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Short communication

Dietary fish oil and flaxseed oil suppress inflammation and immunity in cats

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ABSTRACT

The modulatory activity of dietary n-3 fatty acids on inflammation and immune response in domestic cats is unknown. Mature female cats ($n = 14$/treatment) were fed control, fish oil or flaxseed oil diets with n-6:n-3 fatty acid ratios of 20:1, 5:1 and 5:1, respectively, for 12 wk. Immune response was assessed on wk 0, 6 and 12, and skin hypersensitivity response on wk 6 and 12. Fish oil increased ($P < 0.01$) eicosapentaenoic and docosahexaenoic acids in plasma and skin, whereas flaxseed oil increased α-linolenic acid. Fish and flaxseed oils decreased ($P < 0.01$) skin inflammatory response to histamine. Cats fed fish but not flaxseed oil had higher ($P < 0.05$) skin leukotriene LTB_5, but not LTB_4. Fish and flaxseed oils lowered B, total T and T_h subset populations, and leukocyte proliferative response to PWM ($P < 0.05$). In contrast, there was no change in ConA- or PHA-induced lymphocyte proliferation, Tc and MHC II cell populations, DTH response, NK cytotoxicity, IL-2 production, or plasma IgG concentrations. Therefore, fish and flaxseed oil can reduce skin inflammatory responses in cats, however, flaxseed oil appears less immunosuppressive than fish oil.

1. Introduction

Dietary n-3 polyunsaturated fatty acids (PUFA) lower inflammation in atopic animals (Reinhart and Scott, 1996). In humans, n-3 PUFA lowered heart disease mortalities (Bang et al., 1980) and blood pressure (Kestin et al., 1990), but also decreased plasma lipids cardiovascular disease risk (Rodriguez-Leyva et al., 2010). Omega-3 PUFA have been shown to be beneficial in other diseases, such as Crohn's disease, ulcerative colitis and rheumatoid arthritis (Rugglero et al., 2009). Fish oil (FO) is a good natural source of n-3 PUFA, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) while flaxseed oil (FSO) is high in α-linolenic acid (ALNA, 18:3n-3). These n-3 PUFA are involved in modulating immune functions and producing eicosanoids, such as leukotrienes (LT) and prostaglandins (Seki et al., 2010).

Dietary n-3 PUFA also modulate immune response; they lower Th cell subpopulation in aged dogs (Hall et al., 1999) and increase Tc cell subpopulations in humans (Meydani et al., 1993). Delayed-type hypersensitivity (DTH, hypersensitivity type IV) response was suppressed in dogs (Wander et al., 1997) and rodents (Yoshino and Ellis, 1987; Taki et al., 1992; Fowler et al., 1993) fed n-3 PUFA. Manipulations of dietary n-3 PUFA have been used in recent years to manage inflammatory skin diseases in dogs. However, the possible immunomodulatory and anti-inflammatory action of n-3 PUFA in cats is unknown. This study evaluates these responses in cats fed fish or flaxseed oil.

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2. Materials and methods

2.1. Animals and diets

Female domestic short hair cats (19–20 mo old; average body weight 4.9 ± 0.7 kg; Liberty Research, Waverly, NY) were randomly assigned to control, FO, or FSO diets (n = 14/treatment). All cats were fed an adaptation diet (20:1 n-6:n-3 PUFA ratio) for a 12 wk washout period prior to feeding experimental diets. Poultry fat was the main dietary lipid in the adaptation and control diets (OSM Table 1). Menhaden fish oil and flaxseed oil were added to the FO and FSO diets, respectively, at a n-6:n-3 PUFA ratio of 5:1, and stored at −20 °C. Fresh food was offered daily, and food intake and body weights recorded weekly. Total dietary lipid in Expts. 1 and 2 was 22 (OSM Table 1) and 14% (OSM Table 2), respectively. Cats were group-housed (4–5 cats/pen) in environment-controlled rooms (20 °C, 14 h light). All procedures were approved by the Washington State University Institutional Animal Care and Use Committee.

2.2. Immune assays

Blood was collected on wk 0, 6, and 12. Whole blood was diluted 1:12 with RPMI-1640 and incubated with 1 or 5 mg/L phytohemagglutinin (PHA), 2 or 10 mg/L concanavalin A (ConA), or 0.1 or 0.5 mg/L pokeweed mitogen (PWM) to assess PBMC proliferative response (Kim et al., 2000), expressed as stimulation index (SI).

Leukocytes isolated from whole blood were resuspended in PBS containing 1% FCS (Kim et al., 2000) and incubated with mAb against CD5, CD4, CD8, MHC II and B cell surface markers (Peter Moore, University of California Davis, CA), or $mIgG_1$ (negative control; Serotec, Raleigh, NC), followed by FITC-conjugated goat anti-mIgG (Caltac laboratories, Burlingame, CA). Changes in leukocyte subpopulations were determined through cell distribution analysis with flow cytometry (FACScan, Becton Dickinson, San Jose, CA).

Skin induration response after an i.d. injection with 100 μL of saline, ConA (0.5 g/L), or vaccine (modified live herpesvirus-1, calcivirus, parvovirus, and *Chlamydia psittaci*; Felocell CVR-C™, Pfizer, Exton, PA) was measured on wk 5 and 11 to assess DTH response (Kim et al., 2000). All cats were previously vaccinated with the same vaccine. Skin thickness was measured at 0, 24, 48 and 72 h after injection and DTH response expressed as percent increase in skin thickness.

In addition to DTH response, skin inflammatory response to an i.d. injection of histamine (histamine phosphate; 0.0275 g/L, Greer, Lenoir, NC) was assessed in Expt. 2. Skin thickness was measured at 0, 10, 20, 30, 45 and 60 min after injection. Maximal skin thickness response post-injection also was recorded.

IL-2 production was measured in diluted whole blood stimulated with ConA (5 mg/L) for 48 h. Supernatants were stored at −80 °C until assay, as previously described (Kim et al., 2000).

Natural killer cell cytotoxicity was assessed in Ficoll-separated PBMC. Crandell feline kidney fibroblast cell (CrFK, ATCC CRL-9761; Crandell et al., 1973) were propagated in DMEM (Sigma–Aldrich, St. Louis, MO) containing 10% FBS, 100 U/mL penicillin, and 100 g/L streptomycin sulfate. Cells were prepared at 2×10^5 cells/mL, 100 μL/well added to 96-well flat bottom plates (Nunclon, Denmark) and incubated at 37 °C for 8 h. PBMC at concentrations of 5×10^6/mL, 2.5×10^6/mL and 1.25×10^6/mL and 100 μL cell suspensions added to the CrFK target cells to provide effector:target cell ratios of 25:1, 12.5:1 and 6.25:1. After an 8 h incubation, supernatant was replaced with 100 μL of a 0.25% Rose Bengal solution (Sigma–Aldrich) to stain live target cells. After 5 min at RT, cells were washed twice with PBS, PBS:75% ethanol (1:1, v/v) added, and OD_{550} measured. Data are presented as percent specific cytotoxicity.

Plasma IgG was measured by single radial immunodiffusion using goat antiserum to feline IgG (Sigma–Aldrich) (Kim et al., 2000).

Plasma α-tocopherol was measured by HPLC using the Waters Alliance 2690 HPLC system equipped with a photodiode array detector (Waters, Milford, MA). Plasma was extracted using isopropanol:dichloromethane (2:1, v/v), and samples injected into a 5 μm reverse phase C18 column (3.9 mm × 150 mm, Resolve C18, Waters, Milford, MA). Mobile phase was 47:47:6 (v/v/v) methanol, acetonitrile, and chloroform. The identity of the α-tocopherol was confirmed by comparing absorption spectra to that of a standard compound.

2.3. Skin biopsy

The flank area of the cats were clipped, wiped with alcohol, and three punch samples (6 mm, Henry Schein, Reno, NV) taken. Samples were immediately frozen on dry ice until analysis for fatty acid content using gas chromatography. In addition, LTB_4 and LTB_5 content were measured by HPLC (Vaughn et al., 1994).

2.4. Statistics

Data were analyzed by repeated measures ANOVA using the General Linear Model of SAS. The statistical model was $Y_{ijk} = \mu + \text{treatment}_i + \text{cat}_j(\text{treatment})$ (error term used to test the effects of treatment) + $\text{period}_k + \text{treatment}_i \times \text{period}_k + e_{ijk}$. Differences among treatment means were compared using protected least squares differences and considered statistically significant at $P < 0.05$.

3. Results and discussion

Body weight (4.9 ± 0.4 kg) and food intake (73.3 ± 3.6 g/d) were similar among treatment groups in both experiments. Dietary fatty acids altered fatty acid composition in both plasma and skin when compared to wk 0, with similar fatty acid composition within treatment groups in Expt. 1 (OSM Tables 3 and 4) and 2 (OSM Tables 5 and 6). FO-fed cat plasma had lower ($P < 0.05$) linoleic acid (25.1 ± 0.2%; LA), γ-linolenic acid (0.51 ± 0.01%; GLNA), and consequently, total n-6 PUFA (33.9 ± 0.3%) on wk 6 compared to control (27.9 ± 0.9% LA; 0.72 ± 0.03% GLNA; 38.7 ± 0.8% n-6 PUFA) plasma eicos-

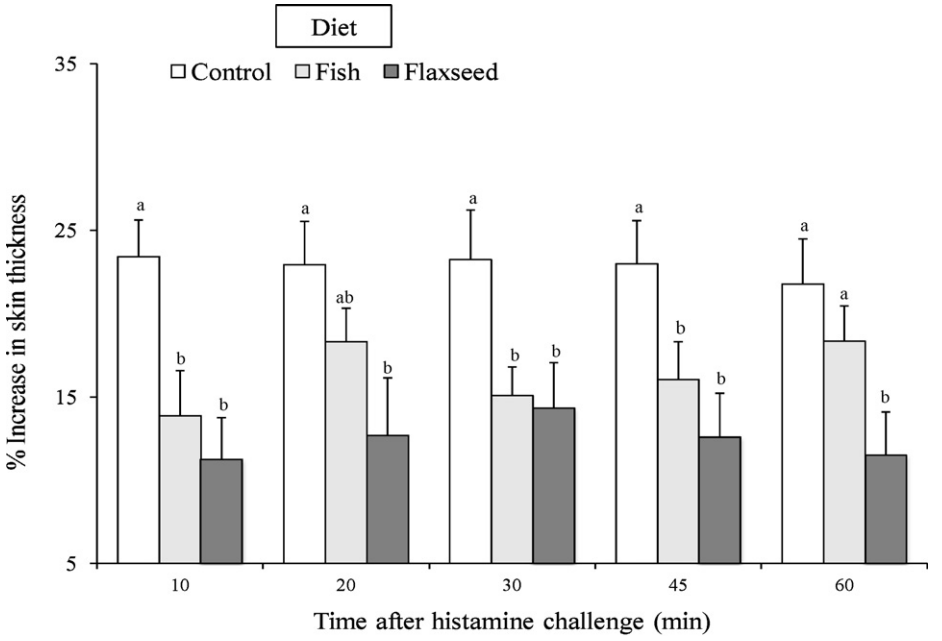

Fig. 1. Concentrations of LTB_4, LTB_5, and LTB_4:LTB_5 ratio in the skin of cats fed the control, fish oil, or flaxseed oil diet for 12 wk (experiment 1). Values are means ± SEM (n = 14 cats/diet) as analyzed by repeated measures ANOVA. Different letters within the same period indicate significant difference, $P < 0.05$.

apentaenoic acid (4.95 ± 0.14%) and docosahexaenoic acid (1.68 ± 0.05%), and therefore total n-3 PUFA (7.67 ± 0.16%) were higher ($P < 0.5$) compared to control (0.12 ± 0.01% EPA; 0.77 ± 0.07% DHA; 1.42 ± 0.07% n-3 PUFA). Plasma arachidonic acid (AA) was not affected by diet, however, ALNA in cats fed FSO (1.6 ± 0.1%) was higher ($P < 0.01$) compared to control (0.30 ± 0.02%). As expected, the plasma n-6:n-3 PUFA ratio was higher in cats fed control (30:1) than FSO (15:1) or FO (4.5:1). Plasma fatty acid composition differences were maintained through wk 12.

Skin fatty acid composition was similar to plasma. Skin LA was lower ($P < 0.05$) in cats fed FO (15.7 ± 0.1%) compared to control (16.2 ± 0.1%), while GLNA and AA did not differ among treatments. ALNA in the skin of cats fed FSO (1.21 ± 0.07%) was twice ($P < 0.05$) that of control (0.67 ± 0.01%) or FO (0.68 ± 0.01%). Cats fed FO had 400–500% higher EPA (0.26 ± 0.03%) and 50% higher DHA (0.37 ± 0.02%) than control (0.04 ± 0.01% EPA; 0.23 ± 0.01% DHA) and FSO (0.05 ± 0.01% EPA; 0.23 ± 0.01% DHA) groups ($P < 0.05$). Total n-3 PUFA was consequently higher ($P < 0.05$) in cats fed FO (1.59 ± 0.06%) and FSO (1.65 ± 0.07%) than control (1.11 ± 0.02%). The n-6:n-3 ratio was highest in cats fed control diet (16:1) compared to FO and FSO (11:1). Even though ALNA increased in the FSO-fed group, the ratio of EPA/DHA in plasma (13.9 ± 0.4 μg/g lipid) and skin (1362 ± 112 μg/g lipid) remained unchanged across the sampling periods.

Although the n-6:n-3 ratio in FO and FSO was similar (5:1), the % n-6:n-3 PUFA in the plasma of cats fed FO reflected that of the diet fed, compared to those fed FSO. This agrees with others who showed that FSO ALNA was less effective than FO EPA/DHA in altering fatty acid composition in rat plasma and tissues (Sanders and Roshanai, 1983; Kelley et al., 1993). The n-6:n-3 ratio in skin of cats fed FO or FSO was similar, suggesting a relatively lower uptake of circulating EPA/DHA by the skin. In contrast, the conversion of ALNA to EPA or DHA was not observed in either plasma or skin (Adam et al., 1986). Feeding ALNA and LA (12 and 4% total energy intake, respectively) for 2 wk showed a five-fold increase in EPA in human plasma, compared to LA alone. Wu et al. (1996) showed significantly higher EPA and DHA when feeding ALNA-enriched diet (3.5–5.3% total energy intake) in non-human primates. Our data suggest that elongation and desaturation enzyme activities for ALNA in the domestic cat may not be as active as in other species.

Skin inflammatory response to histamine decreased during the initial 45 min post-injection by 50% in cats fed FSO ($P < 0.01$), and 20–40% in those fed FO compared to control (Fig. 1). By 60 min post-injection, skin response to histamine in cats fed FSO was still significantly lower than control. Maximal skin thickness response to histamine was 30% lower ($P < 0.05$) in cats fed FO or FSO compared to control.

Diet did not affect skin LTB_4 concentration, although cats fed FO had higher LTB_5 concentrations on wk 12 compared to those fed control or FSO ($P < 0.05$) in both Expt. 1 (Fig. 2) and Expt. 2 (Fig. 3). Consequently, the ratio of LTB_4:LTB_5 in the skin was lowest ($P < 0.05$) in cats fed FO, and was unaffected by FSO. Additionally, FO and FSO n-3 PUFA did not affect NK cell cytotoxic activity in PBMC at any effector:target cell ratio. Specific lysis averaged 23.2 ± 3.9 in Expt. 1 and 22.5 ± 1.4% in Expt. 2 among all cats across all effector:target ratios.

The lower inflammatory response in cats fed FO correlates with the higher production of LTB_5 and unchanged production of LTB_4 in the skin. The n-3 PUFA-derived LTB_5 is 30–100 times less active in stimulating the LTB receptor than the corresponding n-6-derived LTB_4 (Vaughn et al., 1994). Higher concentrations of LTB_5 can competitively inhibit LTB_4-induced neutrophil activation, thereby reduc-

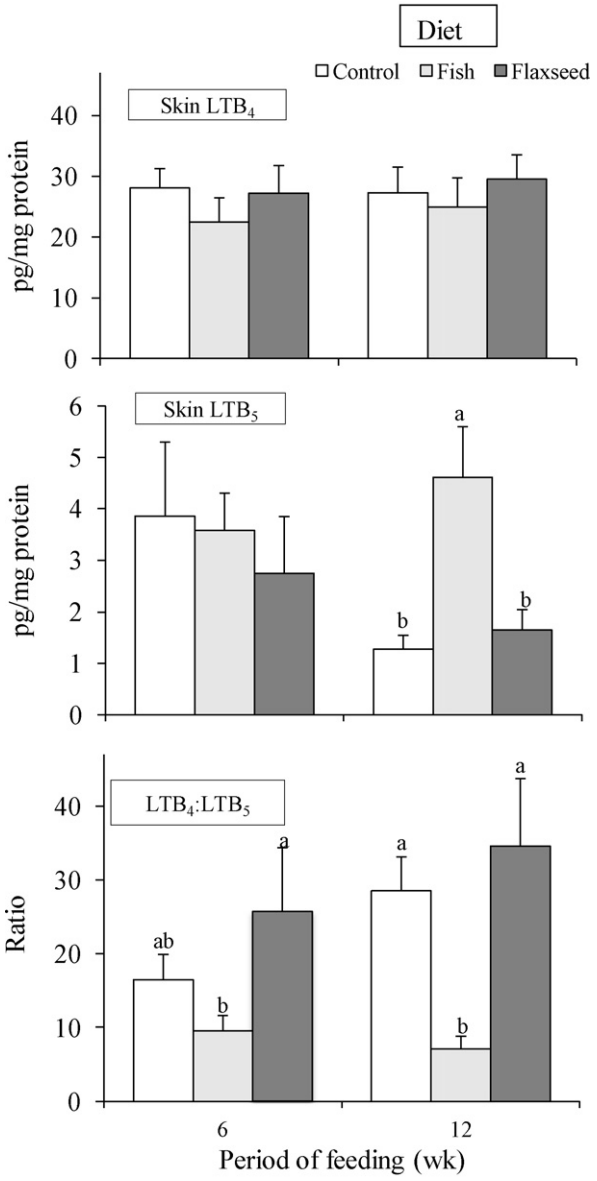

Fig. 2. Hypersensitivity type I skin response to an i.d. injection of histamine in cats fed the control, fish oil or flaxseed oil diet for 11 wk (experiment 2). Values are means ± SEM (n = 14 cats/diet) as analyzed by repeated measures ANOVA. Different letters within the same period indicate significant difference, $P < 0.05$.

ing LTB_4-mediated allergic and inflammatory responses. Dietary FSO did not significantly influence LTB_4 or LTB_5 concentrations in the skin even though FSO and FO were equally active in decreasing inflammatory response, which suggests involvement of other mediators in inflammatory response to histamine injection. No previous studies on the anti-inflammatory activity of n-3 PUFA are available in the cat. Vaughn et al. (1994) reported that dogs fed n-6:n-3 PUFA ratios of 5:1 or 10:1 had lower concentrations of LTB_4 but higher concentrations of LTB_5 in neutrophils and skin compared to pre-feeding levels. A similar increase was not observed in dogs fed higher n-6:n-3 ratios (25:1 to 100:1). Conversely, concentrations of LTB_4 decreased in both neutrophils and skin of dogs fed the higher ratios of n-6:n-3.

Dietary supplementation with FO or purified EPA plus DHA lowered the production of PGE_2, another pro-inflammatory eicosanoid, in rodents (Brouard and Pascaud, 1990) and humans (Meydani et al., 1993). Plant-derived n-3 PUFA had minimal to no effect on PGE_2 production in rodents (Brouard and Pascaud, 1990) but lowered, albeit to a lesser degree than FO, PGE_2 production in non-human primates (Wu et al., 1996). The anti-inflammatory activity of FO in this study is not only similar to that of human, but also mice (Sierra et al., 2008).

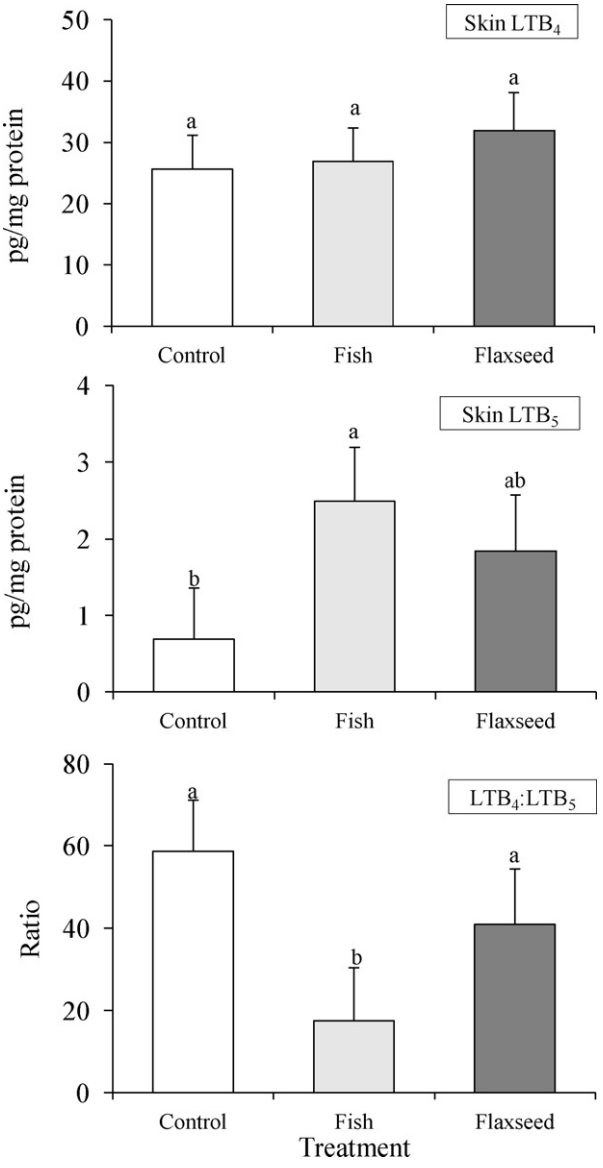

Fig. 3. Concentrations of LTB_4, LTB_5, and LTB_4:LTB_5 ratio in the skin of cats fed the control, fish oil, or flaxseed oil diet for 12 wk (experiment 2). Values are means ± SEM (n = 14 cats/diet) as analyzed by repeated measures ANOVA. Different letters within the same period indicate significant difference, $P < 0.05$.

Table 1
Percentage of peripheral blood leukocyte subsets in cats fed control, fish oil, or flaxseed oil diet for 12 wk (experiment 2).

Subset	Wk	Diet		
		Control	Fish oil	Flaxseed
CD5+ total T cells	0	68.2 ± 19.6	69.3 ± 8.9	66.3 ± 14.0
	6	66.6 ± 25.3	60.5 ± 19.2	66.4 ± 26.6
	12	61.3 ± 8.3[a]	47.8 ± 9.4[b]	56.9 ± 13.7[a]
CD4+ T helper cells	0	22.7 ± 15.9	22.4 ± 10.6	24.0 ± 11.5
	6	24.5 ± 14.3	17.1 ± 8.6	26.3 ± 12.2
	12	21.7 ± 6.8[a]	13.6 ± 7.9[b]	19.1 ± 10.2[a]

Values represent means ± SEM as analyzed by repeated measures ANOVA ($n = 14$). Different letters associated with means within a sampling period denote statistically significance ($P < 0.05$) between dietary treatments.

In Expt. 1, the subpopulations of CD5+ (total T), CD4+ (Th), CD8+ (Tc), and MHC II+ cells were not affected by feeding n-3 PUFA. Percentages across all treatments and periods averaged 61.2 ± 5.5, 23.4 ± 2.2, 10.9 ± 1.1, and 47.3 ± 4.4%, respectively. Conversely, the CD21+ B cell population was lower ($P < 0.05$) on wk 12 in cats fed FO (8.7 ± 1.9%) and FSO (9.3 ± 1.7%) compared to control (14.6 ± 1.7%).

In Expt. 2, on wk 12, cats fed FO had lower ($P < 0.05$) populations of total T and Th cells (Table 1). However, diet did not influence the percent of Tc, MHC II+ and B cells and averaged 26 ± 2, 55 ± 5, and 13 ± 1%, respectively. Production of IgG was also not influenced by feeding FO and FSO and averaged 33.7 ± 3.1 g/L across all treatments and time periods. This agrees with Wander et al. (1997), showing no effects from feeding n-3 PUFA-enriched diets (n-6:n-3 ratios = 5.4:1 or 1.4:1) in dogs. The lower skin response to histamine injection in cats fed the FO and FSO can be explained through these lower subpopulations of B and Th cells, and lower activity of APC with n-3 PUFA as shown by others (Hughes and Pinder, 1997; Dawczynski et al., 2009).

In Expt. 1, FO and FSO decreased ($P < 0.05$) PWM-induced PBMC proliferation on wk 12 but not on wk 6 when compared to control. PWM-induced SI were 47 and 42% lower (0.1 μg/mL PWM), and 78 and 39% lower (0.5 μg/mL PWM), in cats fed FO and FSO, respectively, compared to control cats on wk 12. In contrast, diet did not influence PHA- or ConA-induced PBMC proliferation. The SI for 5 μg/mL PHA and 10 μg/mL ConA averaged 5.3 ± 0.4 and 8.2 ± 0.8, respectively. However, in Expt. 2, dietary FO and FSO also did not significantly PWM-stimulated PBMC proliferation. The SI across all diets and sampling periods averaged 14.7 ± 1.3, 5.7 ± 0.6, and 30.6 ± 9.7 in PBMC stimulated with 10 μg/mL ConA, 5 μg/mL PHA, and 1 μg/mL PWM, respectively. The decreased proliferative response of PBMC to PWM but not to PHA or ConA in Expt. 1 indicates that n-3 PUFA-enriched diets may decrease B, but not T, cell proliferation in cats. In contrast, higher T cell mitogenic responses to dietary n-3 PUFA have been seen in rats (Jeffery et al., 1998), rabbits (Kelley et al., 1988), and non-human primates (Wu et al., 1996). Decreased B cell proliferation in response to PWM may be explained through decreased B, total T, and Th cell numbers in the present study. Decreased APC activity in humans in response to n-3 PUFA (Hughes et al., 1996) may explain this PBMC proliferation response.

FO and FSO did not significantly affect DTH response to ConA or vaccine when compared to control in both the high and low fat studies. Increase in average skin thickness across all diets and periods measured at 48 h post-inoculation with ConA or vaccine was 47 ± 3 and 68 ± 4% in Expt.1 and 59 ± 4 and 74 ± 8% in Expt. 2, respectively. Maximal response to ConA was observed between 24 and 48 h post-injection while maximal response to vaccine was observed at 72 h in all cats. The lack of dietary effects on DTH response is consistent with Th but not MHC class II molecule populations being affected by FO n-3 PUFA but not FSO. Cytokines involved in DTH response include IL-1, IFNγ and TNFα, therefore it is possible that these cytokines, which were not measured, play important roles during the DTH response. Plasma IL-2 production in ConA-stimulated PBMC culture supernatant was not difference at any time period studied, in either Expt. 1 or 2. Concentrations across all treatments and all time periods averaged 12.4 ± 1.4 ng/mL.

The decreased inflammatory response resulting from feeding n-3 PUFA-enriched diets may be of practical importance to atopic individuals (Calder, 1998). Eicosanoids involved in atopic symptoms are mainly derived from n-6 PUFA, especially AA, which can be reduced by feeding n-3 PUFA-enriched diets (Brouard and Pascaud, 1990), thereby lowering the production of AA-derived mediators of inflammation (Volker et al., 2000). However, AA composition in the plasma and skin was not influenced by feeding FO or FSO. Alternatively, the immunosuppressive action of n-3 PUFA may be due to reduced concentrations of α-tocopherol. Rodents (McGuire et al., 1997) and nonhuman primates (Wu et al., 1996) supplemented with n-3 fatty acids lowered blood α-tocopherol concentrations. However, plasma concentrations of α-tocopherol were not significantly influenced by FO or FSO in either Expt. 1 (8.27 ± 0.83 μmol/L) or Expt. 2 (6.1 ± 0.3 μmol/L). FO and FSO appear to mediate anti-inflammatory and immunosuppressive responses through a myriad of mechanisms, including eisocanoid-independent mechanisms that involve intracellular signaling and nuclear transcription (Chapkin et al., 2009).

Moderate suppression of B and T cell function in cats fed diets containing 14 or 20% total lipids with dramatically decreased skin inflammatory response to histamine indicate FO and FSO may be useful in mediating immunosuppression and controlling inflammatory reactions, including allergic reactions, in the domestic cat.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vetimm.2011.02.024.

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