Plasma and skin concentrations of polyunsaturated fatty acids before and after supplementation with n-3 fatty acids in dogs with atopic dermatitis

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Objective—To determine essential fatty acid concentrations in plasma and tissue before and after supplementation with n-3 fatty acids in dogs with atopic dermatitis.

Animals—30 dogs with atopic dermatitis.

Procedure—Dogs received supplemental flaxseed oil (200 mg/kg/d), eicosapentaenoic acid (EPA; 50 mg/kg/d)-docosahexaenoic acid (DHA; 35 mg/kg/d), or mineral oil as a placebo in a double-blind, placebo-controlled, randomized trial. Clinical scores and plasma and cutaneous concentrations of linoleic acid, arachidonic acid, α-linolenic acid (α-LLA), EPA, DHA, prostaglandin E_2, and leukotriene B_4 were determined.

Results—Total plasma concentrations of α-LLA and EPA increased and those of arachidonic acid decreased significantly with administration of EPA-DHA, and concentrations of α-LLA increased with flaxseed oil supplementation; nevertheless, there was no significant change in the concentrations of these fatty acids or eicosanoids in the skin. There was no correlation between clinical scores and plasma or cutaneous concentrations for any of the measured fatty acids or eicosanoids.

Conclusion and Clinical Relevance—Results indicated that at the dose used, neither the concentrations of fatty acids in skin or plasma nor a decrease in the production of inflammatory eicosanoids was a major factor involved in the mechanism of action in dogs with atopy that responded to fatty acid supplementation. (*Am J Vet Res* 2005;66:868–873)

Canine atopic dermatitis is a common disease in small animal practice.[1,2] Management of canine atopic dermatitis is multifaceted and usually combines interventions such as allergen avoidance, allergen-specific immunotherapy, and antimicrobial and anti-inflammatory pharmacotherapy.[1,3,4] Many of these modalities are associated frequently with adverse effects, substantial financial costs, or both. Treatment for atopic dermatitis with essential fatty acids has been the topic of a number of investigations.[5-12] These studies have evaluated clinical variables. Biochemical changes associated with fatty acid supplementation have been studied in clinically normal dogs[13,14] and horses.[15] Recently, Nesbitt et al[16] evaluated the effects of fatty acid supplementation and plasma fatty acid and inflammatory mediator concentrations in dogs with pruritus. The purpose of the study reported here was to determine essential fatty acid concentrations in the skin and plasma of dogs with atopy before and after supplementation with n-3 fatty acids and evaluate whether there was an increase in cutaneous fatty acid concentrations after supplementation and a correlation between clinical improvement and concentrations of fatty acids in plasma or skin.

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Materials and Methods

Thirty privately owned dogs were enrolled in the study during the spring and summer of 2001 and included patients of the dermatology service of the Veterinary Teaching Hospital of Colorado State University and dogs owned by veterinary students and hospital staff. The study protocol was approved by the Animal Care and Use Committee of Colorado State University; informed consent was obtained.

Atopic dermatitis was diagnosed by use of history and physical examination and by ruling out differential diagnoses such as adverse food reactions and scabies with appropriate tests and trials.[1,3,4] Allergens were identified with an ID test. Dogs were fed a variety of diets; however, the recorded type and amount of diet did not change for 8 weeks prior to and during fatty acid supplementation. The total amounts of n-3 and n-6 fatty acids received before and during the trial were calculated for each dog by use of the manufacturer's information, which indicated the fatty acid content of the commercial diet, and by adding the fatty acid content of the administered supplement. No other supplementation was permitted. None of the dogs had received fatty acid supplementation in the 12 months prior to the study.

Dogs were assigned to 1 of 3 groups of 10 dogs each by use of a table of random numbers and simple randomization. Daily, 1 group received flaxseed oil containing 570 mg of **α-linolenic acid** (**α-LLA**; n-3) and 170 mg of **linoleic acid** (**LA**; n-6)/1,000-mg capsule at a dose of 200 mg/kg in 1,000-mg capsules,[a] which equaled 114 mg of α-LLA/kg and 34 mg of LA/kg. A commercial product[b] containing **eicosapentaenoic acid** (**EPA**; 250 mg/capsule, 50 mg/kg) and **docosahexaenoic acid** (**DHA**; 166 mg/capsule, 33 mg/kg) was administered daily to dogs in the second group. The third group received a placebo (200 mg/kg) containing mineral oil. Supplementation was given for 10 weeks. Neither clinicians nor owners were aware of the type of supplementation used in each dog. Clinical scores were obtained from clinicians

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and owners prior to and after supplementation with regard to pruritus and skin lesions, as previously reported.[17] Briefly, clinical scores were obtained by adding the scores for pruritus (obtained with a visual analogue scale from 0 to 30), skin lesions (erythema, papules, or crusts counted separately and graded as mild, moderate, and severe), and medication (topical treatment as 5 points, antihistamines as 10 points, and glucocorticoids as 10 to 40 points [determined by the dose]). The overall maximum clinical score was 117.5 points. At the same time, skin biopsy specimens and blood samples were obtained. After injection of 2% lidocaine as a local anesthetic, 2 skin biopsy specimens from skin on the lateral portion of the thorax were taken with a 6-mm punch. Specimens were stored in cryovials. Blood samples were obtained via jugular venipuncture, and plasma, serum, and skin biopsy specimens were immediately frozen at –80°C until processing. A good response was considered as an improvement of > 50% in clinical scores, and an excellent response was considered complete remission.

Prostaglandin E_2 and leukotriene B_4—Frozen skin specimens were placed on a clean glass slide and quickly cut into small pieces before thawing could occur. Cut pieces were transferred into a 4-mL plastic culture tube, and 2 mL of cold ethyl acetate was added. The specimen was homogenized for 1 minute while the specimen in the culture tube was kept in an ice bath. The specimen and culture tube were removed from the homogenizer, and the homogenizer tip was rinsed with 1 mL of cold ethyl acetate. Rinse medium and the specimen were combined. The culture tube was capped with aluminum foil and set in an ice bath for 30 minutes. After 30 minutes in the ice bath, the tube was capped and centrifuged at 500 × *g* for 10 minutes at 5°C. All supernatant was removed and placed into a 4-mL plastic culture tube. Ethyl acetate was evaporated from the sample with a slow stream of nitrogen gas. A warm water bath (30°C) was used to facilitate drying. The sample residue was resuspended in 500 µL of enzyme immunoassay buffer.[c] The sample was capped with nitrogen gas and stored at –80°C. Samples were analyzed with enzyme immunoassay kits.[c]

Eicosanoid analyses for **leukotriene B_4 (LTB_4)** and **prostaglandin E_2 (PGE_2)** were conducted with commercially available ELISA kits.[c] For PGE_2 analysis, 100 µL of standard or sample, and for LTB_4 analysis, 50 µL of standard or sample was added to a washed plate coated with antibody against the eicosanoid of interest. Fifty microliters of antibody and 50 µL of tracer were added to each standard or sample, and the plate was incubated for 18 hours at room temperature (20°C). After incubation, the plates were washed 5 times and 200 µL of Ellman reagent was pipetted into each well. The plates were placed in the dark on a rotary shaker and allowed to incubate for 60 minutes. The absorbance of each well was read at 405 nm. Eicosanoid concentrations for the samples were calculated for a linear regression by use of the base 10 log of the percentage bound divided by the unbound percentage of each standard.

Polyunsaturated fatty acids in plasma and skin—Plasma samples with EDTA were allowed to thaw and thoroughly mixed. Each sample was analyzed in duplicate. In a 4-mL screw-top vial with a polytef-lined cap,[d] 100 µL of isooctane containing heptadecananoic acid internal standard (2 mg/mL) was added. Under a slow stream of nitrogen gas, the isooctane was evaporated. Two hundred microliters of plasma was measured with a Hamilton gas tight syringe and placed into each vial, followed by 2 mL of a 2:1 mixture of chloroform and methanol. The vial was thoroughly mixed for 1 minute. One milliliter of isotonic saline (0.9% NaCl) solution was then added, and the vial was again mixed for 1 minute and then centrifuged at 2,000 × *g* for 10 minutes.

The resulting solution was composed of an aqueous layer on top, a proteinaceous layer in the middle, and a chloroform layer at the bottom. The aqueous layer was drawn off and discarded. The chloroform layer was subsequently drawn off and placed in a new 15-mL vial with a polytef-lined cap.[d] This chloroform layer was evaporated until dry with a slow stream of nitrogen gas in a 30°C water bath. After the sample was dried, 1 mL of hexane reagent was added to each sample.

Skin samples were weighed and allowed to partially thaw. Each sample was minced on a clean glass slide, placed with 4 mL of PBS solution in a polypropylene test tube, and homogenized for 1 minute. Forty microliters of butylated hydroxytoluene was added, and the solution was frozen at –80°C.

To extract fatty acids from homogenate samples, the samples were allowed to thaw and placed in 50-mL test tubes containing 40 mL of chloroform and methanol (2:1 [vol:vol]). The tube was mixed for 1 minute and filtered through filter paper.[e] To the filtered solution, 1.75 mL of 0.88% KCl was added, and the solution was mixed and centrifuged for 10 minutes at 1,000 × *g*. The top layer was aspirated and discarded, and the chloroform layer was evaporated to dryness in a 37°C water bath under a gentle stream of nitrogen gas. The residue was dissolved in 1 mL of hexane with 100 µg of heptadecanoic acid.

For the methylation procedure for plasma and skin samples, 3 mL of boron trichloride-methanol was added to the hexane in each sample. The vial was capped and placed in a water bath at 60°C for 2 hours. The sample was removed from the water bath and cooled to approximately 22°C, 1 mL of water was added, and the sample was mixed to quench the reaction. The sample was cooled to 4°C for 15 minutes to separate layers. The hexane layer was aspirated and placed in a chromatography vial,[f] and the headspace was flushed with nitrogen gas. Prepared samples were analyzed within 1 week.

Samples were analyzed via gas chromatography[g] with a capillary column,[h] with initial temperature set at 150°C (1 minute), 200°C at 15°C/min, and 250°C (5 minutes) at 2°C/min. Injector and detector temperatures were set at 255°C. Three microliters of each sample was analyzed. Linoleic acid, α-LLA, **arachidonic acid (AA)**, EPA, and docosahexaenoic acid concentrations were determined by first determining the area ratio of each sample (ratio of internal standard to peak of interest) and then comparing results to an external set of standards.[i] Ratios were then matched to corresponding molar concentrations by means of linear regression.

Statistical analyses—Mean ± SD values were calculated for plasma and cutaneous fatty acid concentrations before and after treatment. Data acquired from biochemical analyses of plasma and tissue specimens were evaluated for normality of distribution by use of the Kolmogorov-Smirnov test. Concentrations of fatty acids before and after supplementation were compared with a paired *t* test. Subjective evaluations of pruritus, performed by continuous scale plotting, were quantitated, and the scale distances were evaluated as continuous data for normality of distribution by use of the Kolmogorov-Smirnov test, as were the lesional scores. Normally distributed data were compared over time by use of repeated-measures ANOVA. Group means were compared via post hoc analysis via the Fisher least significant difference test. The Pearson test was used to detect a significant correlation between concentrations of the same fatty acid or eicosanoids in skin and plasma, between individual fatty acids, and between clinical scores and fatty acid concentrations in either skin or plasma. Inter- and intra-assay variability of cutaneous fatty acid determination was calculated by using tissue obtained from the same cutaneous site of a clinically normal dog, dividing it, and analyzing one of those

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Table 1—Mean ± SD concentrations of fatty acids (mg/g) and eicosanoids (pg/g) in the skin of dogs with atopy, before and after treatment with placebo, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), or flaxseed oil (n = 10 dogs/group).

Treatment	LA (mg/g)	α-LLA (mg/g)	AA (mg/g)	EPA (mg/g)	DHA (mg/g)	PGE_2 (pg/g)	LTB_4 (pg/g)
Before placebo	798 ± 266	97 ± 109	344 ± 157	98 ± 98	38 ± 35	31,820 ± 11,879	13,721 ± 10,112
After placebo	764 ± 490	105 ± 117	413 ± 226	140 ± 119	48 ± 36	24,536 ± 9,841	9,324 ± 4,609
Before EPA-DHA	818 ± 225	67 ± 22	305 ± 183	78 ± 89	30 ± 9	24,039 ± 18,292	8,962 ± 7,210
After EPA-DHA	933 ± 843	198 ± 455	398 ± 219	154 ± 304	185 ± 471	30,343 ± 19,627	8,467 ± 5,292
Before flaxseed oil	814 ± 661	204 ± 366	248 ± 66	62 ± 219	234 ± 588	24,525 ± 12,742	7,485 ± 3,693
After flaxseed oil	896 ± 554	115 ± 138	406 ± 438	79 ± 66	58 ± 48	24,820 ± 13,827	9,077 ± 4,804

LA = Linoleic acid. α-LLA = α-Linolenic acid. AA = Arachidonic acid. EPA = Eicosapentaenoic acid. DHA = Docosahexaenoic acid. PGE_2 = Prostaglandin E_2. LTB_4 = Leukotriene B_4.

Table 2—Mean ± SD plasma concentrations (mg/dL) of selected fatty acids in dogs with atopy, before and after treatment with placebo, EPA and DHA, or flaxseed oil (n = 10 dogs/group).

Treatment	LA (mg/dL)	α-LLA (mg/dL)	AA (mg/dL)	EPA (mg/dL)	DHA (mg/dL)
Before placebo	49.6 ± 23.5	4.8 ± 5.6	52.4 ± 23.6	1.3 ± 1.1	3.5 ± 2.5
After placebo	59.4 ± 21.5	13.5 ± 23.1	40.5 ± 19.4	2.3 ± 2.5	4.2 ± 4.4
Before EPA-DHA	56 ± 34.2	2.1 ± 2.6	59.8 ± 24.1	4.3 3 ± 4.5	11.2 ± 9
After EPA-DHA	59.8 ± 21.3	7.9 ± 5.4	39.1 ± 17.1	11.6 ± 8.5	15.3 ± 11.2
Before flaxseed oil	53.3 ± 12.6	3.5 ± 4.1	62.1 ± 23.3	3.1 ± 5	7.5 ± 7.1
After flaxseed oil	59 ± 13.7	6.6 ± 4.3	44.8 ± 18.7	5.5 ± 6.2	6.2 ± 7.4

See Table 1 for key.

samples in duplicate with each assay. The values for LA and AA were determined and results combined to calculate the variability. In addition, all evaluated variables for the dogs that responded completely to therapy were compared with a corresponding number of dogs in the same groups that did not respond to detect possible differences in response. A 2-tailed value of $P = 0.05$ was considered significant.

Results

Clinical scores improved significantly in the dogs supplemented with flaxseed oil and EPA-DHA, but not in the placebo group.[17] Improvement in clinician and owner scores by > 50% was seen in 1 dog in the placebo group and 4 dogs in the group that received flaxseed oil capsules. Five of the dogs that received commercial EPA-DHA capsules improved by more than 50% according to clinicians, and 4 of the 5 dogs improved by more than 50% according to owners. Complete clinical remission with no other treatment except supplementation was seen in 2 dogs that received commercial EPA-DHA capsules and 1 dog given flaxseed oil capsules.

Interassay variability for the skin biopsy specimens was 19%, and intra-assay variability was 10.7%. For plasma concentrations, the interassay coefficient of variability was 14%. The intra-assay coefficient of variability ranged from 0% to 1.6%. Concentrations of individual fatty acids and eicosanoids in the 3 groups before and after treatment were determined **(Tables 1 and 2)**. They were not significantly different at the beginning of the trial. In the placebo group, there was no significant difference between individual fatty acids or eicosanoids in plasma or tissue at the beginning and the end of the trial. The concentrations of most fatty acids and eicosanoids in plasma or tissue were not significantly different before and after supplementation in the other 2 groups. However, there was a significant increase in plasma concentrations of α-LLA ($P = 0.017$) and EPA ($P = 0.004$) and a significant decrease in AA ($P = 0.003$) in the group treated with the commercial preparation. In the flaxseed oil group, the only significant change was an increase in plasma α-LLA($P = 0.023$).

Repeated-measures ANOVA revealed a significant increase after treatment in n-3 intake ($P = 0.03$), n-6 intake ($P = 0.03$), plasma α-LLA ($P = 0.04$), and EPA ($P = 0.001$) and a significant ($P = 0.001$) decrease in AA. A significant difference between group means was detected for the n-6:n-3 ratio and the plasma concentrations of EPA. The n-6:n-3 ratio was significantly different between the group receiving commercial capsules and the placebo group ($P = 0.002$) and between the flaxseed oil and placebo groups ($P = 0.001$). There was a significant ($P = 0.01$) difference in plasma EPA concentrations between the placebo group and the group that received commercial capsules.

There was a positive ($P < 0.001$) correlation between α-LLA and EPA ($r^2 = 0.549$), α-LLA and DHA ($r^2 = 0.832$), and EPA and DHA concentrations ($r^2 = 0.689$) in the skin, but not in the plasma. Skin α-LLA concentrations and n-3 intake were correlated ($r^2 = 0.144$; $P = 0.004$), but not n-3 intake and plasma α-LA-concentrations ($r^2 = 0.02$; $P = 0.9$). In comparison of the dogs in remission after treatment with the corresponding dogs that had no improvement, there were no differences in the concentrations of any of the fatty acids in plasma or skin before treatment or in the concentration increases or decreases after treatment.

Discussion

Polyunsaturated fatty acids can be chemically and physiologically allocated into series on the basis of the location of the double bond nearest to the methyl group at 1 end of the acid. The n-3 series has the first double bond at the third carbon from the methyl group, whereas the n-6 series has the first double bond at the sixth carbon. Although dietary fatty acids are

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subjected to alternating stages of desaturation and elongation, these changes occur only toward the carboxyl end of the molecule and n-3 and n-6 fatty acids are thus never interconverted. Linoleic (n-6) and α-LLA (n-3) cannot be synthesized de novo and are thus considered essential fatty acids.

Polyunsaturated fatty acids have been used in humans with atopic dermatitis[18-21] and allergic asthma[22,23] and modulate the inflammatory response in humans, mice, and rats.[24-28] Orally administered fatty acid supplementation is beneficial for the inflammation and pruritus associated with canine atopy.[5,9,11] These benefits have been related to supplementation with certain n-6 fatty acids (γ-LA),[29] combinations of γ-LA and n-3 fatty acids such as EPA and DHA,[5,11] variable doses of n-3 fatty acids,[9] or n-3 supplemented whole diets with fixed n-6:n-3 ratios.[30] More recently, emphasis has been placed on the use of a higher n-3 fatty acid supplementation dose in the management of canine atopic dermatitis. The EPA-DHA combination supplements administered at approximately 66 mg/kg/d are clinically beneficial to dogs with pruritus.[9] The use of flaxseed oil as a source of potentially beneficial n-3 fatty acids has also been recommended.[31] In our study, supplementation with a commercial product containing EPA and DHA resulted in increased plasma concentrations of α-LLA and EPA and decreased plasma concentrations of AA, indicating that patients indeed received and absorbed the supplemented fatty acids. Similar findings were reported in other studies[14,31] that used flaxseed as n-3 supplementation. The decrease in AA concentration is most likely attributable to competitive inhibition of n-6 elongation and desaturation by higher n-3 concentrations, leading to lesser AA synthesis. Although there was a numerical increase of α-LLA and EPA and a decrease of AA in the plasma of dogs that received flaxseed oil in our study, these changes were less pronounced. Compared with previous reports,[14,31] dogs received less flaxseed oil in our study, which may explain the lack of significance in plasma fatty acid changes in the group that received flaxseed oil. There was a significant increase in plasma EPA concentrations of the dogs that received a commercial preparation containing EPA and DHA, compared with the placebo group. Although mean plasma EPA concentrations in the group that received flaxseed oil were higher than those in the placebo group, this difference did not reach significance, possibly because of the lower dose of flaxseed oil, compared with that in other reports.[31,32] Other possible reasons for the absence of significance may have been the small number of dogs per group and the high variability of the results. In a more recent study,[16] dogs received 4 diets containing different concentrations of fatty acids and different fatty acid ratios. Plasma concentrations of all measured n-3 fatty acids (EPA, DPA, and DHA) also reportedly increased significantly, whereas those of n-6 fatty acids (LA and AA) decreased.

Proposed mechanisms of fatty acid action include modulation of eicosanoid production[9,33] and an increase in epidermal barrier function.[13,14] Eicosapentaenoic acid (n-3) and dihomogamma-LA (n-6) both compete with AA (n-6) as substrate for cyclooxygenase and 5-lipoxygenase, which convert these fatty acids to PGs and LTs, respectively.[34] Proinflammatory PGs such as PGE_2 and $PGF_{2\alpha}$ and LTs such as LTB_4 are derived from AA. Eicosanoids derived from dihomogamma-LA and EPA have less inflammatory or anti-inflammatory action.[35,36] Increases in anti-inflammatory eicosanoids and concurrent decreases in their proinflammatory counterparts presumably decrease cutaneous inflammation. Decreased concentrations of cutaneous LTB_4 and decreased production of LTB_4 by activated neutrophils have been reported in dogs after they received diets with lower n-6:n-3 ratios and diets higher in n-3 fatty acids, respectively.[37,38] Serum PGE_2 concentrations decreased significantly in a recent study[16] after feeding a diet containing total n-3 fatty acids higher than the group supplemented with the commercial product containing EPA-DHA in our study. However, the clinical relevance of these findings and the role of eicosanoids in pruritus observed in canine atopic dermatitis are controversial. In a recent report[39] that reviewed pharmacotherapy of this disease, evidence against the use of LT synthesis inhibitors was presented. In our study, neither cutaneous concentrations of PGE_2 nor of LTB_4 decreased significantly in dogs that responded to fatty acid supplementation. Thus, PGE_2 and LTB_4 may not play a major role in the pathogenesis of canine atopic dermatitis and the associated cutaneous inflammation and pruritus. However, there was a numerical decrease in the cutaneous concentration of PGE_2 in all groups and that of LTB_4 in the placebo group. In particular, the decrease in tissue eicosanoid concentration was puzzling, and further studies are needed to evaluate the effect of fatty acid supplementation on eicosanoid concentrations in atopic skin.

There was an increase in cutaneous concentrations of α-LLA, EPA, and DHA in dogs that received the commercial product containing EPA and DHA. This increase did not reach significance. It is unlikely that these changes were clinically important because they were seen in dogs that responded to fatty acid supplementation as well as in dogs that did not respond to treatment. The strong correlation among α-LLA, EPA, and DHA in the skin, but not in the plasma, may be attributable to the fact that cutaneous EPA and DHA may be at least partly derived from local α-LLA. Alternatively, tissue concentrations may represent a better time-averaged measure of intake than plasma concentrations that have a shorter half-life and may rely equally as much on intake as on tissue elution with time. Concentrations of EPA and DHA in plasma may more directly reflect dietary intake than actual elongation and desaturation of α-LLA. Although an increase in plasma α-LLA was seen in dogs that received n-3 fatty acids, there was no direct correlation between n-3 intake and plasma α-LLA. This may reflect the fact that absorption of fatty acids from the gastrointestinal tract varies from dog to dog and may be dependent on genetic differences, dietary intake of fatty acids, and other factors. Furthermore, plasma α-LLA may be converted, accumulate, or both in tissues. Why, in contrast, the cutaneous concentrations of α-LLA correlated well with intake was unknown. One possibility may

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be that this fatty acid is incorporated preferentially in activated inflammatory cells that accumulate in the skin of dogs with atopic dermatitis and form a substantial source of α-LLA but may not be numerous enough in the blood to alter the concentration significantly.

There was no significant correlation between clinical scores of owners or clinicians and any of the measured fatty acids in plasma or skin. There was no correlation between n-3 intake, n-6 intake, or n-6:n-3 ratio and clinical scores when data from these dogs were recently evaluated.[17] This lack of correlation between fatty acid intake and concentrations of various fatty acids in the plasma and skin and clinical signs indicates that factors other than, or in addition to, fatty acid concentration or ratio in food, plasma, or skin may contribute to the clinical responses seen in some dogs with atopic dermatitis given fatty acid supplementation.

Recently, another possible mechanism of action of fatty acid supplementation has received much attention. A decrease in antigen-presenting function of splenocytes was reported in mice that received n-3 fatty acids.[40] This is most likely caused by the decrease in major histocompatibility complex class II expression reported in humans and mice after n-3 supplementation.[41-43] Lymphocyte proliferation also decreased after such supplementation.[44,45] The underlying mechanism involves both decreased **interleukin (IL)**-2 expression[46] and reduced IL-2 receptor expression.[47] Decreased expression of cell-surface molecules, such as intercellular adhesion molecule 1, leukocyte function-associated antigen 1, and cluster of differentiation 2, which are important for adhesion of lymphocytes to endothelial cells,[48] and decreased production of monocyte-derived cytokines, which are important for chemotaxis of lymphocytes such as tumor necrosis factor and IL-1,[49,50] have been reported. The mechanism of human atopic disease involves presentation of allergens to T cells by antigen-presenting cells and subsequent activation of allergen-specific T cells and cytokine release. Decrease in antigen-presenting function and T-cell activation and exocytosis into the skin caused by n-3 fatty acid supplementation could presumably be at least partly responsible for the clinical efficacy of these supplements in canine atopic dermatitis, although no studies have been published evaluating immunologic variables following fatty acid supplementation in the dog. In the dogs of this study, cutaneous fatty acid concentrations did not increase significantly after supplementation with n-3 fatty acids. However, it should be noted that a recent study[31] revealed rapid incorporation of n-3 fatty acids in plasma phospholipids and triglycerides at the expense of AA in dogs. An increased proportion of n-3 fatty acids in phospholipids of peripheral blood mononuclear cells may be at least partially responsible for immunomodulation and clinical improvement.

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a. Flaxseed oil capsules, Traco Labs, Champaign, Ill.
b. 3V Caps, DVM Pharmaceuticals, Miami, Fla.
c. Caymen, Ann Arbor, Mich.
d. Supelco, Bellefonte, Pa.
e. Schleicher & Schuell filter paper, Aldrich, Milwaukee, Wis.
f. Agilent, San Fernando, Calif.
g. 5890 gas chromatograph, Hewlett Packard, San Fernando, Calif.
h. HP innowax 19091N-213 capillary column, Agilent, San Fernando, Calif.
i. Sigma Chemical Co, St Louis, Mo.

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