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Different ratios of eicosapentaenoic and docosahexaenoic omega-3 fatty acids in commercial fish oils differentially alter pro-inflammatory cytokines in peritoneal macrophages from C57BL/6 female mice

Arunabh Bhattacharya, Dongxu Sun, Mizanur Rahman, Gabriel Fernandes*

Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900, USA

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Abstract

The use of fish oil (FO) as a dietary supplement to prevent or reduce the severity of cardiovascular diseases and autoimmune disorders such as rheumatoid arthritis is receiving much attention. Several recent reports indicate that eating fish often or the use of small doses of FO capsules appears to have benefits against cardiovascular diseases. We have reported in the past that diets enriched with FO protect against renal diseases and prolong the life span of autoimmune-prone mice compared to corn oil (CO) diets. However, the optimum ratio of eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA) in commercially available FOs to reduce the production of various pro-inflammatory cytokines has not been well established. We, therefore, obtained deodorized FO from three sources containing different EPA/DHA contents, fed them to C57BL/6 mice for 8 weeks in a 10% (vol/wt) diet (oil A, 11/10; oil B, 14/9; oil C, 23/14) and compared them with (10%) CO-fed mice as control. TNF-α, IL-6 and IL-1β were measured by enzyme-linked immunosorbent assay in thioglycollate-induced macrophages, 8 and 24 h after lipopolysaccharide treatment. The results showed a significant decrease in TNF-α after only 8 h in oil C. After 24 h, TNF-α, IL-6 and IL-1β levels decreased only in mice fed oil C, although nonsignificant decreases were seen in mice fed oil A compared to mice fed CO. The antioxidant enzymes, catalase and glutathione transferase, were higher in kidneys of mice fed oil C compared to mice fed CO. The study suggests that anti-inflammatory activity may vary among different sources of FO due to variations in EPA/DHA content.

Keywords: Fish oil; Fatty acids; Cytokines; Peritoneal macrophages; Antioxidant enzymes

1. Introduction

Dietary fish oils (FOs) decrease the risk of cardiovascular diseases, autoimmune diseases and malignancy [1–6]. Studies performed in animal models and clinical trials in humans suggest that dietary FOs containing $n-3$ fatty acids, such as eicosapentaenoic acid (EPA; 20:5$n-3$) and docosahexaenoic acid (DHA; 22:6$n-3$), display significant anti-inflammatory properties through inhibition of pro-inflammatory eicosanoids derived from $n-6$ fatty acids, such as arachidonic acid (AA; 20:4$n-6$), and decreasing activities of pro-inflammatory cytokines [7–12]. $n-3$ fatty acids compete with $n-6$ fatty acids for incorporation into biomembranes, leading to decreased production of pro-inflammatory eicosanoids through cyclooxygenase and lipoxygenase pathways. Thus, production of pro-inflammatory eicosanoids, such as prostaglandin (PG) E_2, thromboxane (TX) B_2 and 6-keto-$PGF_1\alpha$, is significantly decreased. These results are particularly interesting considering that $n-6$ fatty acids form a major part of fatty acid intake in Western-style diets [1]. Recent evidence suggests that a higher ratio of $n-6/n-3$ fatty acids may be associated with a higher risk of osteoporosis, and that a higher $n-3/n-6$ ratio may be associated with a lower risk of breast cancer in premenopausal women [13,14].

Some studies have investigated the effect of FOs, purified $n-3$ fatty acids (EPA and DHA) and different $n-3/n-6$ fatty acid ratios on pro-inflammatory mediators in macrophages, both in vivo and in vitro [15–18]. Most of the dietary FO and macrophage pro-inflammatory mediator studies have used ICN Menhaden oil (containing low levels

* Corresponding author. Tel.: +1 210 567 4663; fax: +1 210 567 4592.
E-mail address: fernandes@uthscsa.edu (G. Fernandes).

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of EPA and DHA) as the source of FO [18–22]. However, the effectiveness of concentrated FO containing higher levels of EPA and DHA on pro-inflammatory cytokines in activated macrophages remains to be established when compared to FOs with low EPA and DHA content.

Oxidative stress has been implicated as one of the causes of the pathogenesis of a variety of age-associated disease states, including atherosclerosis, cancer and neurodegeneration [23–25]. Oxidative stress refers to the cytological consequence of a mismatch between the production of free radicals and reactive oxygen species (ROS), and the ability of the cell to defend against them by an array of free radical scavenging antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione transferase (GST)] and antioxidant molecules such as glutathione [26]. Although the association between oxidative stress and inflammatory response is not fully understood, it is believed that oxidative stress has a definitive role in the inflammatory process through the transcriptional factor, nuclear factor κB, which is involved in regulating the expression of cytokines and other mediators that participate in acute inflammatory responses, many of which are associated with increased generation of ROS [27–30]. We have consistently shown that FO supplementation significantly increases the activities of antioxidant enzymes and decreases lipid peroxidation (LPO) in different tissues of normal and autoimmune kidney disease-prone NZB/W and MRL/lpr mice [31–36]. A recent in vitro study showed increased glutathione activity and decreased levels of intracellular peroxides in DHA-treated murine macrophages, providing further evidence regarding the antioxidant-enhancing activity of $n-3$ fatty acids [37]. However, the effect of concentrated FO with higher EPA and DHA content on antioxidant enzyme activity remains to be established in animal models.

Therefore, the present study was designed to compare the effect of FO with high EPA and DHA content to two commercially available FOs on pro-inflammatory cytokine production in endotoxin-challenged and activated murine macrophages, with corn oil (CO) as control. Since oxidative stress is implicated in the inflammatory process, activities of antioxidant enzymes (SOD, CAT, GPX and GST) and LPO were also analyzed in these mice. Serum and kidney fatty acids were also measured to account for differences in fatty acid composition in mice fed these different FOs.

2. Materials and methods

2.1. Animals and experimental diets

Four-week-old female C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Weight-matched mice were housed in a laboratory animal care facility in cages (5 mice/cage) and fed standard laboratory chow diet. At 6 weeks of age, mice were divided into four dietary groups of 10 mice each and fed semipurified AIN-93M diets containing 10% CO or 10% of one of the three deodorized FOs varying in EPA/DHA ratios. In FO diets, 1% CO was added to 9% FO to prevent essential fatty acid deficiency. The FOs used for the study were: (A) ICN Menhaden (EPA/DHA=10/11; EPA+DHA=21%; MP Biomedicals, Irvine, CA); (B) Omega Pure (EPA/DHA=14/9; EPA+DHA=23%; Omega Protein, Inc., Reedville, VA); and (C) Ocean Nutrition (EPA/DHA=23/14; EPA+DHA=37%; Ocean Nutrition Canada Ltd., Mulgrave, NS, Canada). The diets were supplemented with equal amounts of antioxidant supplements (vitamin E and tertiary butyl hydroxyquinone) to prevent peroxidative damage during storage. The composition of the semipurified diet per kilogram of diet was: 140 g of casein, 424.3 g of corn starch, 145 g of dextronized corn starch, 90 g of sucrose, 50 g of fiber, 35 g of AIN-93 mineral mix, 10 g of AIN-93 vitamin mix, 1.8 g of L-cystine and 2.5 g of choline bitartrate. Fresh diet was provided, and leftover food was removed daily to prevent rancidity. Diets were prepared weekly and stored in aliquots at −20°C. Oils used in the study were always stored under nitrogen to prevent oxidation. The fatty acid compositions of CO and different FOs are presented in Table 1. The animals were maintained on a 12-h light/12-h dark cycle. The National Institutes of Health guidelines provided in "The Guide for the Care and Use of Laboratory Animals" were strictly followed, and all studies were approved by the Institutional Laboratory Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

2.2. Collection of blood and kidney tissues

After 8 weeks of dietary treatment, six mice per group were sacrificed by cervical dislocation; blood was collected for the isolation of serum by centrifugation at 3000 rpm for 15 min. Kidneys were collected, instantly frozen in liquid nitrogen and stored at −80°C until the time of study.

Table 1
Selected fatty acid composition of dietary oils[a]

Fatty acids	CO[b]	Oil A[c]	Oil B[c]	Oil C[c]
14:0	0.14	9.10	8.14	5.83
16:0	10.50	17.02	18.90	12.40
16:1 ($n-9$)	ND	12.87	10.29	6.22
18:0	2.06	2.78	3.14	3.10
18:1 ($n-9$)	27.76	8.08	9.16	9.02
18:2 ($n-6$)	56.06	1.27	2.08	3.37
18:3 ($n-3$)	1.27	1.67	1.81	1.29
18:4 ($n-6$)	ND	3.64	3.41	3.06
20:4 ($n-6$)	ND	0.81	0.60	1.02
20:5 ($n-3$) EPA	ND	14.27	10.14	23.26
22:5 ($n-3$)	ND	2.05	1.63	3.00
22:6 ($n-3$) DHA	ND	8.70	11.04	13.81
EPA+DHA	0	23	21	37

ND=not detected.
[a] Expressed as percentage of total fatty acids.
[b] CO diets contained 100 g of corn oil per kilogram of diet.
[c] FO diets contained 90 g of fish oil+10 g of corn oil per kilogram of diet.

2.3. Isolation and activation of peritoneal macrophages

After 8 weeks of dietary treatment, six mice per group were injected intraperitoneally with 1 ml of Brewer's thioglycollate broth to facilitate the migration of macrophages to peritoneal cavity [19]. Four days later, the mice were killed by cervical dislocation. Peritoneal exudates cells were collected by washing the peritoneal cavity with 5 ml of phosphate-buffered saline (PBS), and cells were collected by centrifugation. Cells were suspended in RPMI 1640 culture medium containing 10% fetal calf serum (FCS), 2 mM glutamine and antibiotics at a concentration of 1×10^6 cells/ml medium. Macrophages were purified in 96-well plates by adherence to the surface of tissue culture plates at 37°C for 1 h in 5% CO_2. Nonadherent cells were discarded by washing plates twice with PBS. Macrophages (1×10^6 cells) were incubated in 1 ml of RPMI 1640 culture medium supplemented with 10% FCS, 2 mM glutamine, antibiotics and lipopolysaccharide (LPS; 1 mg/l). After 8 and 24 h, the medium was removed, and concentrations of TNF-α, IL-6 and IL-β were determined by standard enzyme-linked immunosorbent assay (ELISA).

2.4. Cytokine measurements

Cytokines released upon LPS stimulation in peritoneal macrophages were measured by standard ELISA techniques using commercially available BD OptEIA ELISA kits for TNF-α, IL-6 and IL-1β (BD Biosciences, San Diego, CA), as described previously [38]. Each well of flat-bottom 96-well microtiter plates was coated overnight with 50 μl of purified anti-TNF-α, anti-IL-6 and anti-IL-1β antibodies (4 μg/ml in binding solution) at 4°C. The plates were rinsed four times with washing buffer and then samples were added, followed by incubation for 2 h at room temperature. The plates were washed four times with washing buffer, followed by the addition of biotinylated anticytokine antibodies. The plates were incubated in room temperature for 1 h and then washed four times with washing buffer. Streptavidin–alkaline phosphatase conjugate was added, and the plates were incubated for 30 min at room temperature. The plates were again washed four times with washing buffer, and chromogen substrate was added. The plates were then incubated at room temperature to achieve the desired maximum absorbance and were read at 410 nM in an ELISA reader (Dynex Technologies, UK).

2.5. Fatty acid analysis of serum and kidneys

Serum and kidney total lipids were extracted by the method of Folch et al. [39] using chloroform:methanol (2:1). The organic phase, containing total lipid extracts, was dried under a stream of nitrogen, and the residue was methylated according to the method of Kates [40]. Fatty acid methyl esters were separated and quantified by gas–liquid chromatography using a Hewlett-Packard 5890A series II gas chromatograph, equipped with a DB225MS capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector. The injection and detector port temperatures were 225°C and 250°C, respectively. The oven temperature was maintained at 170°C for 1 min and then increased to 215°C at a rate of 5°C/min. Helium was used as carrier gas. The running time of each sample was approximately 36 min. Fatty acid methyl esters were identified by comparison of retention times with fatty acid methyl ester standard (FIM-FAME-7) from Matreya, Inc. (Pleasant Gap, PA). Quantification was performed by an integrator (Hewlett-Packard 3396 series II) attached to a GLC machine, and results were expressed as area percentages. Oil samples were treated similarly beginning on the methylation step.

2.6. Preparation of kidney homogenates

Frozen kidneys were rinsed in ice-cold physiological saline and minced with scissors. Ten percent homogenates were prepared in 0.01 M Tris–HCl buffer (pH 7.4) and centrifuged at $10{,}000\times g$, and supernatants were used for antioxidant enzyme assays. For the determination of LPO, kidneys tissues were homogenized in 1.15% KCl solution to obtain a 10% (wt/vol) homogenate. The protein content of kidney supernatants was determined by the microplate procedure of the bicinchoninic acid protein assay, as described by the supplier (Pierce Chemical Co., Rockford, IL).

2.7. Determination of kidney antioxidant enzyme activity

Antioxidant enzyme activities were measured as described previously [36]. The activity of CAT was measured using its peroxidative function according to the method of Johansson and Borg [41]. SOD activity was determined through the inhibition of cytochrome *c* reduction by the method of Flohe and Otting [42]. GPX activity was measured following NADPH oxidation using a coupled reaction system consisting of glutathione, glutathione reductase and cumene hydroperoxide [43]. GST activity was measured by the catalytic reaction of 1-chloro-2, 4-dinitrobenzene with the sulfhydryl group of glutathione [44].

2.8. Determination of LPO in kidney tissues

Thiobarbituric acid reactive substances (TBARS) were measured by a modification of the method of Bhattacharya et al. [35] and Ohkawa [45]. For each sample to be assayed, four tubes — containing 100, 150, 200 and 250 μl of tissue homogenate; 100 μl of 8.1% sodium dodecyl sulfate; 750 μl of 20% acetic acid; and 750 μl of 0.8% aqueous solution of TBA — were set up. The volume was made up to 4 ml with distilled water, mixed thoroughly and heated at 95°C for 60 min. After cooling, 4 ml of *n*-butanol was added to each tube. The contents were mixed thoroughly then centrifuged at 3000 rpm for 10 min. The absorption of the clear upper (*n*-butanol) layer was read at 532 nm. 1,1,3, 3-Tetraethoxy propane (97%) was used as external standard. Results are expressed as nanomoles of TBARS per milligram of protein.

Table 2
Selected fatty acids of serum total lipids[y]

Fatty acids[z]	CO	Oil A	Oil B	Oil C
16:0	16.98±0.32[a]	21.05±0.83[b]	23.19±1.12[b]	21.44±0.42[b]
16:1*n*−9	2.41±0.11[a]	5.64±0.22[b]	5.02±0.18[b]	4.83±0.21[b]
18:0	11.81±0.44[a]	8.78±0.27[b]	8.53±0.76[b]	9.31±0.27[b]
18:1*n*−9	13.00±0.98	11.51±0.41	11.69±0.93	12.26±.18
18:2*n*−6	22.30±0.57[a]	9.73±0.25[b,d]	10.40±0.77[c,d]	8.20±0.36[b]
20:3*n*−6	1.01±.10[a]	0.36±0.02[b]	0.52±0.04[b]	ND
20:4*n*−6	26.94±1.36[a]	7.19±0.23[b]	6.08±0.59[b]	8.57±0.35[b]
20:5 (*n*−3) EPA	ND	21.60±0.60[a]	17.59±1.03[b]	21.40±1.16[a]
22:5*n*−3	ND	0.81±0.01	0.79±0.08	0.77±0.03
22:6 (*n*−3) DHA	4.48±0.51[a]	10.89±0.58[b]	12.91±0.97[b]	12.44±0.28[b]

Means in a row with superscripts without a common letter differ ($P<.05$).
ND=not detected.
Oil A, ICN Menhaden FO; oil B, Omega Pure FO; oil C, Ocean Nutrition FO.
[y] Expressed as percentage of total fatty acids.
[z] Results are expressed as mean±S.E.M. ($n=5$).

2.9. Statistics

Results are expressed as mean±S.E.M. Data were analyzed statistically by two-way ANOVA using Graphpad Prism 4 software. Newman–Keuls multiple comparison test was used to test the differences among groups. Differences were considered significant at $P<.05$.

3. Results

There was no difference in body weight in mice fed different FOs.

3.1. Fatty acid composition of serum

The different FO diets, compared to the CO diet, significantly decreased the linoleic acid (LA) and AA contents of serum with a concomitant increase in DHA levels and incorporated EPA (Table 2). However, there was no difference in AA and DHA content between the different FOs. EPA content was higher in oil-A-fed and oil-C-fed mice compared to oil-B-fed mice. LA content was lowest in oil C, although results were significant only when compared to oil B. D-homo-γ-linolenic acid (DGLA; 20:3*n*−6) levels were undetectable in oil-C-fed mice.

3.2. Fatty acid composition of kidney tissues

FO-fed mice had significantly lower LA and AA content and significantly higher 22:5*n*−3 and DHA content. There was no difference in any of these fatty acids between the different FOs. EPA was undetectable in CO-fed mice and was significantly higher in oil-A-fed and oil-C-fed mice compared to oil-B-fed mice (Table 3). There was no difference in DGLA levels between the different FO groups.

3.3. LPS-induced pro-inflammatory cytokine production (8 and 24 h)

Oils A, B and C decreased TNF-α compared to CO at 8 h, but results were significant only for oil C. IL-6 and IL-1β decreased in oils A and C, but the results were not

Table 3
Selected fatty acids of kidney total lipids[y]

Fatty acids[z]	CO	Oil A	Oil B	Oil C
14:0	0.65±0.07[a]	0.89±0.05[b]	0.96±0.06[b]	1.02±0.12[b]
16:0	17.96±0.47[a]	20.80±0.86[b]	20.78±0.16[b]	20.34±0.44[b]
16:1 (*n*−9)	2.34±0.52	2.45±0.70	2.54±0.60	2.49±0.50
18:0	11.54±0.94[a]	15.01±0.89[b]	14.98±0.67[b]	15.22±0.77[b]
18:1 (*n*−9)	20.43±2.77[a]	11.74±0.51[b]	12.23±2.11[b]	11.44±1.43[b]
18:2 (*n*−6)	16.98±0.91[a]	5.86±0.11[b]	5.54±0.21[b]	5.92±0.13[b]
18:3 (*n*−3)	0.17±0.01	0.18±0.01	0.20±0.02	0.22±0.01
20:3 (*n*−6)	0.59±0.06[a]	0.42±0.01[b]	0.45±0.02[b]	0.41±0.02[b]
20:4 (*n*−6)	14.23±1.80[a]	7.32±0.32[b]	7.20±0.40[b]	7.57±0.41[b]
20:5 (*n*−3) EPA	ND	9.82±0.56[a]	7.23±0.23[b]	9.99±0.69[a]
22:5 (*n*−3)	0.20±0.00[a]	1.40±0.08[b]	1.38±0.10[b]	1.49±0.07[b]
22:6 (*n*−3) DHA	7.30±1.05[a]	15.87±1.10[a]	16.12±0.89[a]	16.66±1.01[a]

Means in a row with superscripts without a common letter differ ($P<.05$).
ND=not detected.
Oil A, ICN Menhaden FO; oil B, Omega Pure FO; oil C, Ocean Nutrition FO.
[y] Expressed as percentage of total fatty acids.
[z] Results are expressed as mean±S.E.M. ($n=5$).

Table 4
Effect of different FOs on pro-inflammatory cytokine production in LPS-activated (8 and 24 h) murine resident peritoneal macrophages[y]

Time (h)	Parameters	Groups			
		CO	Oil A	Oil B	Oil C
8	TNF-α	1017.0±75.9[a]	807.6±101.1[a,b]	923.1±64.8[a,b]	706.7±68.9[b]
	IL-6	537.6±63.7	451.5±73.7	516.9±47.5	420.5±52.9
	IL-1β	621.8±53.7	521.5±57.7	616.0±51.4	481.3±50.3
24	TNF-α	937.8±70.9[a]	759.1±89.0[a,b]	909.9±44.3[a]	677.1±65.9[b]
	IL-6	603.1±24.9[a]	503.3±54.7[a,b]	580.9±26.9[a]	459.2±32.3[b]
	IL-1β	633.3±52.5[a]	549.5±56.9[a]	601.5±37.2[a]	474.0±36.9[b]
		Oil	Time	Interaction	
P[z]	TNF-α	<.05	.29	.96	
	IL-6	<.05	.22	.98	
	IL-1β	<.05	.90	.97	

Means in a row with superscripts without a common letter differ (P<.05).
Oil A, ICN Menhaden FO; oil B, Omega Pure FO; oil C, Ocean Nutrition FO.
[y] Results are expressed as mean±S.E.M. (n=6).
[z] From two-way ANOVA for significant main effects.

statistically significant. Oil C, compared to CO, decreased the level of all three pro-inflammatory cytokines at 24 h after LPS stimulation (Table 4). Two-way ANOVA for main effects revealed a statistically significant effect of oil for all cytokines. There was, however, no statistically significant effect of Time and Time×Oil interaction.

3.4. Antioxidant enzymes in kidneys

The three dietary FOs had differential effects on antioxidant enzyme activities. Oils A and C, compared to CO, significantly increased SOD activity, but there was no effect of oil B. CAT activity increased in all dietary FOs compared to CO, but oil C maintained levels higher than those of oils A and B. Although GST activity increased with all dietary FOs, it was only significant for oil C compared to CO. GPX activity was higher in FO diets, but was highest in oil B (Table 5).

3.5. Lipid peroxidation in kidneys

Lipid peroxidation, measured as TBARS, increased in all dietary FO groups compared to CO control. However, oil B had higher LPO than those of oils A and C, which had similar levels (Table 5).

4. Discussion

Our results show that concentrated FOs containing higher levels of EPA and DHA may be far more effective in decreasing pro-inflammatory cytokine production in murine peritoneal macrophages derived from C57BL/6 female mice, compared to regular FOs with low EPA and DHA levels. Our results further show that, although FOs may enhance antioxidant enzyme activities, they may vary depending on EPA/DHA content. The present study suggests that FOs with high EPA and DHA content are likely to act more effectively in preventing inflammatory disorders, such as arthritis, osteoporosis and systemic lupus erythematosus (SLE), than regular FOs. Indeed, in a separate ongoing study, when we fed diets containing 10% oil A, oil B or oil C to autoimmune-prone NZB/W female mice since weanling, mice fed concentrated oil C had a higher median life span compared to mice fed oils A and B and control mice fed CO (unpublished observation).

While the effects of $n-3$ fatty acids or FOs on pro-inflammatory eicosanoids such as PGE_2, TXB_2 and 6-keto-$PGF_1\alpha$ are consistent with most in vivo and in vitro studies showing inhibition [9,16–18], there is, however, considerable variation in their effects on pro-inflammatory cytokines

Table 5
Effect of different FOs on the activity of antioxidant enzymes and LPO in kidney tissues[z]

Parameters	Groups			
	CO	Oil A	Oil B	Oil C
SOD (U/mg protein)	5.96±0.06[a]	7.41±0.08[b]	6.22±0.13[a]	7.45±0.10[b]
CAT (U/mg protein)	2085.0±91.6[a]	2744.0±17.7[b]	2819.0±106.7[b]	3259.0±77.5[c]
GPX (U/mg protein)	0.314±0.000[a]	0.341±0.001[b]	0.348±0.001[c]	0.338±0.001[b]
GST (U/mg protein)	0.225±0.005[a]	0.247±0.007[a,b]	0.245±0.006[a,b]	0.264±0.005[b]
LPO (nmol/mg protein)	168.3±2.7[a]	209.2±1.8[c]	246.9±9.9[b]	213.4±7.1[c]

Means in a row with superscripts without a common letter differ (P<.05).
Oil A, ICN Menhaden FO; oil B, Omega Pure FO; oil C, Ocean Nutrition FO.
[z] Results are expressed as mean±S.E.M. (n=6).

[12,21,46–49]. ICN Menhaden oil (oil A in our study), the most commonly used oil in animal studies, has been shown to either elevate or decrease pro-inflammatory cytokine levels in macrophages [19,21]. However, the studies differed in terms of the amount of oil used and the feeding duration. Our present study suggested that, although oil A may have inhibitory effects on TNF-α, IL-1β and IL-6 production in murine macrophages, the results were not statistically significant. Considerable variation between animal studies may be ascribed to differences in the strains of mice used, time of study, composition of diet and percentage of oil used for the different studies. In humans fed FO, peripheral blood mononuclear cells have regularly shown decreased IL-1β and TNF-α production when stimulated with mitogens [10,12].

FO has been previously shown to enhance the activity of antioxidant enzymes in different tissues. An earlier study from this laboratory showed that FO supplementation significantly increased the activities and mRNA expression of CAT, SOD and GPX enzymes and decreased LPO in autoimmune-prone NZB/W mice [31]. We proposed that one of the mechanisms by which $n-3$ lipids delay the onset of autoimmune diseases in this autoimmune-prone strain of mice may be through the maintenance of higher activity and expression of hepatic antioxidant enzymes. More recently, we showed that FO, compared to CO, significantly increased the activities of SOD, CAT and GPX in kidneys of 4- and 8-month-old lupus-prone B/W mice [32]. Since both studies showed significantly higher life span and lower kidney disease in FO-fed mice, the antioxidant-enzyme-enhancing properties of FO and correspondingly decreased pro-inflammatory cytokines may be involved in the amelioration of this inflammatory disorder. In a mouse model of arthritis-prone MRL/lpr mice, FO increased the activity of antioxidant enzymes in spleen tissues, which correlated with lower arthritis and bone loss, providing further evidence that FO may be beneficial in preventing inflammatory disorders through upregulation of antioxidant enzymes [36]. Omega-3 fatty acids at hypotriglyceridemic doses are known to enhance the activities of the hepatic antioxidant enzymes SOD, CAT, GPX and GST [50]. In fact, FO or $n-3$ fatty acids enhance antioxidant activity in other tissues as well, suggesting that their effects are not tissue-specific [33,34]. In the present study, all FOs selectively enhanced the activities of antioxidant enzymes. However, oil C seemed to be more effective among all the FOs. Interestingly, lipid peroxides were higher in all FOs groups, but were highest in oil B. Lower SOD activity observed in oil B could explain the high superoxide radical generation in this group. Although GPX activity was significantly higher in oil B, this elevation may not be sufficient to prevent hydroxyl radical generation and, subsequently, higher lipid peroxide formation. Higher SOD activity, together with higher CAT activity in oils A and C, suggests a higher conversion of hydrogen peroxide to water and oxygen and, consequently, low generation of lipid peroxides. Increase in lipid peroxides is not necessarily associated with compensatory increase in the activity of antioxidant enzymes. We and others have previously shown a higher activity of antioxidant enzymes and lower lipid peroxides in the same tissues of mice fed FOs [31,33–35].

The fatty acid composition of different tissues reflects the fatty acid composition of dietary lipids. CO-fed mice exhibit higher levels of $n-6$ fatty acids, whereas FOs significantly elevate $n-3$ fatty acids at the expense of $n-6$ fatty acids [35,36,51]. Very low levels of DGLA were detected in the serum of mice fed FOs A and B compared to the serum of mice fed CO, whereas no detectable levels were observed in oil-C-fed mice. DGLA is a downstream product of LA and converts to AA through the activity of enzyme Δ^5-desaturase. A higher level of AA (although not statistically significant) in oil-C-fed mice could indicate a higher activity of this enzyme in these mice. AA levels were lower in oils A and B where DGLA was found to be within detectable limits. EPA level was higher in oils A and C compared to oil B, but no difference was observed in DHA content. A higher EPA content of oils A and C compared to oil B and CO may lead to a higher production of the low-inflammatory PGE_3 through the cyclooxygenase pathway by competition with AA. LA was found to be lowest in oil-C-fed mice, but no difference was observed in AA level between FOs, which suggests that they may not differ in their effects on eicosanoids since AA is the precursor for pro-inflammatory mediators. Based on these observations, the anti-inflammatory activity of oil C may be ascribed only to its effects on pro-inflammatory cytokines. However, this needs to be confirmed with further studies by measuring AA-derived eicosanoids and other inflammatory mediators such as nitric oxide.

In conclusion, our results suggest that FO that is high in EPA/DHA content (oil C) may be more anti-inflammatory compared to regular FOs and CO in C57BL/6 female mice by decreasing the levels of pro-inflammatory cytokines in macrophages. Based on these encouraging results in this mice strain, more studies are urgently needed to establish the role and mechanism of action of concentrated FOs that are high in EPA and/or DHA in the prevention of inflammatory disorders, such as SLE and arthritis, and malignancy.

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