Effects of Supplementation with a Docosahexaenoic Acid-Enriched Salmon Oil on Total Plasma and Plasma Phospholipid Fatty Acid Composition in the Cat

Charles R. Filburn, PhD*
David Griffin, MS, DVM†

*Research and Development Laboratory
†Division of Veterinary Medicine
Nutramax Laboratories, Inc.
Edgewood, MD

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ABSTRACT

Cats lack an adequate liver capacity to synthesize the longer-chain omega (n-6 and n-3) polyunsaturated fatty acids (LCPUFAs) that, upon release into the blood, are incorporated into membrane phospholipids in many tissues and serve essential structural and signaling functions. The necessity and importance of a dietary source of the n-3 LCPUFA docosahexaenoic acid (DHA) prompted an evaluation of the effects of supplementation of a typical cat diet with a DHA-enriched salmon oil on the n-3 LCPUFAs in plasma and the relative levels of n-6 and n-3 LCPUFAs. Supplementation resulted in rapid increases in both DHA and eicosapentaenoic acid (EPA) in total plasma fatty acids and in plasma phospholipids. These changes produced shifts in the overall n-6:n-3 ratio present in plasma fatty acids while markedly shifting the relative levels of arachidonic acid (AA) to DHA (AA:DHA) and arachidonic acid to EPA (AA:EPA) ratios to substantially lower values and a more favorable balance. The importance of providing DHA and EPA to the cat is reviewed and the potential benefits are discussed.

INTRODUCTION

Mammals have a dietary requirement for both n-6 and n-3 polyunsaturated fatty acids (PUFAs) due to an inability to synthesize the n-6 PUFA linoleic acid (LA) and the n-3 PUFA -linolenic acid (ALA). In cats and dogs these PUFAs are important for skin and normal haircoat luster, especially LA, which is a component of lipids needed for normal water permeability and skin texture.[1,2] In addition, they also serve as precursors for the synthesis of longer-chain, more unsaturated fatty acids (LCPUFAs) that may start in the liver but end in organs with high LCPUFA levels.[3,4] In a process of desaturation and elongation, LA (designated 18:2n-6 for its 18-carbon length and 2 double bonds beginning at the 6th carbon) is converted to the n-6 LCPUFA arachidonic acid (AA; 20:4n-6) and ALA to the n-3 LCPUFAs

EPA (20:5n-3) and DHA (22:6n-3). DHA is normally present at higher levels in the brain and retina, where it is critical to normal function.[5–7] AA is also present at relatively high levels in the brain, but is also present in many other organs and tissues.

Synthesis of AA begins in the liver with addition of a double bond to LA by a -6 desaturase, which can also desaturate ALA in the first of several steps leading to synthesis of DHA. Since dietary intake of LA frequently far exceeds that of ALA, synthesis of AA in many mammalian species is adequate to meet the requirements of many tissues. However, the cat is especially inefficient in synthesis of AA and requires dietary AA for support of reproduction and optimal health.[8,9] Feline desaturation of ALA is even less efficient, even with substantial amounts of ALA added to the diet,[4] in part due to competition by LA for the desaturase. The -6 desaturase acts a second time, catalyzing addition of the sixth double bond in synthesis of DHA, which is absent in the cat liver.[4] In the cat a key aspect of the inefficient conversion of precursors to AA, EPA, and DHA is the very low level of the -6 desaturase in the liver. Use of a very sensitive technique that follows utilization of deuterium-labeled LA and ALA allowed the demonstration that a low level of -6 desaturase is present in the liver. LA was converted as a low rate to 18:3n-6 and other elongated metabolites, including AA up to 22:4n-6 (docosatetraenoic acid, DTA). ALA was also metabolized at a low rate, but only up to 22:5n-3 (docosapentaenoic acid, n-3 DPA), which appeared in the plasma.[4] In contrast to the liver, the brain was shown to accumulate the deuteurium-labeled, longer chain, more unsaturated 22:5n-6 (n-6 DPA) and 22:6n-3 (DHA), respectively.[4] Thus, while the liver produces at least some AA, it produces a limited amount of longer-chain transport forms that may be converted to n-6 DPA or DHA in certain tissues, such as the brain and retina, that appear to have sufficient desaturase activity to meet the minimal requirements for DHA.

Although AA is considered essential for normal reproduction,[9,10] less is known about the essentiality of DHA. Studies with the cat and other species suggest that the cat may be in a state of DHA deficiency, especially during early development. Upon developing a state of DHA deficiency, rats exhibit learning and memory deficits, which can be reversed with supplementation with DHA.[11] Infants that are breast-fed or have DHA added to their formulas show improved visual acuity and more rapid neurological development than unsupplemented, non-breast-fed controls.[12] Even puppies exhibit enhanced trainability when supplemented with DHA.[13] Maternal provision of DHA and AA occurs during both gestation and lactation, but the levels of DHA supplied during rapid neuronal development may be less than optimal depending on the maternal diet. The levels of DHA observed in juvenile cat brains were substantially lower than the level found in adult cats.[14,15]

In rats a state of DHA deficiency results in replacement of DHA in brain phospholipids with n-6 DPA, with the n-6 DPA:DHA ratio indicative of the degree of deficiency.[16] This substitution of n-6 DPA for DHA, especially in phosphatidylserine, is associated with behavioral deficits that can be reversed with DHA supplementation,[16–19] indicating that n-6 DPA is unable to fulfill the roles that depend upon the unique biochemical characteristics of DHA.[20] In the brains and retina of juvenile cats maintained on an LA-rich, ALA-poor diet the n-6 DPA level actually exceeds DHA, with an n-6 DPA:DHA ratio up to 3.4 compared with 0.32 when DHA is supplemented.[15] Even this low ratio (0.32) in supplemented juvenile cats is higher than the ratio observed in the brain of adult cats[14] and the very low ratio of rats, which have a higher liver capacity for synthesis of DHA and normally very low levels of brain n-6 DPA.[16] Therefore, the n-6 DPA:DHA ratio may exhibit species differences, with cats having a comparatively high value that is clearly diet dependent. In the cat DHA may

be conditionally essential, especially during neuronal development when it is unlikely that optimal levels are present in the absence of supplementation.

The importance of DHA and EPA to the cat may extend to many other aspects of normal physiology and pathophysiology during all stages of the life cycle. Both n-6 and n-3 LCPUFAs are incorporated into membrane phospholipids where they play fundamental but different roles in regulating membrane properties and signal transduction from membrane receptors. Upon release from membranes by phospholipases, AA and EPA may be converted to prostaglandins, leukotrienes, or thromboxanes, with AA-derived eicosanoids having more inflammatory activity than the EPA-derived metabolites.[3] DHA serves as a critical component of phospholipids involved in membrane receptor signaling by receptors different from those linked to AA release. DHA increases membrane fluidity important to many membrane functions, affects the localization and activities of membrane-associated receptors and enzymes, and may also be released by specific phospholipases.[7,20] As a free fatty acid DHA may be metabolized to recently discovered "docosanoids" or bind to cytoplasmic receptors and regulate gene expression.[21-24]

In view of these various functional activities of DHA and the fact that many mammals consume diets that are n-6 rich but n-3 poor, utilize ALA poorly even when it is present in the diet, and usually have a low dietary intake of n-3 LCPUFAs, it should not be surprising that a growing list of pathological conditions may benefit from DHA supplementation. These range from age-associated cognitive impairment[25-28] to cardiovascular[29] and renal conditions,[30] skin disorders,[31] skeletal abnormalities,[32] and diabetes and obesity,[33] with many of these conditions having an inflammatory component. Compared to dogs, rodents, and humans, there are only a limited number of studies of the effects of n-3 LCPUFA supplementation in the cat, including skin conditions,[34,35] the immune system,[36] platelet function[37] and insulin resistance in obese cats.[33] A case-control study showing a significantly higher AA intake in cats with chronic renal failure than controls[38] suggests that feline renal failure may also benefit from n-3 LCPUFAs as seen in dogs.[30] Because of the potential benefits of providing cats with a DHA-enriched supplement, a study was conducted in cats over a 4-week period on the effects of a DHA-enriched salmon oil on the fatty acid profiles of plasma total fatty acids as well as plasma phospholipids. Plasma phospholipid fatty acid levels reflect dietary LCPUFA and liver fatty acid and phospholipid metabolism, while also serving as an index of the potential for affecting phospholipids in various organs. The AA:DHA and AA:EPA ratios, which have come to be recognized as more physiologically relevant than overall n-6:n-3 ratios,[39,40] were also determined as indices of changes that more closely relate to the effects of n-3 LCPUFAs.

MATERIALS AND METHODS

The protocol was reviewed and approved prior to study initiation by an Institutional Animal Care and Use Committee (IACUC) and complied with the Animal Welfare Act. Amendments were reviewed and approved by the IACUC chairperson prior to their initiation.

Cats were fed Purina Cat Chow Complete Formula, which contains 31.5% crude protein, 11% crude fat, a minimum of 1.25% LA and minimum 0.02% AA, plus 35 IU/kg vitamin E. Cats weighing between 2.4 and 7.1 kg (mean 4.9 kg) were orally administered a daily dose of 1.5 mL of DHA-enriched salmon oil (Welactin, Nutramax Laboratories, Inc., Edgewood, MD, USA) at feeding, resulting in 180 mg DHA and 117 mg EPA/animal.

A complete blood count (CBC) and serum chemistry panel were run at baseline and day 28 (N = 16). Blood was collected prior to daily supplement administration. Blood samples were used for measurement of fatty acid levels in plasma and plasma

Table 1. Effect of Supplementation with Docosahexaenoic Acid (DHA)/Eicosapentaenoic Acid (EPA) on Cat Plasma Lipid Profiles

	Plasma Total Fatty Acids (% total fatty acids)		Plasma Phospholipids (% total fatty acids)	
	Day 0	Day 28	Day 0	Day 28
Fatty Acid				
LA*	29.10 ± 0.47	25.20 ± 0.51†	21.36 ± 0.23	15.76 ± 0.46†
ALA	0.39 ± 0.02	0.32 ± 0.02†	0.20 ± 0.01	0.16 ± 0.01†
AA	8.34 ± 0.19	8.70 ± 0.14	9.15 ± 0.45	9.55 ± 0.44
EPA	0.86 ± 0.06	4.77 ± 0.31†	2.75 ± 0.19	4.71 ± 0.40†
DPA	0.37 ± 0.03	0.72 ± 0.05†	0.51 ± 0.05	1.04 ± 0.06†
DHA	0.90 ± 0.11	3.32 ± 0.31†	1.40 ± 0.20	4.73 ± 0.42†
Total n-6	39.49 ± 0.39	35.40 ± 0.39†	32.90 ± 0.58	27.13 ± 0.63†
Total n-3	2.54 ± 0.17	9.15 ± 0.39†	4.90 ± 0.37	10.66 ± 0.50†
n-6/n-3	15.96 ± 0.92	9.98 ± 0.67†	6.93 ± 0.40	2.59 ± 0.13
AA/DHA	10.04 ± 0.98	2.74 ± 0.19†	7.23 ± 0.77	2.10 ± 0.16†
AA/EPA	9.98 ± 0.67	1.88 ± 0.13†	3.41 ± 0.23	2.10 ± 0.14†

*LA indicates linoleic acid; ALA, -linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.
†Significantly different from basal value at day 0, $P < 0.05$.

phospholipids for cats at days 0, 7, 21, and 28 of the study (N = 8). Blood used for fatty acid analysis was collected in a lavender top (EDTA) tube. All samples for fatty acid analysis were sent via courier for overnight delivery to Peroxisomal Diseases Laboratory, Kennedy Krieger Institute, Baltimore, MD, USA.

The plasma was separated from red blood cells (RBCs) and the samples stored at -80°C until analysis. A 0.5-mL aliquot of plasma or packed RBCs was Folch extracted[41] and the lipids redissolved in hexane:benzene 6:4. The phospholipids were isolated using a silicic acid column, 1.2 cm inside diameter by 3 cm height, by the method of Vance and Sweely.[42] After addition of appropriate standards and methanolysis, the total fatty acid or phospholipid samples were analyzed using capillary gas chromatography-electron capture negative-ion mass spectrometry (GC/MS). A 100-μL aliquot of plasma was taken for direct measurement of the total lipid fatty acids by GC/MS. The individual fatty acids were calculated as percentage of total fatty acids identified in each analysis.

Fatty acids were analyzed using a repeated measures analysis of variance (ANOVA) to evaluate changes in fatty acids over time at an alpha of 0.05. Hematology and serum chemistry results were analyzed using an ANOVA to evaluate any changes between day 0 and day 28 at an alpha of 0.05.

RESULTS

Prior to supplementation the plasma PUFA profile was predominated by the n-6 PUFAs, with a 16-fold excess over n-3 PUFAs in total fatty acids and a 7-fold excess in phospholipids (Table 1). LA accounted for most of the n-6 content, with 21% in phospholipids and 29% in total fatty acids, while AA was present at 8%–9% in both. This value for AA is higher than has been reported in other studies,[4,14,15,34] perhaps due to the AA included in the diet. In contrast, ALA was present at the lowest level of any of the n-3 fatty acids, all of which were present at less than 1% in the total fatty acid pool. Higher levels were found in plasma phospholipids, especially for EPA (2.8%), which was 2-fold greater than DHA (1.4%) and opposite to the pattern typically seen in human and rodent plasma.[16,26]

Following supplementation for 28 days with the DHA-enriched salmon oil both EPA and DHA increased by 5.5-fold and 3.7-fold, respectively, in total fatty acids while DPA increased 2-fold (Table 1). In plasma phos-

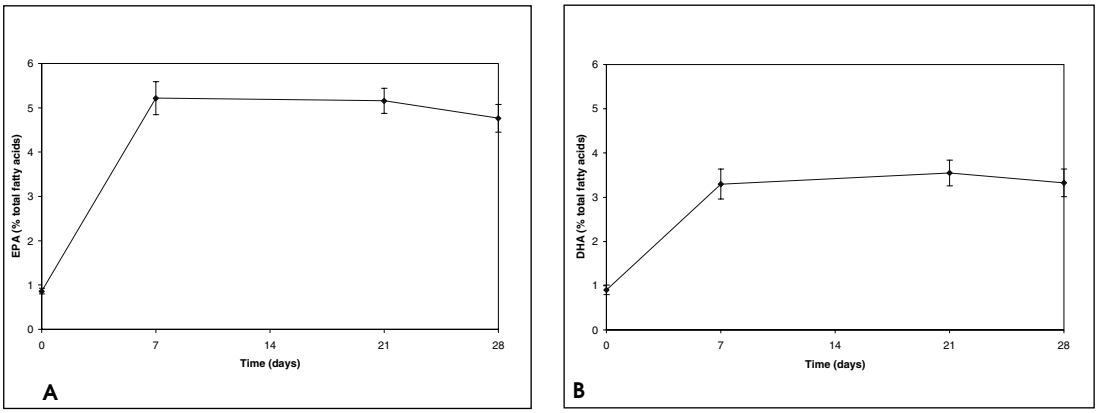

Figure 1. Effect of docosahexaenoic acid (DHA)-enriched salmon oil supplementation on eicosapentaenoic acid (EPA) and DHA levels in plasma total fatty acids. Panel A: EPA; panel B, DHA. Values are means ± standard error. N = 8.

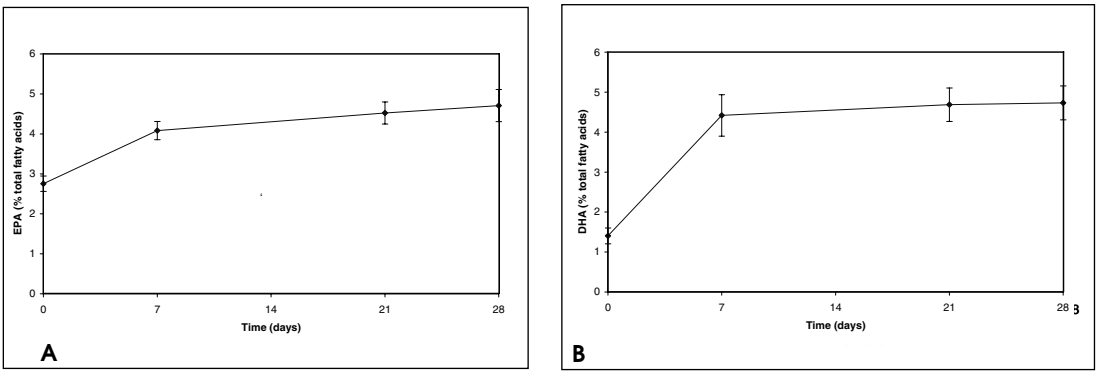

Figure 2. Effect of docosahexaenoic acid (DHA)-enriched salmon oil supplementation on eicosapentaenoic acid (EPA) and DHA levels in plasma phospholipids. Panel A: EPA; panel B, DHA. Values are means ± standard error, N = 8.

pholipids EPA increased 70% and DHA by 3.4-fold from their basal levels to the same 4.7%; DPA again increased 2-fold from its low basal level to 1.0% of the fatty acids present in plasma phospholipids. These increases in total fatty acids and plasma phospholipids occurred rapidly, with near maximal values observed by the seventh day, followed by a gradual rise to slightly higher values through 28 days (Figures 1 and 2). As these n-3 LCPUFAs were increasing, LA decreased by 13%–18%, while no change was observed in AA (Table 1). The net result of these shifts was a reduction in the overall n-6:n-3 ratio by 75% in the total fatty acid pool and 64% in the phospholipid pool. The key AA:DHA ratio decreased over 70% in both pools, while the AA:EPA ratio decreased by 79% in total fatty acids and 38% in phospholipids.

Supplementation with salmon oil produced small, statistically significant changes in 7 parameters of the CBC/serum chemistry (N = 16). On the CBC there was a slight decrease in mean cell hemoglobin concentration (MCHC) and a slight increase in platelet count. On the serum chemistry, decreases in urea nitrogen, calcium, sodium, chloride, and urea nitrogen/creatinine ratio were observed. None of these values were clinically significant, as they remained within the normal laboratory references ranges.

DISCUSSION

The present study shows that supplementation with a DHA-enriched salmon oil elicits a rapid shift in the n-6:n-3 balance of LCPUFAs in cat plasma, reflected in both total fatty acids and phospholipids. This shift in the n-6:n-3 ratio resulted from sub-

stantial increases in both DHA and EPA, especially in the plasma phospholipids. While the overall n-6:n-3 ratio may reflect the degree of LCPUFA balance, recent studies have emphasized the more physiologically meaningful AA:DHA and AA:EPA ratios.[39,40] The shorter-chain precursors and intermediates in synthesis may contribute substantially to an overall n-6:n-3 ratio but do not serve the unique roles fulfilled by DHA, AA, and EPA. Both of these ratios decreased substantially upon supplementation with a DHA-enriched salmon oil.

Supplementation studies of PUFAs or n-3 LCPUFAs in cats have examined antithrombotic effects,[34,37] cellular responses involved in immunity and inflammation,[36] dermatitis,[35] insulin sensitivity,[33] and conversion of LA and ALA to AA and DHA.[4,15,33] Some of these studies do not adequately describe the dietary intake of the n-3 fatty acids to allow comparisons of blood levels of n-3 LCPUFAs. In one carefully documented study, much higher doses of EPA and DHA (1.1–1.7 g/d and 0.6–0.9 g/d, respectively) than used in the current study (0.12 g/d and 0.19 g/d, respectively) were shown to elicit changes in plasma fatty acids comparable to those observed here without major effects on platelet aggregation or bleeding time.[34] In another study with an EPA-enriched fish oil added to achieve a dietary content of 0.35% EPA and 0.15% DHA, no clinically significant effects were observed on a large number of immune parameters, while final plasma EPA reached levels comparable to those shown here.[36] No clinically significant differences in a large number of serum chemistry and blood cell parameters were observed in the current study, further supporting the safety of supplementation with a DHA-enriched salmon oil.

Much remains to be learned about the actual benefits of providing cats with n-3 LCPUFAs via supplementation. Some improvement in long-term glucose control, with a decrease in plasma insulin concentration, was observed when cats fed ad libitum became obese while consuming a diet enriched in DHA and EPA.[33] Effects on skin conditions may occur but need further study, with more clearly defined intakes than was previously described.[35] A much larger number of canine studies on n-3 LCPUFAs have shown benefits involving the cardiovascular system, renal function, skin conditions, as well as neuronal development. While it does not appear to be as deficient in -6 desaturase activity as the cat, the dog's limited ability to utilize ALA to synthesize DHA and EPA accounts for the various positive effects that have been shown with supplementation of n-3 LCPUFAs. Consequently it is likely that the cat may also respond in a similar manner. Further studies are needed, particularly on kittens and on aging cats, where the need for DHA is likely to be the greatest.

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