	0.23 $\pm$ 0.07	4.64 $\pm$ 1.44
24 h				
C	1.51 $\pm$ 0.71 †	6.48 $\pm$ 1.55	0.36 $\pm$ 0.14 †	2.92 $\pm$ 1.67
FO	1.44 $\pm$ 0.57 †	8.83 $\pm$ 1.89 *, †	0.29 $\pm$ 0.17	4.43 $\pm$ 1.44
SO	1.19 $\pm$ 0.25	7.53 $\pm$ 1.39	0.30 $\pm$ 0.09	5.79 $\pm$ 2.02 *

\* Mean values significantly differed from those of the C group at the same time point:  $P < .05$ .

† Mean values significantly differed from those of the other time points in the same groups:  $P < .05$ .



inflammatory and immune responses. Circulating TNF- $\alpha$  is associated with a significant pathologic change, possibly leading to mortality [31], suggesting that TNF- $\alpha$  synthesis must be controlled. In this study, we found that TNF- $\alpha$  concentrations were lower in the FO group than the SO group at the early stage of sepsis. This finding is consistent with MCP-1 levels in PLF in the FO group. MCP-1 increases with the inflammatory response and is involved in the recruitment of peripheral blood leukocytes to the peritoneal cavity [9,11]. An in vitro study showed that both EPA and DHA effectively decreased lipopolysaccharide-induced MCP-1 expression, and PPAR- $\gamma$  activated by EPA and DHA may be one of the underlying mechanisms responsible for the beneficial effects of fish oil [32]. The lowered TNF- $\alpha$  and MCP-1 levels observed in this study suggest that FO supplementation reduced the inflammatory response in the high-fat, high-cholesterol-induced hypercholesterolemic mice with sepsis.

Cytokines are produced by various cells of the immune system and act as mediators of the immune and inflammatory responses. The cytokine profiles are related to the severity of different types of infection and are determined by two functional subsets of T lymphocytes, Th1 and Th2. Th1 cytokines, including IL-2 and IFN- $\gamma$ , enhance cell-mediated immunity, whereas Th2 cytokines, including IL-4 and IL-10, enhance humoral immunity. Studies have shown that a marked depression in cellular immunity occurs after the onset of sepsis, and the Th2 cytokine, IL-4, is responsible for the immunosuppression and death associated with polymicrobial sepsis [24,33]. We directly measured intralymphocyte IFN- $\gamma$  and IL-4 production to investigate the effect of fish oil on Th1- and Th2-type responses in hypercholesterolemic mice with sepsis. The results showed that the ratio of IFN- $\gamma$ /IL-4 increased after fish oil administration, indicating that the Th1 response was not suppressed. Previous study demonstrated that MCP-1 promotes Th2 development and increases the production of IL-4 [34]. In this study, we found that FO feeding resulted in lower MCP-1 levels that may reduce IL-4 production and consequently led to a lower IFN- $\gamma$ /IL-4 ratio.

CD11a/CD18 is exclusively expressed on leukocytes and CD11b/CD18 is abundant in PMNs [6]. Excessive expression of these integrins may induce an inflammatory response and tissue injury [7]. ICAM-1 is a cell-surface protein expressed on the vascular endothelium which plays an important role in transendothelial migration of leukocytes through binding to  $\beta$ 2 integrin [35]. Plasma ICAM-1 levels are considered to be a biochemical marker for the risk of cardiovascular disease in healthy individuals [36]. A study by Miles et al. [37] showed that dietary fish oil reduced ICAM-1 expression on murine peritoneal macrophages and may ameliorate macrophage-induced plaque formation. Also, an in vitro study showed that ECs treated with n-3 FAs inhibited cytokine-induced expression of adhesion molecules [38]. In this study, we found that the CD11a, CD11b and ICAM-1 were activated in hypercholesterolemic

mice with sepsis. Compared with mice fed SO, FO administration resulted in lower integrin and ICAM-1 expressions in the early stage of sepsis. These findings indicate that CAM-mediated cell interactions may be attenuated when FO is administered at an early stage of sepsis in hypercholesterolemic mice.

MPO predominantly originates from activated neutrophils and monocytes. MPO plays an important role in leukocyte-mediated vascular injury and exerts potent proatherogenic effects [23,39]. This enzyme has been identified in human vascular plaques and elevated MPO levels can be used to predict the risk of coronary artery disease in healthy adults [40]. In the present study, we found that hypercholesterolemia resulted in an increase of MPO activities in different organs, but no differences in MPO activities in various organs were found between the SO and FO groups. This finding may indicate that hypercholesterolemia results in the infiltration of neutrophils into organs, and the progress of sepsis may even promote the accumulation of neutrophils in tissues. However, the influence of FO administration on organ MPO activities in hypercholesterolemic mice with sepsis seemed obscure.

In summary, this study demonstrated that feeding fish oil to hypercholesterolemic mice did not result in immunosuppression when complicated with sepsis. Fish oil administration reduced adhesion molecule expressions and inflammatory-related mediators at the site of injury at an early but not a late stage of sepsis. However, compared with the SO groups, the influences of fish oil on MPO activities in various organs were not obvious in hypercholesterolemic mice with sepsis.

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## References

- [1] Fan J, Watanabe T. Inflammatory reactions in the pathogenesis of atherosclerosis. *J Atheroscler Thromb* 2003;10:63–71.
- [2] Hulthe J, Wikstrand J, Mattsson-Hultén L, Fagerberg B. Circulating ICAM-1 (intercellular cell-adhesion molecule 1) is associated with early stages of atherosclerosis development and with inflammatory cytokines in healthy 58-year-old men: the Atherosclerosis and Insulin Resistance (AIR) study. *Clin Sci (Lond)* 2002;103:123–9.
- [3] Han SN, Leka LS, Lichtenstein AH, Ausman LM, Meydani SN. Effect of a therapeutic lifestyle change diet on immune functions of moderately hypercholesterolemic humans. *J Lipid Res* 2003;44:2304–10.
- [4] Shimizu Y, Newman W, Tanaka Y, Shaw S. Lymphocyte interactions with endothelial cells. *Immunol Today* 1992;13:106–12.
- [5] Williams TJ, Hellewell PG. Endothelial cell biology. Adhesion molecules involved in the microvascular inflammatory response. *Am Rev Respir Dis* 1992;146:S45–S50.
- [6] Henderson RB, Lim LH, Tessier PA, Gavins FN, Mathies M, Perretti M, et al. The use of lymphocyte function-associated antigen (LFA)-1-deficient mice to determine the role of LFA-1, Mac-1, and  $\alpha$ 4



- integrin in the inflammatory response of neutrophils. *J Exp Med* 2001;194:219–26.
- [7] Ulbrich H, Eriksson EE, Lindbom L. Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacol Sci* 2003;24:640–7.
- [8] Nolte D, Kuebler WM, Muller WA, Wolff KD, Messmer K. Attenuation of leukocyte sequestration by selective blockade of PECAM-1 or VCAM-1 in murine endotoxemia. *Eur Surg Res* 2004;36:331–7.
- [9] Turler A, Schwarz NT, Turler E, Kalff JC, Bauer AJ. MCP-1 causes leukocyte recruitment and subsequently endotoxemic ileus in rat. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G145–55.
- [10] Deo R, Khera A, McGuire DK, Murphy SA, Meo Neto Jde P, Morrow DA, et al. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *J Am Coll Cardiol* 2004;44:1812–8.
- [11] Bossink AW, Paemen L, Jansen PM, Hack E, Thijs LG, Van Damme J. Plasma levels of the chemokines monocyte chemoattractant proteins-1 and -2 are elevated in human sepsis. *Blood* 1995;86:3841–7.
- [12] Grimm H, Mayer K, Mayser P, Eigenbrodt E. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. *Br J Nutr* 2002;87(Suppl 1):S59–S67.
- [13] Trebble TM, Wootton SA, Miles EA, Mullee M, Arden NK, Ballinger AB, et al. Prostaglandin E2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation. *Am J Clin Nutr* 2003;78:376–82.
- [14] Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* 2003;38:343–52.
- [15] Zhang P, Smith R, Chapkin RS, McMurray DN. Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. *J Nutr* 2005;135:1745–51.
- [16] Calder PC. n-3 polyunsaturated fatty acids and cytokine production in health and disease. *Ann Nutr Metab* 1997;41:203–34.
- [17] Virella G, Fourspring K, Hyman B, Haskill-Stroud R, Long L, Virella I, et al. Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. *Clin Immunol Immunopathol* 1991;61:161–76.
- [18] Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J Leukoc Biol* 1993;54:599–603.
- [19] Chang HR, Dulloo AG, Vladoianu IR, Piguet PF, Arsenijevic D, Girardier L, et al. Fish oil decreases natural resistance of mice to infection with *Salmonella typhimurium*. *Metabolism* 1992;41:1–2.
- [20] Fritsche KL, Shahbazian LM, Feng C, Berg JN. Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with *Listeria monocytogenes*. *Clin Sci (Lond)* 1997;92:95–101.
- [21] Lanza-Jacoby S, Flynn JT, Miller S. Parenteral supplementation with a fish-oil emulsion prolongs survival and improves rat lymphocyte function during sepsis. *Nutrition* 2001;17:112–6.
- [22] Esmon T. Why do animal models (sometimes) fail to mimic human sepsis? *Crit Care Med* 2004;32:S219–22.
- [23] Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol* 2005;77:598–625.
- [24] Ayala A, Deol ZK, Lehman DL, Herdon D, Chaudry IH. Polymicrobial sepsis but not low-dose endotoxin infusion causes decreased splenocyte IL-2/IFN-gamma release while increasing IL-4/IL-10 production. *J Surg Res* 1994;56:579–85.
- [25] Hillegass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. *J Pharmacol Meth* 1990;24:285–95.
- [26] Morlion BJ, Torwesten E, Lessire H, Sturm G, Peskar BM, Furst P, 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;45:1208–13.
- [27] Maier S, Traeger T, Entleutner M, Westerholt A, Kleist B, Huser N, et al. Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. *Shock* 2004;21:505–11.
- [28] Petit V, Arnould L, Martin P, Monnot MC, Pineau T, Besnard P, et al. Chronic high-fat diet affects intestinal fat absorption and postprandial triglyceride levels in the mouse. *J Lipid Res* 2007;48:278–87.
- [29] Moller S, Lauridsen C. Dietary fatty acid composition rather than vitamin E supplementation influence ex vivo cytokine and eicosanoid response of porcine alveolar macrophages. *Cytokine* 2006;35:6–12.
- [30] Broughton KS, Wade JW. Total fat and (n-3):(n-6) fat ratios influence eicosanoid production in mice. *J Nutr* 2002;132:88–94.
- [31] DiPiro JT. Cytokine networks with infection: mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. *Pharmacotherapy* 1997;17:205–23.
- [32] Li H, Ruan XZ, Powis SH, Fernando R, Mon WY, Wheeler DC, et al. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. *Kidney Int* 2005;67:867–74.
- [33] Ferguson NR, Galley HF, Webster NR. T helper cell subset ratios in patients with severe sepsis. *Intens Care Med* 1999;25:106–9.
- [34] Rollins BJ, Pober JS. Interleukin-4 induces the synthesis and secretion of MCP-1/JE by human endothelial cells. *Am J Pathol* 1991;138:1315–9.
- [35] Issekutz AC, Rowter D, Springer TA. Role of ICAM-1 and ICAM-2 and alternate CD11/CD18 ligands in neutrophil transendothelial migration. *J Leukoc Biol* 1999;65:117–26.
- [36] Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clin Chim Acta* 2006;368:33–47.
- [37] Miles EA, Wallace FA, Calder PC. Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis* 2000;152:43–50.
- [38] De Caterina R, Libby P. Control of endothelial leukocyte adhesion molecules by fatty acids. *Lipids* 1996;31(Suppl):S57–S63.
- [39] Daugherty A, Dunn JL, Rateri DL, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 1994;94:437–44.
- [40] Meuwese MC, Stroes ES, Hazen SL, van Miert JN, Kuivenhoven JA, Schaub RG, et al. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. *J Am Coll Cardiol* 2007;50:159–65.