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Fish oil supplementation maintains adequate plasma arachidonate in cats, but similar amounts of vegetable oils lead to dietary arachidonate deficiency from nutrient dilution

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ABSTRACT

Because fatty acid (FA) metabolism of cats is unique, effects of dietary fish and vegetable oil supplementation on plasma lipids, lipoproteins, lecithin/cholesterol acyl transferase activities, and plasma phospholipid and esterified cholesterol (EC) FAs were investigated. Cats were fed a commercial diet supplemented with 8 g oil/100 g diet for 4 weeks using either high-oleic-acid sunflower oil (diet H), Menhaden fish oil (diet M), or safflower oil (diet S). When supplemented, diet M contained sufficient arachidonate (AA), but diets H and S were deficient. We hypothesized that diet M would modify plasma lipid metabolism, increase FA long-chain n-3 (LCn-3) FA content but not deplete AA levels. Also, diet S would show linoleic acid (LA) accumulation without conversion to AA, and both vegetable oil supplements would dilute dietary AA content when fed to meet cats' energy needs. Plasma samples on weeks 0, 2, and 4 showed no alterations in total cholesterol or nonesterified FA concentrations. Unesterified cholesterol decreased and EC increased in all groups, whereas lecithin/cholesterol acyl transferase activities were unchanged. Diet M showed significant triacylglycerol lowering and decreased pre–β-lipoprotein cholesterol. Plasma phospholipid FA profiles revealed significant enrichment of 18:1n-9 with diet H, LA and 20:2n-6 with diet S, and FA LCn-3FA with diet M. Depletion of AA was observed with diets H and S but not with diet M. Diet M EC FA profiles revealed specificities for LA and 20:5n-3 but not 22:5n-3 or 22:6n-3. Oversupplementation of some commercial diets with vegetable oils causes AA depletion in young cats due to dietary dilution. Findings are consistent with the current recommendations for at least 0.2 g AA/kg diet and that fish oil supplements provide both preformed LCn-3 polyunsaturated FA and AA.

1. Introduction

Fatty acid (FA) metabolism of cats is unique among mammalian species. Rivers et al [1] concluded that cats have extremely low or nonexistent Δ6 desaturase activity after observing essential FA deficiency signs while maintaining them for approximately 15 months on diets containing only linoleic acid (LA) as the predominant n-6 FA. Consequently,

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexanoic acid; EC, esterified cholesterol; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LCAT, lecithin/cholesterol acyltransferase; LCn-3 PUFA, long-chain n-3 polyunsaturated fatty acid; LP-C, lipoprotein cholesterol; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; TC, total cholesterol; TG, triacylglycerol; UC, unesterified cholesterol; VLDL, very-low-density lipoprotein.

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conversion of α-linolenate (ALA) to longer-chain n-3 polyunsaturated fatty acid (PUFA) is also expected to be minimal in this species.

As a naturally occurring model of Δ6 desaturase deficiency, cats provide a unique species for investigating FA metabolism. Aspects of lipid metabolism relating to cholesterol, the lecithin/cholesterol acyl transferase (LCAT) system, and its fatty acyl specificity have not been studied extensively in cats and may provide additional insight into comparative lipid metabolism including that of humans. Using an artificial proteoliposome substrate, Butterwick et al [2] determined feline LCAT activities of 239 ± 44 nmol esterified cholesterol (EC) formed mL^{-1} h^{-1} with higher values for intact females than either intact or castrated males. In a separate study, Plantinga and Beynen [3] found that docosahexanoic acid (DHA; 22:6n-3) is not incorporated into feline plasma EC even when present in significant amounts in the diet. Contrary to this, Liu et al [4] found DHA in feline plasma EC but did not specify the diet fed. In light of how little is known about feline LCAT, it was hypothesized that LCAT shows low fatty acyl specificity for DHA, but dietary PUFA does not alter total LCAT activities. Specifically, the objective was to determine plasma LCAT activities, cholesterol, and EC FA profiles in cats fed diets enriched in n-3, n-6, and n-9 FAs.

In addition, it is generally believed that cats may benefit from dietary long-chain n-3 (LCn-3) PUFA, given their limited ability to convert ALA to longer-chain n-3 derivatives [5]. However, the possibility exists that the substitution of eicosapentaenoic acid (EPA) for arachidonate (AA) when LCn-3 PUFAs are fed may lead to AA depletion even at several multiples of minimally recommended dietary amounts. To evaluate this possibility, a second hypothesis of this study was that dietary fish oil supplementation contains sufficient AA to meet the feline requirement, modifies plasma lipids, and provides LCn-3 PUFA enrichment. By contrast, vegetable oil supplements high in LA would only accumulate LA without conversion to AA. Furthermore, consumption of a vegetable oil–supplemented commercial feline diet originally formulated to meet minimal AA requirements would lead to the depletion of this FA due to dietary dilution when fed to meet cats' energy needs. To address this hypothesis, plasma phospholipid (PL) FA profiles of cats fed a commercial diet deficient in AA due to vegetable oil supplementation were determined. Fatty acid profiles of plasma PLs and EC are representative of tissue content and, thus, used as biomarkers in this study [6].

2. Methods and materials

2.1. *Experimental design*

Twenty-nine young, intact female domestic short-hair cats (ages 7-9 months) were randomly divided into 3 diet groups, 2 consisting of 10 cats each and 1 with 9 cats. These cats were showing sexual maturity and were used to minimize hormonal differences with males due to sexual maturation effects on lipid metabolism and because males do not appear to require AA for reproduction [7]. Cats were individually housed at the Laboratory Animal Research and Resources facility, cared for by its resident veterinarian and staff, and fed by members of the Companion Animal Nutrition Lab in the College of Veterinary Medicine and Biomedical Sciences (College Station, Texas). Daily food consumption was monitored during the experimental period, and body weights were measured weekly as an indication of adequate food intake. The cats were fed a calculated amount of kilocalories to maintain healthy growth according to the following equation: metabolizable energy (kJ) = 561 × [body weight (kg)]$^{0.67}$. Cats were judged to be 80% grown at the beginning of the study and showing signs of sexual maturity [8]. Start dates for the cats were staggered to make the acquisition of samples manageable. The protocol for this study was approved by the Texas A&M University Laboratory Animal Use Committee. Before the study, the cats had been maintained on a commercial diet that contained 24.3% total fat on an as-fed basis (Hill's Science Diet Kitten Original Hill's Pet Products, Topeka, Kansas). To begin the experiment, a commercially available, low-fat adult cat diet (basal diet, Kit 'N Kaboodle; Nestle Purina, St Louis, Missouri) was top dressed daily with 1 of 3 dietary oils (8 g oil/100 g diet) and fed for 4 weeks. A 10-day period was used to transition the cats from the Hill's diet to the basal diet so that the animals were eating only basal diet for 6 days before beginning any oil supplements on day 0. The basal diet was analyzed using a commercial laboratory (Midwest Laboratories, Inc, Omaha, Nebraska). Composition (and methods) used were 32% crude protein Association of Official Analytical Chemists (AOAC) 990.03),11.6% fat (AOAC 922.06), 1.36% crude fiber (ANKOM, Macedon NY, Filter bag, AOCS Ba6a-05), 6.75% ash (AOAC 942.05), and 8.3% moisture (AOAC 930.15) as-fed. The oil supplements increased the total fat content not to exceed 18% as-fed. High-oleic sunflower (diet H; Clear Valley, Cargill, Minneapolis, Minnesota), menhaden fish (diet M; Virginia Prime Gold, Omega-Protein, Houston, Texas), and safflower (diet S; Oilseeds International, San Francisco, California) oils provided supplemented diets containing high oleic acid, EPA/DHA, and LA, respectively (Table 1). Diet M contained sufficient AA (0.93 g/kg diet), but diets H and S were deficient (0.10 and 0.17 g/kg diet, respectively) (Table 1). The recognized requirement of cats for AA is 0.2 g/kg diet [8]. The same lot of each oil supplement and basal diet was fed throughout the experimental period and stored at –4°C. Diet H was used as a comparison because of its low dietary LA and marginal AA contents. Diet S was studied because of its increased LA and marginal AA contents. Diet M provided both AA and LCn-3 PUFA.

2.2. *Blood sample collection and lipid analyses*

After withholding food overnight, blood samples (7 mL) were obtained on weeks 0, 2, and 4 via a saphenous vein into an evacuated tube containing EDTA (1.5 mg/mL) as an anticoagulant. Plasma was harvested by centrifugation at 1825 × *g* for 15 minutes at 4°C and stored in aliquots at –80°C for subsequent analysis.

Plasma samples were analyzed in triplicate for triacylglycerol (TG) using a glycerophosphate oxidase enzymatic method with quantitation at 490 nm using a microplate reader (Molecular Devices Corp, Menlo Park, California) and its software (SoftMax Pro; Molecular Devices Corp Sunnyvale, California), as described previously [9]. Plasma total