Dietary fish oil enhances adhesion molecule and interleukin-6 expression in mice with polymicrobial sepsis

Chun-Sen Hsu[1], Wan-Chun Chiu[2], Chiu-Li Yeh[2], Yu-Chen Hou[2], Szu-Yuan Chou[1] and Sung-Ling Yeh[2]*

[1]*Department of Obstetrics and Gynecology, Taipei Medical University Associated Wan-Fang Hospital, 111 Hsing-Long Road, Sec 3, Taipei, Taiwan 116, ROC*
[2]*School of Nutrition and Health Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan 110, ROC*

This study investigated the effects of fish oil (FO) diet on plasma intercellular adhesion molecule 1 (ICAM-1) levels and leucocyte integrin expression in polymicrobial sepsis. Mice were randomly assigned to a control group and an FO group. The control group was fed a medium-fat diet containing soyabean oil, whereas in the FO group, 70 % of the soyabean oil was replaced by FO for 3 weeks. After that, sepsis was induced by caecal ligation and puncture (CLP) in the experimental groups and mice were killed at 0, 6, 12 and 24 h, respectively, after CLP. Results showed that compared with the control group, plasma ICAM-1 levels were higher in the FO group 6 h after CLP. Intra-lymphocyte interferon-γ expression in the FO group was lower, whereas IL-4 expression was higher than in the control group 12 and 24 h after CLP. The expression of leucocyte integrin was significantly higher in the FO group 12 and 24 h after CLP. The FO group had higher IL-6 levels at 12 h in the lungs, at 6 and 12 h in the kidneys, and at 6, 12 and 24 h in the intestines after CLP. The survival rate did not differ between the two groups after CLP. The present findings suggest that pretreatment with an FO diet enhances adhesion molecule and inflammatory cytokine expressions during sepsis, which might aggravate the inflammatory reaction and increase neutrophil infiltration into tissues. In addition, FO diet promotes the Th2-type response and suppresses cellular immune response in polymicrobial sepsis.

Fish oil: Polymicrobial sepsis: Adhesion molecules: CD11a/CD18: CD11b/CD18: Myeloperoxidase

Sepsis resulting from surgery, burns and trauma is a substantial cause of morbidity and the leading cause of death in intensive care units. In order to achieve optimal protection against most naturally acquired infections, some vulnerability to collateral damage during overwhelming sepsis as a result of inflicted injuries may be inevitable. During sepsis, immune cells are activated, and their subsequent overproduction of pro-inflammatory mediator was thought to result in tissue damage that precedes organ failure (Oberhoffer *et al.* 1999; Groeneveld *et al.* 2003). Neutrophil sequestration during polymicrobial sepsis and endotoxaemia were proved to be associated with increased adhesion molecule expression. Overexpression of adhesion molecules facilitates leucocyte–endothelial cell interactions and thus aggravates polymorphonuclear leucocyte (PMN) accumulation and tissue damage (Ulbrich *et al.* 2003; Nolte *et al.* 2004). Studies have shown that the concentration of plasma intercellular adhesion molecule 1 (ICAM-1) increases in patients with septic multiple organ failure (Endo *et al.* 1995; Whalen *et al.* 2000). Clinical trials have also shown that increased plasma ICAM-1 levels are correlated with the development of multiple organ dysfunction syndrome and death in patients with sepsis (Kayal *et al.* 1998; Whalen *et al.* 2000).

Cytokines are produced by cells of the immune system that act as mediators of the immune response and the response of tissue to injury. The release of cytokines is significantly altered during diseases. The cytokine profiles are determined to some extent by two functional subsets of T lymphocytes, Th1 and Th2. Th1 cytokines enhance cell-mediated immunity, while Th2 cytokines enhance humoral immunity (DiPiro, 1997). The effects of Th1 and Th2 lymphocytes are counter-regulatory. In some cases, the Th1/Th2 balance was an important indicator of the disease state (Diehl & Rincon, 2002). A shift from Th1 to Th2 cytokine profiles in many clinical interventions was shown to result in improvement in some diseases (Calder & Grimble, 2002).

Fish oils (FO) are rich sources of *n*-3 fatty acids, especially EPA and docosahexaenoic acid. A number of clinical trials have shown that FO supplementation has immune modulatory effects (Grimm *et al.* 2002). Dietary FO has been shown to have beneficial clinical effects on Th1-mediated diseases including rheumatic arthritis, ulcerative colitis, Crohn's disease, multiple sclerosis and insulin-dependent diabetes mellitus (Calder, 1997; Calder & Grimble, 2002). A study by Zhang *et al.* (2005) showed that the anti-inflammatory effect of FO was due to the suppression of Th1 development. A shift away from a Th1 response was considered to be the

mechanism that resulted in the benefits of FO (Wallace *et al.* 2001). Previous studies have shown that cell-mediated immunity is markedly depressed after the onset of sepsis, and that Th2 cytokines are responsible for the suppression and death associated with polymicrobial sepsis (Ayala *et al.* 1994; Ferguson *et al.* 1999). We hypothesized that an FO diet alters the Th1/Th2 balance toward a Th2-type response and may have an adverse effect on polymicrobial sepsis. Since sepsis is an inflammatory reaction with high adhesion molecule expression, the aim of the present study was to investigate the effects of dietary FO on the expressions of adhesion molecule associated with leucocyte–endothelial cell interaction and inflammatory cytokine IL-6 as well as intra-lymphocyte Th1/Th2 cytokine production in sepsis. Myeloperoxidase (MPO) is a leucocyte-derived enzyme that catalyses the formation of a number of reactive oxidant species. Although it is an integral component of the innate immune response, evidence has emerged that MPO-derived oxidants contribute to tissue damage during inflammation (Klebanoff & Seymour, 2005). In the present study we analysed MPO activity in organs as an indicator for identifying the extent of tissue injury during sepsis.

Materials and methods

Animals

Male ICR mice weighing 20–25 g were used in the present study. All mice were housed in stainless steel cages maintained in a temperature- and humidity-controlled 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. The care of the laboratory animals was established by Taipei Medical University, and protocols were approved by the Animal Care Committee.

Study protocol

Mice were randomly assigned to a control group (*n* 40) and FO (*n* 42) group. The control group was maintained for 3 weeks on a medium-fat (10 %, w/w) diet containing soyabean oil (Taiwan Sugar Co., Taipei, Taiwan), while that of the FO group had 70 % FO (Denofa Co., Fredrikstad, Norway) and 30 % soyabean oil (Table 1). The soyabean oil was used to prevent essential fatty acid deficiency. The FO contained 34 % EPA, 27 % docosahexaenoic acid and 72 % total *n*-3 fatty acids, while the mixed tocopherol was 2·4 mg/g according to the manufacturer. This amount of FO was comparable to that used in our previous studies (Chao *et al.* 2000; Chyi & Yeh, 2000) and provided 10 % of total energy as *n*-3 fatty acids. After feeding the mice their respective diets for 3 weeks, polymicrobial sepsis was induced in mice in the experimental groups by caecal ligation and puncture (CLP). CLP was performed as previously described by Ayala *et al.* (1994). Mice were lightly anaesthetized with ether. A midline incision (1·5–2·0 cm) was made below the diaphragm, exposing the internal organs. The caecum was isolated, and a 3-0 silk ligature was placed around it, ligating the caecum just below the ileocaecal valve. The caecum was then punctured in two places with a 22-G needle. The caecum was slightly compressed until a small drop of stool appeared.

Table 1. Composition of the experimental diets

Component (g/kg)	Control	Fish oil
Soyabean oil	100	30
Fish oil	–	70
Casein	200	200
Salt mixture*	35	35
Vitamin mixture†	10	10
Methyl cellulose	31	31
Choline chloride	1	1
Methionine	3	3
Maize starch	620	620

* The salt mixture contained the following (mg/g): calcium phosphate diabasic, 500; sodium chloride, 74; potassium sulphate, 52; potassium citrate monohydrate, 20; magnesium oxide, 24; manganese carbonate, 3·5; ferric citrate, 6; zinc carbonate, 1·6; curpric carbonate, 0·3; potassium iodate, 0·01; sodium selenite, 0·01; chromium potassium sulphate, 0·55.

† 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-α-tocopherol acetate, 20; cholecalciferol, 0·25; menaquinone, 0·005.

The abdominal wound was closed in two layers. Mice in the experimental groups were killed 0, 6, 12 and 24 h after CLP, respectively. All mice were anaesthetized and killed by cardiac puncture. Blood samples were collected in tubes containing heparin for analysis. Tissues including the lungs, kidneys, liver and intestines were rapidly harvested, rinsed with saline and stored at −70°C for further analysis.

Measurements of plasma intercellular adhesion molecule 1 concentrations during sepsis

Concentrations of plasma ICAM-1 were measured by a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA). Antibodies specific for mice ICAM-1 were coated on to the wells of the microtitre strips provided. Procedures followed the manufacturer's instructions. Readings of the optical densities were made on an ELISA reader (Multiskan RC; Labsystems, Helsinki, Finland). The amount of ICAM-1 was determined from a standard curve and expressed as pg/ml. The minimum detectable dose for ICAM-1 was 30 pg/ml. The within-assay CV was 5·3 % in the present study.

Analysis of the CD11a/CD18 distribution in lymphocytes and polymorphonuclear leucocyte expression of CD11b/CD18

Fresh blood (100 μl) was incubated with 10 μl fluorescein isothiocyanate (FITC)-conjugated rat monoclonal anti-mouse CD11a (I21/7) and phycoerythrin (PE)-conjugated rat anti-mouse CD18 (C71/16; Serotec, Oxford, UK) for 15 min at 4°C. FITC-conjugated rat IgG2a and PE-conjugated rat IgG2a (Serotec) were used for isotype control. Afterwards, erythrocytes were lysed with lysing buffer (Serotec). The proportions of CD11a/CD18 expressed on lymphocytes were analysed by flow cytometry (Coulter, Miami, FL, USA). Fluorescence data were collected, and the results are presented as a percentage of CD11a-presenting cells in 1×10^5 lymphocytes. To determine CD11b/CD18 expressions on PMN, FITC-conjugated rat monoclonal anti-mouse CD11b (M1/70·15) and PE-conjugated rat anti-mouse CD18 (C71/16) were added to 100 μl PMN suspension. FITC-conjugated rat

IgG2b (Serotec) and PE-conjugated rat IgG2a were used for isotype control. Fluorescence data were collected on 1×10^5 viable cells which were also analysed by flow cytometry. The results are presented as a percentage of CD11b-presenting cells in 1×10^5 PMN. Lymphocytes and PMN were gated by their characteristic forward and orthogonal light scatter characteristics, and analysed for the expressions of CD11a/CD18 and CD11b/CD18, respectively. Non-specific fluorescence was determined on cells incubated with isotype and fluorochrome-matched control antibodies.

Analysis of IL-4 and interferon-γ distributions in lymphocytes

Populations of lymphocyte IL-4 and interferon-γ (IFN-γ) expressions in fresh blood were analysed by flow cytometry. After killing the mice, 50 μl fresh blood were immediately incubated with 100 μl leucoperm (Serotec) reagent A for 15 min at room temperature to fix the leucocytes, then 5 ml PBS was added and centrifuged for 5 min at 300g. After discarding the supernatants, 100 μl leucoperm reagent B was added to the cell pellets to penetrate the leucocytes, then 10 μl fluorescein-conjugated rat monoclonal anti-mouse IFN-γ (XMG1.2; Serotec) and 5 μl PE-conjugated rat monoclonal anti-mouse IL-4 (BVD6-24G2; Serotec) were incubated together for 30 min at room temperature. FITC-conjugated rat IgG1 and PE-conjugated rat IgG1 (Serotec) were used for isotype control. Cells were washed with PBS. After removing the supernatant and resuspending cells in the sheath fluid, lymphocytes were gated on the basis of their forward scatter and side scatter profile. Lymphocyte IL-4 and IFN-γ expressions were assessed using dual intracellular cytokine staining and flow cytometry. The results are presented as a percentage of cytokine-producing cells in 1×10^5 lymphocytes. Non-specific fluorescence was determined on cells incubated with isotype and fluorochrome-matched control antibodies.

Measurements of IL-6 concentrations in organ tissues

The lungs, kidneys, liver and intestines, including the proximal, middle and distal small intestine, were removed and parts of the samples (0·5 g) were placed in 10 ml lysing buffer containing protease inhibitors (2 mmol/l phenylmethylsulphonyl fluoride, 2 μg/ml leupeptin, pepstatin A and aprotinin; Sigma, St Louis, MO, USA) at 4°C. Samples were homogenized and ultracentrifuged at 15 000 rpm for 45 min at 4°C. The concentrations of IL-6 in the supernatants were determined with a commercially available ELISA kit. Antibodies specific for mice IL-6 were coated on to the wells of the microtitre strips provided (Bio-Source, Camarillo, CA, USA). Readings of the optical densities were made on a microplate reader (Multiskan RC). The amount of IL-6 was determined from a standard curve. The detection limit for IL-6 was < 7 pg/ml. The within-run CV was 7·4 % in the present study. The IL-6 concentrations were expressed as pg/mg tissue.

Myeloperoxidase activities in organs

MPO activities were measured using a method as previously described (Hillegass *et al.* 1990). Liver, lung, kidney and intestinal samples were homogenized in 50 mM-potassium phosphate buffer (pH 6·0), and centrifuged at 20 000g, 4°C for 15 min. The supernatants were discarded, and the pellets were suspended in 50 mM-potassium phosphate buffer containing 0·5 % hexadecyltrimethylammonium bromide. After freezing and thawing for three cycles, the iced homogenate was then sonicated to disrupt the granules and solubilize the MPO in hexadecyltrimethylammonium bromide. Samples were centrifuged at 20 000g for 15 min. Aliquots (0·3 ml) were added to 2·3 ml reaction mixture containing 50 mM-potassium phosphate buffer, *o*-dianisidine and 20 mM-H_2O_2 solution. MPO activity is presented as U/mg tissue protein. The concentrations of tissue protein were measured by Lowry's method. The absorbance at 460 nm was immediately measured for 3 min, and the rate of change in the absorbance was used to calculate the activities of MPO.

Statistical analysis

Data are expressed as means and standard deviations. Differences among groups were analysed by two-way ANOVA with Duncan's test. The survival rate at different time-points was determined by χ^2 test. $P < 0.05$ was considered statistically significant.

Results

Food intake and body weight

The food intake did not differ among the groups. There were no differences in initial body weight and body weight after feeding the diets for 3 weeks between the control and the FO groups (Table 2).

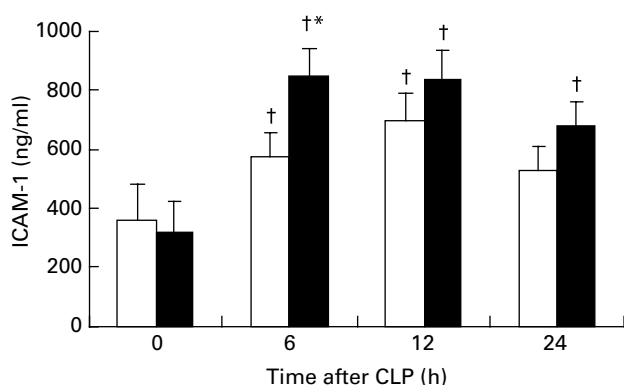### Plasma intercellular adhesion molecule 1 levels

Plasma concentrations of ICAM-1 increased with the progression of sepsis in both groups, then decreased by 24 h after CLP in the control group. Compared with the control group, plasma ICAM-1 levels were significantly higher in the FO group 6 h after CLP (Fig. 1).

CD11a/CD18 expressions on lymphocytes and CD11b/CD18 expressions on polymorphonuclear leucocytes

The expressions of lymphocyte CD11a/CD18 were significantly higher in the FO group 12 and 24 h after CLP than those of the corresponding control group, whereas no significant changes were observed in the control group at various time-points after CLP. Compared with 0 h, CD11b/CD18 expressions on PMN significantly increased in both groups by 6 and 12 h after CLP and even at 24 h in the FO group.

Table 2. Food intake and body weight of the control and fish oil groups
(Mean values and standard deviations)

	Food intake		Initial weight		Final weight	
	Mean	SD	Mean	SD	Mean	SD
Control (*n* 40)	5·7	0·5	23·5	1·0	29·7	1·2
Fish oil (*n* 42)	5·3	0·8	23·6	0·5	28·9	1·8

Fig. 1. Concentrations of plasma intercellular adhesion molecule 1 (ICAM-1) during sepsis. For details of procedures and diets, see p. 856−857 and Table 1. Values are means with their standard deviations depicted by vertical bars. Mean values were significantly different from those of the control group at the same time-point: *$P<0.05$. Mean values were significantly different from time 0 h in the same group: †$P<0.05$. According to a two-way ANOVA, the P value of diet effect was 0·05 and time effect was 0·01. CLP, caecal ligation and puncture. ■, Diet containing fish oil; □, control diet.

PMN expressions of CD11b/CD18 in the FO groups were higher than those in the control group at 12 and 24 h after CLP (Table 3).

Intra-lymphocyte interferon-γ and IL-4 distributions

Intra-lymphocyte expressions of IFN-γ decreased whereas IL-4 expressions increased in both groups as sepsis progressed. Lymphocyte IFN-γ expressions in the FO groups were significantly lower, and IL-4 levels were higher than those in the control group at 12 and 24 h after CLP (Fig. 2).

Table 3. Expressions (%) of lymphocyte CD11a/CD18 and neutrophil CD11b/CD18 during sepsis§

(Mean values and standard deviations)

	CD11a/CD18		CD11b/CD18	
	Mean	SD	Mean	SD
0 h				
Control	4·87	1·61	10·78	0·26
Fish oil	5·12	0·84	10·58	0·74
6 h				
Control	3·60	1·51	19·58†‡	1·25
Fish oil	4·79	1·56	18·94†‡	1·94
12 h				
Control	3·06*	1·79	13·68*†	0·54
Fish oil	9·14†	1·5	19·65†‡	1·06
24 h				
Control	6·13*	2·45	9·83*	0·59
Fish oil	21·49†‡	2·28	22·53†‡	1·69
P value of the effects				
Diet effect	<0·001		<0·001	
Time effect	<0·001		<0·001	

Mean values were significantly different from those of the fish oil group group at the same time-point: *$P<0.05$.
Mean values were significantly different from time 0 h in the same group: †$P<0.05$.
Mean values were significantly different from other time-points in the same group: ‡$P<0.05$.
§ For details of procedures and diets, see p. 856−857 and Table 1. There were eight mice in each group at various time-points.

### IL-6 levels in the liver, lungs, kidneys and intestines

IL-6 levels in lung, kidney and intestinal homogenates in both groups were significantly higher at 6, 12 and 24 h after CLP than at 0 h. The levels of IL-6 reached a peak at 6 h in the lungs and kidneys and at 6 and 12 h in the intestines after CLP in both groups. The FO group had higher IL-6 levels at 12 h in the lungs, at 6 and 12 h in the kidneys and at 6, 12 and 24 h in the intestines than those of the control group. Contrary to results for the lungs, kidneys and intestines, IL-6 levels decreased as sepsis progressed in the liver regardless of whether FO was administered or not (Table 4).

Myeloperoxidase activities in various organs

The activities of MPO in the lungs, liver, kidneys and intestines increased in both groups as sepsis progressed and reached a peak at 6 or 12 h after CLP. The FO groups had higher MPO activities at various time-points after CLP than those of the control group in the organs (Table 5).

Survival rate between the groups

All mice survived at 0 and 6 h after CLP in both groups. The survival rates did not differ among the two groups at 12 h (FO: 8/13 v. control: 8/12, $P>0.05$) and 24 h (FO: 8/15 v. control: 8/14, $P>0.05$) after CLP.
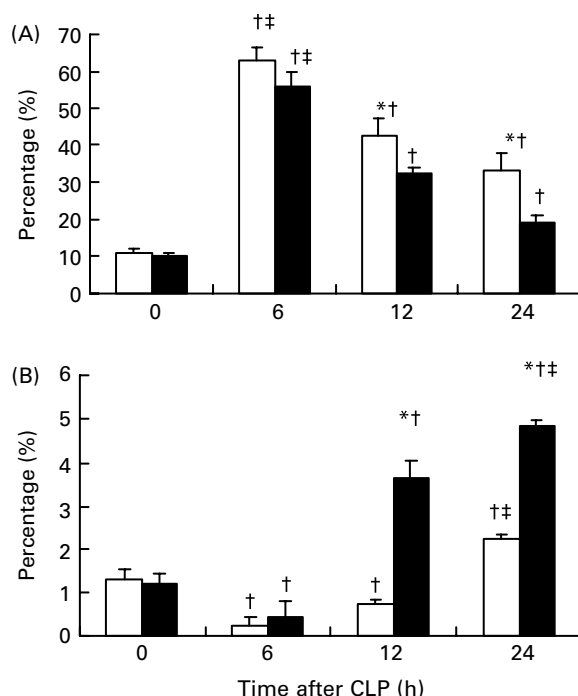
Fig. 2. Percentage of intra-lymphocyte interferon-γ (A) and IL-4 (B) expressions at the time indicated after caecal ligation and puncture (CLP). For details of procedures and diets, see p. 856−857 and Table 1. Values are means with their standard deviations depicted by vertical bars. Mean values were significantly different from those of the control group at the same time-point: *$P<0.05$. Mean values were significantly different from time 0 h in the same group: †$P<0.05$. Mean values were significantly different from other time-points in the same group: ‡$P<0.05$. According to a two-way ANOVA, the P values of diet effect and time effect in interferon-γ and IL-4 were 0·001. ■, Diet containing fish oil; □, control diet.

Table 4. Concentrations of IL-6 (pg/mg tissue) in lung, kidney, liver and intestinal tissue homogenates during sepsis§

(Mean values and standard deviations)

	Lung		Kidney		Intestines		Liver	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 h								
Control	3·2	1·2	12·4	0·9	1·1	0·77	26·1	3·2
Fish oil	3·5	0·8	11·6	1·1	0·99	0·59	25·9	3·3
6 h								
Control	26·6†‡	2·1	32·6†‡	1·07	25·5†‡	0·9	10·3†	1·7
Fish oil	24·1†‡	1·8	40·6*†‡	1·2	33·3*†‡	1·5	11·2†	1·5
12 h								
Control	12·5†	1·1	26·9†	1·1	4·2†	1·6	8·4†	0·7
Fish oil	20·7*†	0·9	36·2*†	1·9	29·1*†‡	1·4	8·6†	1·2
24 h								
Control	14·1†	1·2	20·4†	0·7	3·4†	0·8	7·0†	2·0
Fish oil	13·4†	1·5	21·2†	1·4	13·5*†	1·7	8·1†	1·3
P value of the effects								
Diet effect	<0·01		<0·001		<0·001		NS	
Time effect	<0·001		<0·001		<0·001		<0·01	

Mean values were significantly different from those of the control group at the same time-point: *$P < 0.05$.
Mean values were significantly different from time 0 h in the same group: †$P < 0.05$.
Mean values were significantly different from other time-points in the same group: ‡$P < 0.05$.
§ For details of procedures and diets, see p. 856–857 and Table 1. There were eight mice in each group at various time-points.

Discussion

In the present study, we used CLP as a sepsis model, because this experimental model most closely resembles bowel perforation with devitalized tissue leading to polymicrobial infection similar to the clinical scenario of acute peritonitis (Heuer *et al.* 2004). Sepsis is a systemic inflammatory response to infection, which involves various pathogenic changes in many host systems. The pathophysiologic sequence for sepsis involves cytokine release and endothelial and neutrophil activation, initiating a cascade of leucocyte–endothelium interactions and adhesions (Kayal *et al.* 1998). ICAM-1 is a cell surface protein which plays an important role in transendothelial migration of leucocytes through its expression on the vascular endothelium and binding to β_2 integrin. β_2 integrin is a heterodimer composed of a common β subunit encoded by the CD18 gene. CD11a and CD11b are members of the leucocyte adhesion molecule β_2 integrin family. CD11a/CD18 is exclusively expressed on leucocytes and CD11b/CD18 is abundant in PMN (Henderson *et al.* 2001). Integrin binding to ICAM-1 is particularly important for firm attachment and migration across the endothelium (Weber, 2003). Excessive expression of these integrins may induce an inflammatory response and tissue injury (Henderson *et al.* 2001; Ulbrich *et al.* 2003).

Table 5. Activities of myeloperoxidase (U/mg protein) in organ homogenates during sepsis§

(Mean values and standard deviations)

	Lung		Liver		Kidney		Intestines	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 h								
Control	0·74	0·18	0·54	0·10	0·70	0·12	0·28	0·11
Fish oil	0·74	0·10	0·54	0·09	0·76	0·21	0·29	0·08
6 h								
Control	2·06†‡	0·29	1·09†	0·05	1·36†	0·19	0·51†‡	0·11
Fish oil	4·55*†‡	0·25	1·37†	0·40	1·42†	0·14	0·90*†‡	0·08
12 h								
Control	2·85†‡	0·33	1·09†	0·07	3·15†‡	0·27	0·44‡	0·15
Fish oil	4·78*†‡	0·43	1·10†	0·23	5·86*†‡	0·21	0·72*†	0·11
24 h								
Control	1·26	0·45	0·63‡	0·13	1·83†	0·29	0·22	0·03
Fish oil	3·71*†‡	0·55	1·95*†‡	0·09	2·82*†	0·21	0·70*†	0·07
P value of the effects								
Diet effect	<0·001		<0·01		<0·001		<0·001	
Time effect	<0·001		<0·001		<0·001		<0·001	

Mean values were significantly different from those of the control group at the same time-point: *$P < 0.05$.
Mean values were significantly different from time 0 h in the same group: †$P < 0.05$.
Mean values were significantly different from other time-points in the same group: ‡$P < 0.05$.
§ For details of procedures and diets, see p. 856–857 and Table 1. There were eight mice in each group at various time-points.

A previous study showed that the expression of CD11b/CD18 was significantly increased in patients with septic shock (Brunialti *et al.* 2006). In the present study, we observed that plasma concentrations of ICAM-1 were significantly higher in the FO group than in the control group at 6 h after CLP, which is consistent with the higher CD11a/CD18 and CD11b/CD18 expressions on leucocytes. The present finding indicates that adhesion molecule-mediated cell interactions may be aggravated when FO is administered in a septic condition.

In the present study, we directly measured intra-lymphocyte IFN-γ and IL-4 production to investigate the effect of FO on the Th1/Th2-type response during sepsis. IFN-γ is produced by Th1 lymphocytes which enhance cell-mediated immunity. IL-4 is a Th2 cytokine that enhances humoral immunity. The results showed that in accordance with the progression of sepsis, a predominant Th2-type response occurred in both groups. FO administration produced more pronounced IFN-γ suppressive and IL-4 enhancing effects during sepsis than seen in the control group. The present finding suggests that a more predominant Th2 response occurred in the FO groups which may lead to a greater extent of suppression of cellular immunity. IL-6 is an important mediator in the early phase of infection and is primarily secreted by macrophages during sepsis (Ayala & Chaudry, 1996). Although IL-6 is considered an integral mediator of the physiologic acute phase response to injury, excessive and prolonged post-injury elevation of IL-6 are associated with morbidity and mortality (Biffl *et al.* 1996). A study by Ishimura *et al.* (1998) showed that IL-6 expression in the lungs and kidneys was enhanced under a septic condition. In the present study, we found that IL-6 levels in the lungs, kidneys and intestines in the FO groups were higher than those of the control group at various time-points. A study by Rincon *et al.* (1997) found that IL-6 promotes Th2 differentiation by inducing the expression of IL-4 producing CD4 T cells. The higher IL-6 levels in the FO groups observed in non-hepatic organs were comparable to the predominant Th2 response in these groups. Whether FO administration activates macrophages and thus enhances IL-6 secretion after sepsis requires further investigation. We found that hepatic IL-6 levels were reduced as sepsis progressed in both groups. The present finding is consistent with our previous report (Yeh *et al.* 2005). In our studies, we ligated and punctured the caecum of the mice twice. This model of peritonitis results in irreversible hepatic dysfunction (Deutschman *et al.* 1997) and may possibly impair the function of hepatic IL-6 production.

MPO is exclusively synthesized by neutrophil and monocyte precursor cells. It functions not only in host defence by mediating efficient microbial killing but also contributes to progressive tissue damage in inflammatory states (Klebanoff & Seymour, 2005). A study showed that MPO activity in tissues was increased by sepsis (Iseri *et al.* 2005) and tissue MPO activity correlates significantly with the number of PMN determined histochemically in inflamed tissues (Bradley *et al.* 1982). Some reports used tissue-associated MPO activity as an indication of accumulation of neutrophils (Gaut *et al.* 2001; Iseri *et al.* 2005). The results of the present study showed that compared with 0 h, MPO activities in the various organs increased as sepsis progressed, indicating that an inflammatory reaction was induced by sepsis. Compared with the control group, the FO groups had higher MPO activities at different time-points after CLP in the organs. The present finding may indicate that mice with FO administration have greater neutrophil infiltration during sepsis in these organs.

The results of the present study appeared to show that FO administration had adverse effects in septic mice. Sepsis is a condition with Th2 predominant immune response. The Th1/Th2 ratio in septic patients was much lower than both non-septic controls and healthy subjects (Ferguson *et al.* 1999). A study by Ayala *et al.* (1994) also showed that prolonged sepsis caused a marked suppression of Th1, while enhancing Th2 cytokine production. Since FO alters the Th1/Th2 balance towards a Th-2 predominate response, the administration of FO may deteriorate the condition of sepsis. However, the present results are inconsistent with previous studies which used the same CLP model as we did. Those studies with beneficial results in sepsis provided 2–4 % of total energy as *n*-3 fatty acids (Muakkassa *et al.* 1991; Johnson *et al.* 1993; Lanza-Jacoby *et al.* 2001), while in the present study, 10 % of total energy was provided by *n*-3 fatty acids. We speculate that in addition to promoting Th2 response, a high dose of *n*-3 fatty acids results in excessive suppression of prostaglandin E_2 which may consequently lead to overexpression of inflammatory cytokines (Somers & Reickson, 1994). A study by our laboratory also showed that compared with the control group, the FO group had higher IL-1β and TNF-α and lower prostaglandin E_2 concentrations in peritoneal lavage fluid in a diabetic rat model of intraperitoneal sepsis (Chyi & Yeh, 2000). CLP is a model with live multiplying bacteria in the blood. It is possible that immunosuppression results from a reduced Th1 response leading to a more severe inflammatory reaction as observed in the present study. We did not observe a higher mortality in the FO group 12 and 24 h after CLP. Since survival was only observed for 24 h in the present study, determining whether the FO diet affects survival over a longer period requires further investigation.

In summary, the present study demonstrated that compared with the control group, pretreatment with an FO diet promoted leucocyte integrin expression, increased plasma ICAM-1 levels, and enhanced IL-6 as well as MPO activities in various organs in polymicrobial sepsis. In addition, FO administration reduced intra-lymphocyte IFN-γ and enhanced IL-4 expressions during sepsis. The present findings indicate that pretreatment with a high-dose FO diet aggravates the inflammatory reaction and increases neutrophil infiltration into tissues. Moreover, a more pronounced Th2-type response was observed under the present experimental septic condition. The present results suggest that FO administration in a critical condition, especially in Th-2 skewed diseases, should be carefully evaluated.

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