COMPARATIVE ASPECTS OF CHOLESTEROL METABOLISM AND LECITHIN:CHOLESTEROL ACYLTRANSFERASE ACTIVITY IN DOGS AND CATS

A Thesis

by

REBECCA JOYCE ANGELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2007

Major Subject: Nutrition

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Approved by:

Chair of Committee,	John E. Bauer
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ABSTRACT

Comparative Aspects of Cholesterol Metabolism and Lecithin:Cholesterol Acyltransferase Activity in Dogs and Cats. (December 2007)

Rebecca Joyce Angell, B.S., Texas A&M University

Chair of Advisory Committee: Dr. John E. Bauer

Little research has focused on the relationship between lecithin:cholesterol acyltransferase (LCAT) activity and cholesterol metabolism in dogs and cats. To study weight loss and cholesterol metabolism in dogs, four experimental weight-loss diets were fed to 12 obese female beagles for 8 wk in a partial crossover design (n = 6). High- (HGI) or low-glycemic index (LGI) starch and diacylglycerol or triacylglycerol oil were combined to compose diets with similar fatty acid (FA) profiles. Body weight was measured weekly. Fasted blood samples were drawn at wk1, wk4, and wk8 to measure plasma total (TC), unesterified (UC), and esterified cholesterol (EC) concentrations, LCAT activity, and FA composition of the phospholipid (PL) and EC fractions. All groups lost weight. UC increased from wk1 to wk4 ($p < 0.05$). LCAT activity increased from wk1 to wk4 and remained elevated at wk8 ($p < 0.05$). Plasma PL FA profiles reflected the diets fed with few diet or time effects. Plasma EC FA profiles reflected the specificity of LCAT for linoleic acid (LA) with minimal diet or time effects. We conclude that weight reduction in dogs occurs in conjunction with increased LCAT activity and altered plasma cholesterol fractions but not changes in plasma PL or EC FA profiles. To measure the activity and demonstrate the FA specificity of LCAT in felines fed varying types of fat, 29 female cats were fed diets enriched with high-oleic

sunflower (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil (8g oil/100g kibble) for 4 wk. Fasted blood samples were drawn at d0, d14, and d28 for determination of the blood parameters mentioned previously. LCAT and TC showed no time or diet effects. UC decreased at d28 compared to d0 and d14, while EC increased at d28 compared to d0 and d14 (all $p < 0.05$). Plasma EC FA profiles reflected the specificity of LCAT for LA with many diet and time effects but contained no docosahexanoic acid (DHA). We conclude that feline LCAT has no measurable affinity for DHA, but both feline and canine LCAT demonstrated specificity for LA regardless of diet fed.

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CHAPTER I

INTRODUCTION

Dog Study

Overweight and obesity are common problems among companion animals, affecting an estimated 25 to 40% of dogs and cats seen in private veterinary practice (1). Significant health repercussions such as greater risk for earlier morbidity, shortened lifespan, insulin resistance, and osteoarthritis are associated with excess body weight in dogs (2, 3). Typically, this is addressed by decreasing calorie intake and increasing energy expenditure to promote weight loss (4). Another approach proposed recently is the replacement of the typical form of fat found in the diet, triacylglycerol (TAG), with a form of fat normally found in very low quantities in the diet, diacylglycerol (DAG), especially 1,3-DAG (5).

Animal studies report suppression of diet-induced body weight and visceral fat gain in rats and obesity-prone mice fed diets greater than 10% by weight 1,3-DAG vs. TAG (5). Such findings have led to human studies, which have demonstrated a decrease in visceral fat mass in normal weight, overweight, and obese subjects associated with 1,3-DAG vs. TAG intake (5). Because DAG and TAG oils have been observed to have similar digestibilities, energy content per gram, and absorption, it has been hypothesized that the metabolic differences observed may be due to the structural differences between the two lipids (6, 7).

This thesis follows the style of Lipids.

During digestion of TAG, the fatty acids at the sn-1 and sn-3 positions are hydrolyzed, resulting in 2-monoacylglycerols (2-MAG) and free fatty acids. After absorption into the intestinal mucosal cell, these components are re-esterified into TAG, packaged into chylomicrons, and eventually enter general circulation. In contrast, the digestion of 1,3-DAG produces free fatty acids and 1(3)-MAG, which exhibit less efficient re-esterification into TAG than 2-MAG and free fatty acids in the intestinal mucosal cells (6, 8). As with the TAG oil components, the fatty acids from DAG that are re-esterified into TAG are packaged into chylomicrons and eventually enter general circulation. Less clear is the fate of the fatty acids that are not re-esterified in the intestinal mucosal cells after digestion of 1,3-DAG and absorption of its components. One possibility is that these fatty acids are oxidized within intestinal mucosal cells. Indeed, animal studies have demonstrated an increase in small intestinal β-oxidation in lean and obese mice fed diets high in DAG (9, 10).

One effect of obesity often observed in canines is increased plasma cholesterol (11). This phenomenon has also been seen in cats, as well as a reduction in plasma cholesterol in conjunction with weight loss (12, 13). This decrease in plasma cholesterol during weight loss could be explained by an increase in the activity of lecithin:cholesterol acyltransferase (LCAT) and efficient scavenging of the resultant esterified cholesterol as part of reverse cholesterol transport (RCT).

Lecithin:cholesterol acyltransferase facilitates RCT by creating a concentration gradient of unesterified cholesterol between peripheral cells and high density lipoprotein (HDL) particles. After being synthesized in the liver, LCAT is released into general

circulation where it is incorporated into HDL particles and, to a much lesser extent, low density lipoprotein (LDL) particles (14). Once bound to HDL, LCAT is located on the surface of the particle and can be stabilized in an active conformation by apolipoprotein A-I (apoA-I), the principal activating cofactor of LCAT. As the HDL particle circulates, LCAT preferentially catalyzes the transfer of an unsaturated acyl group from the sn-2 position of phosphatidylcholine (PC) located on the surface of the HDL particle to unesterified cholesterol from peripheral tissues, resulting in the formation of lysophosphatidylcholine (LPC) and esterified cholesterol (14) (Figure 1). The newly formed EC is transferred into the core of the HDL particle and will eventually be released into the liver for processing. The LPC diffuses into solution and binds to albumin for transport back to the liver. With the conversion of unesterified cholesterol into esterified cholesterol, LCAT decreases the concentration of unesterified cholesterol in circulation relative to the concentration of unesterified cholesterol in the peripheral cells, maintaining the concentration gradient. In this way, LCAT facilitates reverse cholesterol transport (15).

Current knowledge of canine LCAT activity is limited. No studies have reported the relationship between LCAT activity and weight loss in dogs, and few have reported the activity of canine LCAT in general. One study reported an increase in LCAT activity that was associated with an increase in plasma cholesterol concentrations in mixed-breed dogs fed a high-fat diet (16). These data support the concept of a relationship between LCAT activity and cholesterol concentrations, which is not counterintuitive as enzyme activities are often influenced by the availability of substrate.

The main objective of this dietary trial was to determine the efficacy of four experimental weight loss diets differing in starch type (high- or low- glycemic index) and oil type (DAG or TAG). Secondary to this and a main focus of this thesis, the relationship between weight loss, cholesterol concentrations, and LCAT activity were investigated, as well as the effects of dietary DAG vs. TAG on plasma phospholipid and esterified cholesterol fatty acid compositions. We hypothesized that obese dogs would exhibit decreased plasma total cholesterol concentrations during weight loss and that if this occurred, it would do so in conjunction with an increase in plasma LCAT activity.

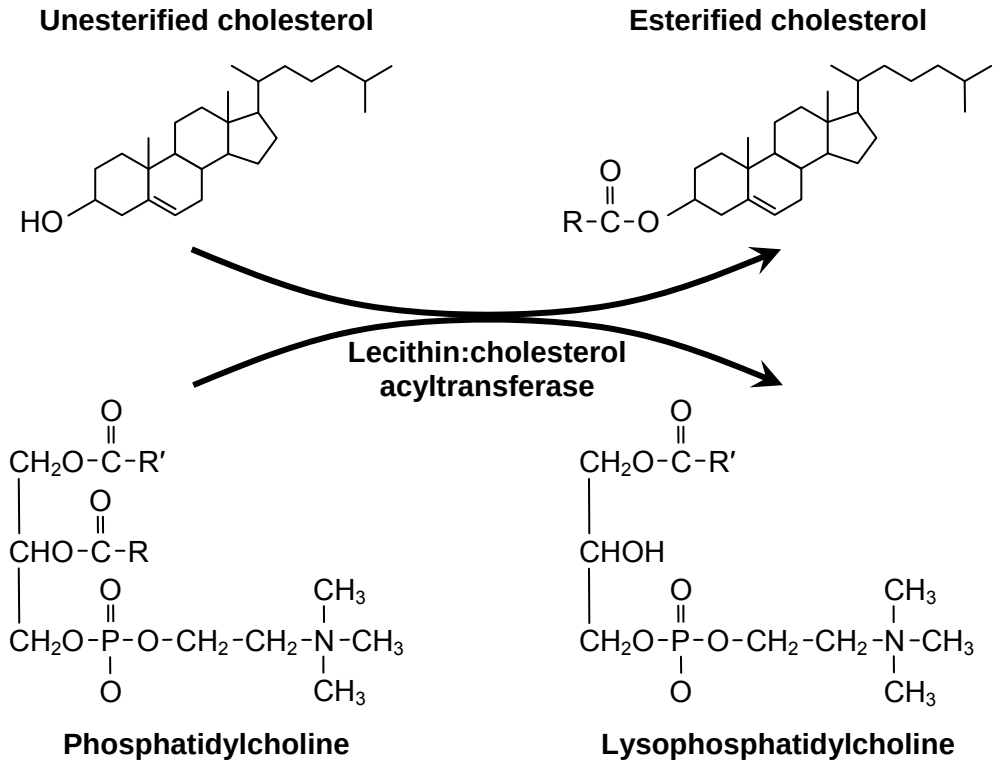

Figure 1 Diagram of the reaction catalyzed by lecithin:cholesterol acyltransferase (LCAT).

Cat Study

It has been generally accepted that cats lack the Δ6 desaturase enzyme that is required for the formation of long-chain polyunsaturated fatty acids (LCPUFAs) from the typical essential fatty acid (EFA) precursor for other mammalian species, linoleic acid (LA, 18:2n-6). In species that express Δ6 desaturase, LA is elongated and desaturated to form arachidonic acid (AA, 20:4n-6), which is subsequently metabolized to form other LCPUFAs and metabolites necessary for normal biological functioning. Rivers et al demonstrated in 1975 that cats have extremely low or nonexistent Δ6 desaturase activity by maintaining cats on diets containing LA as the predominant fatty acid without any other long chain fatty acids for an average of 15 months (17). Despite the high levels of LA fed, only very low amounts of AA were detected in plasma phospholipids, indicating that despite an abundance of substrate, the Δ6 desaturase enzyme was minimally active. The observation of extremely limited or no Δ6 desaturase activity in cats has been confirmed by other researchers and has led to the current view that cats require dietary AA due to an inability to synthesize it from precursors (18).

Contrary to this, more recent studies indicate that cats may have limited, but existent, Δ6 desaturase activity. The presence of small amounts of $20{:}3\Delta^{5,8,11}$ observed in EFA deficient cats indicates low activity of Δ6 desaturase, since this fatty acid likely is a product of elongation and Δ6 desaturation of oleic acid (OA, 18:1n-9) (19). This suggests that cats might have the capacity to synthesize AA from LA; however, due to the excessively small amount of $20{:}3\Delta^{5,8,11}$ detected, authors maintained that such a low

activity of Δ6 desaturase would be metabolically insignificant and that AA would be nutritionally essential for cats (19). More definitive evidence of Δ6 desaturase activity in cats has been demonstrated using stable isotope techniques combined with gas chromatography and mass spectrometry. Pawlosky et al found that 48 hours after oral administration of deuterium-labeled LA to cats that had been maintained on an EFA-deficient diet, labeled 18:3n-6 was present in plasma, but only at 0.06% of the amount administered (20). In concert, these studies demonstrate that Δ6 desaturase activity can be observed in felines, but only to a small degree. The primary objective of this dietary trial was to determine the ability of cats to convert LA to AA after feeding diets rich in LA (from safflower oil), OA (from high-oleic sunflower oil), or LCPUFA (from menhaden fish oil).

A feline enzyme that has received much less attention than Δ6 desaturase is LCAT. Very little research has focused on the activity of LCAT in cats. Butterwick et al utilized an artificial proteoliposome substrate to determine LCAT activity in cats and measure differences between life stages and sexes. For adolescent (age 32 weeks) cats, this study reported an activity of 239 ± 44 nmol of EC formed/ml/h and greater activity in intact females than in either intact or castrated males (21). Slightly more data are available describing the fatty acid specificity of feline LCAT as shown by measuring the fatty acid profile of plasma EC. Platinga et al found that docosahexanoic acid (DHA, 22:6n-3), a LCPUFA present in fish oils, is not incorporated into feline plasma EC even when present in significant amounts in the diet (22). Contrary to this, Liu et al demonstrated the presence of DHA in feline plasma EC (diet not reported) (23). In light

of how little is known about feline LCAT, we measured its activity in plasma and determined the FA profile of plasma EC as part of the larger study. We hypothesized that dietary fat type would not impact LCAT activity in adolescent cats, but it would modulate the fatty acid composition of the plasma EC.

CHAPTER II

MATERIALS AND METHODS

Dog Study

Experimental Design. This research project was part of a larger project investigating the effects of different types of dietary carbohydrate and oil on weight loss, metabolism, and lipid parameters in obese dogs. The protocol for this study was approved by the Texas A&M University Laboratory Animal Use Committee. The dogs were housed at the Laboratory Animal Research and Resources (LARR) 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. Twelve adult intact female obese beagles were divided into four diet groups of three dogs each. After a four week acclimation period to the gruel-based diet form, the groups were then fed their respective experimental weight loss diets for 8 weeks. During a 17-week washout period, the dogs were fed a weight-gain diet to attain their initial levels of obesity. Afterward, the acclimation period and experimental diet feedings were repeated with each group receiving a different diet than it had received previously. During the experimental feeding period, the dogs were fed the amount of kcal required to maintain their starting obese body weight as determined by feeding records during stable obesity. Food intake was recorded daily and body weight was measured weekly. Start dates for the dogs were staggered to make the acquisition of samples manageable.

The experimental diets differed in the type of starch and type of oil they contained. A high- or low- glycemic index starch (waxy corn (W) or high amylose corn (A), respectively) was paired with diacylglycerol (DAG) or triacylglycerol (TAG) oil and a protein and vitamin source to compose each diet, designated as waxy-DAG (WD), waxy-TAG (WT), amylose-DAG (AD), and amylose-TAG (AT). The starches and oils were provided by Kao Corporation (Tokyo, Japan). The DAG and TAG oils had similar fatty acid profiles, which can be found in Appendix A, Table A-1. Specifically, the dry diet mixture consisted of chicken by-product meal (Tyson Foods, Inc., Oklahoma City, OK), starch, and a vitamin and mineral premix (Akey, Lewisburg, OH). On the day of feeding these ingredients were combined with the specified oil and sufficient water to form a gruel that was fed to the dogs. The ingredients and fatty acid profiles of the experimental diets can be found in Appendix A, Tables A-2 and A-3. The amount of water added to each diet was adjusted to form a palatable texture, according to the differing characteristics of the starches. Due to the high amylopectin content of the waxy corn starch, more water was required to form a palatable diet with this ingredient than with the high amylose corn starch. To acclimate the dogs to a gruel-based diet, a dry mixture of chicken by-product meal, 50/50 starch blend (50% waxy corn / 50% high amylose corn by weight), and a vitamin and mineral premix was combined with an oil mixture (50% canola / 50% vegetable oil by volume) and water and fed to the dogs. The fatty acid profile of the acclimation diet can be found in Appendix A, Table A-4.

Blood Sample Collection. Blood was drawn on the first day of the experimental diet period (week 1) before the diets were fed and at weeks 4 and 8 during this period.

The dogs were fasted overnight and blood was obtained from a jugular catheter. Approximately 7 ml of blood were collected into tubes containing EDTA (1.5 mg/ml) as an anticoagulant. Plasma was harvested by low speed centrifugation at 100 × g for 10 min and stored in aliquots at -20°C for subsequent analysis.

Plasma Cholesterol Analysis. Plasma samples were analyzed for total (TC) and unesterified cholesterol (UC) concentrations enzymatically using a microplate reader (Molecular Devices Corporation, Menlo Park, CA) and its software (SoftMax® Pro, Molecular Devices Corporation, Sunnyvale, CA). This data was then used to calculate esterified cholesterol (EC) concentrations. Six (6) µl of plasma and 200 µl of reagent (described below) were applied in triplicate to microplate wells and mixed by the microplate reader. The plate was incubated at 37°C for 30 min, allowed to sit at room temperature for another 30 min, and the absorbance was read at 490 nm. A standard curve was generated by replacing sample plasma in the above procedure with 6 µl of calibrator standards (100, 200, and 400 mg/dl) (Sigma Diagnostics, St. Louis, MO), also in triplicate. Distilled water served as a blank. TC and UC concentrations were calculated by the microplate software based on the slope of the standard curve and expressed as mmol/l. EC concentrations were calculated as the difference between TC and UC.

Cholesterol Reagents. The enzymatic cholesterol reagents were prepared according to the procedure described by Warnick (24). One (1) L of 50 mM PIPES buffer (pH 6.9) was prepared using 17.3 g of disodium salt (Sigma-Aldrich, St. Louis, MO) to form the buffer and 1.292 g of sodium cholate and 1 ml of Triton X-100 (Sigma-

Aldrich, St. Louis, MO) to extend the shelf life. Reagent A was formed by mixing 0.102 g of 4-aminoantipyrine and 1.492 g of KCl (Sigma-Aldrich, St. Louis, MO) with 100 ml of the PIPES buffer. Reagent B was formed by dissolving 0.08 g of 2-hydroxy-3,5-dichlorobenzenesulfonic acid, sodium salt (Research Organics, Inc., Cleveland, OH) in 100 ml of the PIPES buffer. Reagents A and B were mixed in equal parts along with the enzymes cholesterol esterase (0.5 U/ml), cholesterol oxidase (0.5 U/ml), and horseradish peroxidase (10 U/ml) (Sigma-Aldrich, St. Louis, MO) to form reagent C. The procedure was repeated with the omission of cholesterol esterase to form reagent C′.

TC was measured using reagent C. When added to the sample plasma, reagent C catalyzed the following series of reactions: the hydrolysis of EC to UC and fatty acids by cholesterol esterase; the oxidation of UC into cholest-4-en-3-one and H_2O_2 by cholesterol oxidase; and the synthesis of quinoneimine dye from 4-aminoantipyrine, 2-hydroxy-3,5-dichlorobenzensulfonic acid, and H_2O_2 by horseradish peroxidase. Quinoneimine dye has a red color that can be measured at 490 nm. In this way, reagent C allows the quantitation of EC+UC in plasma. UC was measured using reagent C′. Because this reagent lacks cholesterol esterase, it only allows the detection of endogenous UC.

LCAT Activity Assay. Plasma samples were assayed for LCAT activity according to the procedure described by Gillett & Owens (25). This technique utilizes endogenous substrate with which radiolabeled tracer is equilibrated. Results are based on the percent of tracer esterified as an index of endogenous UC esterified. As a control, plasma from week 1 was pooled, mixed, and stored in aliquots at -20°C. Each time the

LCAT assay was performed, a control sample was treated as a plasma sample except that it was not run in duplicate. Once LCAT activities had been determined for all samples and controls, a median control value was determined and used to normalize the data.

^{14}C Substrate Preparation. A 5% (w/v) bovine serum albumin (BSA) (SeraCare Diagnostics, Milford, MA) solution in 0.2 M NaH_2PO_4 buffer (pH 7.4) (Sigma-Aldrich, St. Louis, MO) was prepared and incubated at 56°C for 30 min to destroy any endogenous LCAT activity. It was transferred to a clean vial and weighed. For each 1 g of BSA solution, 2 µCi of ^{14}C-unesterified cholesterol (UC) (PerkinElmer, Boston, MA) solution was prepared. This was done by placing the calculated amount of UC solution into a clean vial and evaporating it to dryness under N_2. The UC was then resuspended in 100 µl of acetone and added dropwise to the BSA solution and mixed using a vortex mixer to bind the UC to the BSA. The acetone was evaporated from the UC/BSA emulsion under N_2 for 30 min with mixing every 10 min. The emulsion was weighed and dH_2O was added until the original weight of the BSA solution was achieved to replace any water lost due to evaporation.

Sample Preparation. Plasma samples were allowed to thaw at 6°C for approximately 15 min. Each sample was prepared in duplicate with a blank. In each tube, 200 µl of sample and 40 µl of DTNB solution (1.4 mM dithionitrobenzoic acid in 0.2 M NaH_2PO_4 buffer, pH 7.4) (Sigma-Aldrich, St. Louis, MO) were pre-incubated in a 37°C water bath for 30 min to reversibly inhibit LCAT activity. Then 60 µl of the UC/BSA emulsion was added and the mixture was mixed using a vortex mixer and

incubated in a 37°C water bath for 4 hr to equilibrate the ^{14}C-UC with endogenous plasma UC.

Enzyme Assay. Forty (40) µl of mercaptoethanol solution (0.1 M mercaptoethanol in 0.2 M NaH_2PO_4 buffer, pH 7.4) (Sigma-Aldrich, St. Louis, MO) was added to each sample and duplicate in 20-second intervals to reverse the inhibition of LCAT by DTNB and activate the reaction. Forty (40) µl of saline (0.9% NaCl) was added to each blank to act as a negative control. After another 30-min incubation at 37°C, 4 ml of chloroform:methanol (2:1, v/v) with 0.2% acetic acid was added in 20-second intervals in the same order as the mercaptoethanol and saline solutions to stop the reaction and extract total lipids. The samples were stored under N_2 at -20°C overnight.

Lipid Extraction and Separation. After adding 2 ml of dH_2O and shaking for 5 min, the samples were centrifuged at 1400 × g for 20 min. The lower chloroform phase was transferred to clean test tubes and evaporated to dryness under N_2. Fifty (50) µl of chloroform was added to each tube to resuspend the lipids applied in narrow streaks to 20 × 20 silica gel G TLC plates (Analtech, Newark, DE) that had been washed by developing in chloroform:methanol (2:1, v/v) with 0.2% acetic acid and dried in a glassware dryer overnight. Three (3) samples plus 20 µl of a TLC standard (#18-5A, Nu-Check Prep, Inc., Elysian, MN) to aid in visualizing the lipid subfractions were applied per plate. The plates were developed in hexane:ether:glacial acetic acid (80:20:1, v/v/v) until the solvent front reached 1 cm from the top of the plate to separate the lipid subfractions. After the plates were dry, the lipid subfractions were visualized in an I_2

chamber and the UC and EC fractions were marked and scraped into labeled scintillation vials with 5 ml of liquid scintillation fluid (UltimaGold, PerkinElmer, Boston, MA).

Liquid Scintillation Counting. A Packard 1900 TR Liquid Scintillation Analyzer (Packard Instruments Co., Downers Grove, IL) was used to count the radioactivity in the UC and EC fractions. The ratio of radioactivity in the EC fraction to the radioactivity in the TC of the samples minus the ratio of radioactivity in the EC fraction to the radioactivity in the TC of the blanks was used as a measure of the percent esterification of endogenous UC by LCAT. Results were transformed from percent UC esterified to nmol UC esterified per ml of plasma per hour using the concentration of UC in that plasma sample as determined by the plasma cholesterol assay. See Appendix B for the equations used to calculate LCAT activity.

Lipid Extraction. Five hundred (500) µl of plasma was combined with 9 ml of chloroform:methanol (2:1, v/v) with 0.2% acetic acid and shaken for 20 min. Two (2) ml of dH_2O were added and the samples were shaken for an additional 10 min. After centrifugation at 1400 × g for 10 min to separate the lipid fraction, the bottom layer was transferred to a clean test tube and 5 ml of chloroform:methanol:water (3:48:47, v/v/v) was added with subsequent shaking for 10 min as a wash. The samples were centrifuged again at 1400 × g for 10 min and the bottom layer was transferred to a clean teflon-lined screw capped test tube and stored under N_2 at -20°C overnight.

Thin Layer Chromatography. Extracted plasma samples were evaporated to dryness under N_2 and 100 µl of $CHCl_3$ was added to each tube. Fifty (50) µl of each sample was applied in narrow streaks to 20 × 20 silica gel G TLC plates (Analtech,

Newark, DE) which had been washed in chloroform:methanol (2:1, v/v) with 0.2% acetic acid and dried in a glassware dryer overnight. Three (3) samples plus a TLC standard (#18-5A, Nu-Check Prep, Inc., Elysian, MN) to aid in visualizing the lipid subfractions were applied to each TLC plate, which was developed in hexane:ether:glacial acetic acid (80:20:1) until the solvent front reached 1 cm from the top of the plate to separate the lipid subfractions. The plates were allowed to dry, then the phospholipid fraction was scraped into a clean test tube. The plates were then visualized in I_2 and the EC fraction was marked and scraped into a clean test tube.

Methylation. After the addition of 2 ml of 4% H_2SO_4 in methanol to each sample, they were mixed and incubated for 1 hr in a water bath at 90°C to catalyze the formation of methyl ester derivatives of the fatty acids that were incorporated into the phospholipids and esterified cholesterol. After standing at room temperature for 5 min and the addition of 3 ml of hexane to each sample, they were mixed, centrifuged at 1400 × g for 15 min, and the top layer was transferred to a clean test tube to isolate the fatty acid methyl esters (FAMEs). The samples were stored under N_2 at -20°C overnight until the time of further analysis.

Gas Chromatography. The FAMEs samples were concentrated by evaporating them to dryness under N_2 and adding back 40 µl of hexane, 20 µl of which was loaded into a gas chromatography (GC) vial insert and placed on a Hewlett Packard 6890 Series Injector. Three (3) µl of each sample were injected onto a FAMEWAX™ fused silica capillary column (30 m long, 0.25 µm thickness, and 0.32 mmID) (Restek, Bellefonte, PA) in a Hewlett Packard 5890 Series II Gas Chromatograph (Hewlett Packard Co., Palo

Alto, CA) with a flame ionization detector. Helium was used as the carrier gas at an initial velocity of 28.3 cm/sec and flow of 1.25 ml/min. Initial oven temperature was held at 160°C for 10 min, then increased at a rate of 1.0°C/min until a final temperature of 220°C was reached. Results were generated with Hewlett Packard ChemStation software package. Authentic FAMEs standards (#68-A, plus 17:0, 18:1n7, 20:5n3, and 22:4n6, Nu-Check Prep, Inc., Elysian, MN) were used to identify individual fatty acid peaks based on retention times.

Data Analysis. Statistical analyses were performed using Statistix8 (Analytical Software, Tallahassee, FL) for Windows. The Shapiro-Wilks test was used to determine normal distribution of data. Non-normally distributed data were either transformed using log, square root, or inverse to achieve normal distribution or analyzed with the Kruskal-Wallis one-way ANOVA. Repeated measures ANOVA was used to test LCAT and cholesterol data for time effects, main effects of starch and oil type, and for interactions. One-way ANOVA was used to test plasma EC FA profile and FA type distribution data for diet effects at each time point and time effects within each diet. For all data, a p-value < 0.05 was considered significant and multiple comparisons were performed where appropriate.

Cat Study

Experimental Design. This research project was part of a larger project to investigate the ability of cats to convert linoleic acid to arachidonic acid. The protocol for this study was approved by the Texas A&M University Laboratory Animal Use Committee. The cats were housed at the Laboratory Animal Research and Resources (LARR) 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. Twenty-nine adolescent (ages 7 to 9 months) intact female cats were randomly divided into three diet groups, two consisting of 10 cats each and one consisting of 9 cats. Cats were individually housed during the experimental period and daily food consumption was monitored. Body weight was measured weekly as an indication of adequate intake. The cats were fed the calculated amount of kcal to maintain healthy growth according to the following equation: Metabolizable Energy (kcal) = 134 × [body weight (kg)]$^{0.67}$. Cats were assumed to be 80% grown at the beginning of the study (26). Start dates for the cats were staggered to make the acquisition of samples manageable.

A commercially available, low-fat adult cat diet (Kit ‘N Kaboodle®, Nestle Purina, St. Louis, MO) was top-dressed with one of three oils (8 g oil/100 g kibble) to compose each diet, which was fed for 4 wk. High-oleic sunflower (H) (Clear Valley®, Cargill, Minneapolis, MN), menhaden fish (M) (Virginia Prime Gold®, Omega Protein, Houston, TX), and safflower (S) (Oilseeds International, San Francisco, CA) oils were

used to provide oleic acid, eicosapentanoic acid (EPA, 20:5n-3)/DHA, or LA, respectively. The fatty acid profiles of the pre-experimental diet (Hill's Science Diet® Kitten Original), Kit 'N Kaboodle®, each oil, and each diet can be found in appendix C, Tables C-1 and C-2. The same lot of each diet constituent was used throughout the experimental period.

Blood Sample Collection. Fasted blood samples (7 ml) were taken on d 0, 14 and 28. Food was withheld overnight prior to the samples being taken, which were obtained via saphenous vein into an evacuated tube containing EDTA (1.5 mg/ml) as an anticoagulant. Plasma was harvested by centrifugation at 100 × g for 10 min and stored in aliquots at -20°C for subsequent analysis.

Plasma Analyses. Plasma cholesterol analysis, LCAT activity assay, lipid extraction, thin layer chromatography, methylation, and gas chromatography were performed as described previously.

Data Analysis. Statistix8 (Analytical Software, Tallahassee, FL) for Windows was used to perform the Shapiro-Wilks test for normal distribution on all data. Non-normally distributed data were either transformed using log, square root, or inverse to achieve normal distribution or analyzed with the Kruskal-Wallis one-way ANOVA. Using SAS, repeated measures ANOVA was performed on transformed LCAT and cholesterol data using PROC MIXED to test for main effects of time and diet and for interactions. Statistix8 was used to perform one-way ANOVA testing on plasma EC FA profile and FA type distribution data for diet effects at each time point and time effects

within each diet. For all data, a p-value < 0.05 was considered significant and multiple comparisons were performed where appropriate.

CHAPTER III

RESULTS

Dog Study

Animals and Diets. Daily monitoring of diet consumption revealed that the dogs consumed an average of only 68 ± 4(SEM)% of the diets offered overall per day on a weight basis. Considering that the dogs were fed the estimated amount of kcal necessary to maintain their starting obese body weights, it is not surprising that this voluntary restriction resulted in all dogs losing weight independent of diet. A starch effect was seen, however, on the average percent of starting obese body weight lost per week. Dogs on the high amylose corn (LGI) diets lost an average of 2% body weight per week whereas dogs on the waxy corn (HGI) diets lost an average of 1% body weight per week. This coincides with lower total digestibilities of the high amylose corn diets (AD 79.8 ± 5.0% and AT 82.1 ± 4.3%, mean ± SEM) vs. the waxy corn diets (WD 95.0 ± 3.0% and WT 93.7 ± 4.5%, mean ± SEM). Thus, even though all dogs were consuming a similar amount of their respective diet on a weight basis, the metabolizable energy derived from the diets varied due to starch type.

Plasma Cholesterol. Inspection of the EC data revealed that values for some dogs were negative. Because this value was calculated using the measured values for TC and UC, a negative value for EC indicates an error in the measurement of either TC, UC, or both. In these instances, the TC, UC, and EC data for that dog at that time period were eliminated from further analysis and SAS PROC MIXED was used to perform

repeated measures analysis on the resulting unbalanced data set. In all, 6 sets of cholesterol data were disregarded as follows: from wk 1, 1 dog each from diet groups WD and WT; from wk 4, 1 dog each from diet groups AD and WT and 2 dogs from diet group AT. Statistical analysis of the abbreviated data set revealed an overall starch effect for both TC and EC concentrations with values for waxy corn groups higher than those for high amylose groups in both cases (Appendix A, Figure A-1). An overall time effect was seen on UC concentrations, which were elevated at wk 4 compared to wk 1 although both measurements were similar to values at wk 8 (Appendix A, Figure A-1).

LCAT Activity. Repeated measures analysis revealed a main time effect but no diet effect or diet × time interaction for LCAT activity (Figure 2). LCAT activity increased from wk 1 to wk 4 and remained elevated at wk 8.

Plasma Esterified Cholesterol Fatty Acid Composition. Few differences were observed between starch groups, oil groups, and diet (starch × oil) groups in the plasma EC fatty acid profiles (Table 1). An overall oil effect was seen for 20:1n-9, but multiple comparisons did not show group differences. At wk 4, 18:3n-3 concentrations were greater in TAG groups than DAG groups. At wk 8, 14:1n-5 concentrations were greater in DAG groups than TAG groups. Interestingly, there was an overall starch effect for 16:1n-7 with concentrations being greater in high amylose groups than waxy corn groups. There was also a starch effect at wk 4 for 20:5n-3, although multiple comparisons did not reveal any significant differences. Diet effects were seen at wk 4 for 20:1n-9 and wk 8 for 16:1n-7, 18:1n-7, and 20:3n-6, but multiple comparisons indicated no significant differences. Similarly, time effects were not widely seen. A

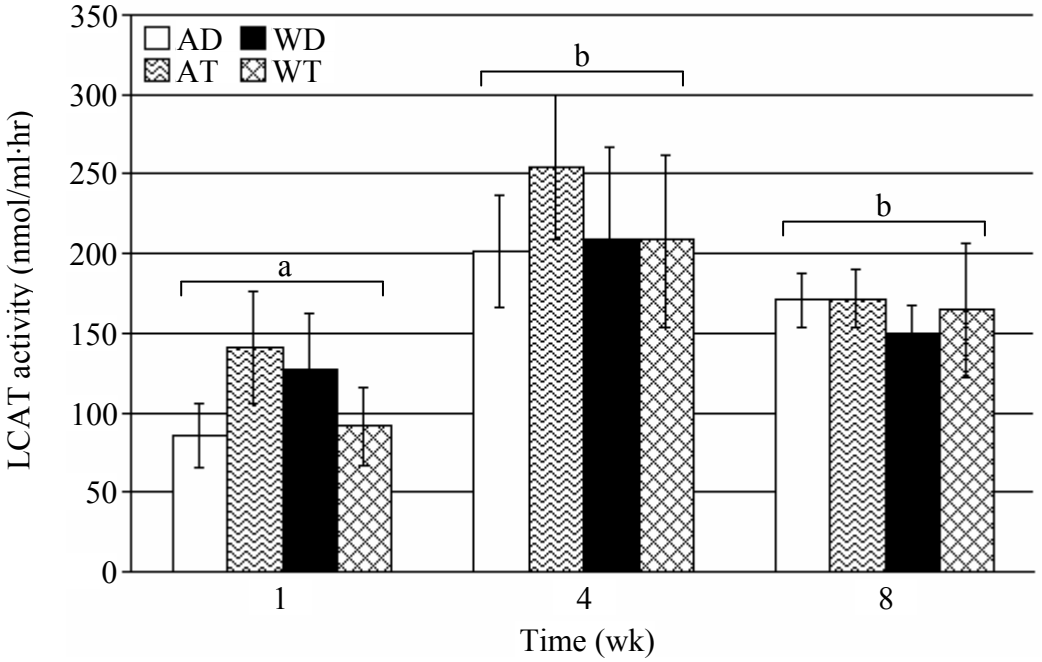

Figure 2 LCAT activities of dogs fed diets differing in starch type (high amylose [A] or waxy corn [W]) and oil type (diacylglycerol [D] or triacylglycerol [T]) (n = 6/group). Values are mean ± SEM. Letters not in common indicate a significant difference between weeks by repeated measures ANOVA, $p < 0.05$.

Table 1 Dog study plasma esterified cholesterol fatty acid composition (relative %)

FA	AD			AT			WD			WT		
	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8
14:0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.1	0.2±0.0	0.2±0.0
14:1n-5	TR	0.2±0.1	0.1±0.0	TR	0.1±0.1	TR	TR	0.1±0.1	TR	TR	0.1±0.0	ND
16:0	8.0±1.1	9.6±0.8	8.1±0.1	9.0±1.3	8.4±0.4	6.7±1.0	7.7±1.2	8.3±1.4	6.6±0.7	8.5±1.6	7.8±0.7	7.3±1.0
16:1n-7	1.0±0.1	1.4±0.2	1.2±0.1	1.2±0.2	1.2±0.1	0.8±0.1	1.2±0.3	1.1±0.2	0.8±0.1	1.2±0.3	1.0±0.1	1.0±0.1
17:0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	TR	TR	0.1±0.0	0.2±0.1	0.2±0.1	0.4±0.2[a]	0.3±0.2[ab]	TR[b]
18:0	5.1±1.4	3.1±0.9	2.7±0.8	4.8±0.7	3.1±0.7	3.1±1.2	3.2±0.9	3.7±1.5	2.4±0.6	5.1±0.9	4.7±2.1	2.4±0.9
18:1n-7	4.1±0.3	4.3±0.5	3.8±0.1	4.1±0.5	3.9±0.4	3.0±0.2	3.8±0.4	3.8±0.4	3.3±0.2	3.9±0.5	3.4±0.1	3.6±0.3
18:1n-9	11.1±0.9	11.3±1.3	11.3±0.2	12.4±1.5	11.4±0.5	9.7±0.6	10.7±1.4	10.3±1.0	10.1±0.3	11.5±1.5	9.9±0.4	10.0±0.4
18:2n-6	47.1±2.1	50.5±3.1	53.8±1.3	47.8±1.7	52.4±2.0	50.0±3.2	46.6±2.7	49.0±3.1	50.7±2.3	44.8±3.5	48.2±3.2	52.4±2.1
18:3n-3	0.2±0.0	0.1±0.0	0.2±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.1
20:0	0.1±0.1	0.2±0.1	TR	0.2±0.1	0.3±0.2	0.1±0.1	0.3±0.2	TR	TR	0.2±0.2	TR	0.3±0.3
20:1n-9	ND	TR	TR	ND	ND	ND	TR	ND	ND	ND	ND	TR
20:2n-6	ND	ND	ND	ND	TR	ND	ND	TR	ND	ND	TR	TR
20:3n-3	ND	ND	TR	ND	ND	ND	TR	ND	ND	ND	ND	TR
20:3n-6	0.4±0.1[a]	0.2±0.0[b]	0.2±0.1[b]	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.1	0.2±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.2±0.1
20:4n-6	13.4±1.7	9.6±1.2	12.1±1.0	11.8±1.7	11.2±0.5	11.8±0.5	14.2±2.0	11.4±0.7	14.6±1.4	13.2±2.3	13.1±1.3	13.5±1.3
20:5n-3	TR	ND	ND	TR	TR	TR	TR	TR	TR	0.1±0.0	TR	TR
22:0	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	ND	0.2±0.2	TR	TR	TR	0.4±0.2	TR	TR
22:1n-9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	TR
22:4n-6	0.1±0.1	TR	TR	0.1±0.0	TR	TR	TR	TR	TR	TR	0.1±0.1	1.4±1.3
22:5n-3	0.3±0.1	0.2±0.1	0.1±0.1	0.3±0.1	0.1±0.1	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.3±0.1	0.3±0.1	0.1±0.1
22:6n-3	ND	ND	ND	TR	ND	ND	TR	TR	ND	TR	ND	TR
24:0	ND	ND	ND	TR	ND	ND	TR	TR	ND	TR	ND	TR
24:1	7.1±1.2	7.8±2.5	5.3±1.4	6.3±1.3	6.0±1.4	12.5±4.8	8.1±1.9	9.5±2.9	9.9±3.8	6.8±2.2	7.7±1.8	6.1±1.7
UI	1.3±0.9	1.2±0.7	0.7±0.3	0.6±0.2	1.1±0.3	1.0±0.4	2.3±1.2	1.5±0.5	0.3±0.1	2.6±1.4	2.6±2.4	0.8±0.5

Values given are mean ± SEM; all diet groups n = 6.
FA = fatty acid; AD = high amylose/DAG; AT = high amylose/TAG; WD = waxy corn/DAG; WT = waxy corn/TAG; ND = not detected; TR = trace (< 0.1%); UI = unidentified.
Superscript letters not in common in a row within a diet group indicate a significant difference between weeks by ANOVA, $p < 0.05$.

main time effect for 14:1n-5 showed that concentrations increased from wk 1 to wk 4, although wk 8 was similar to both previous weeks. Main time effects for 17:0, 18:0, and 22:5n-3 revealed that their concentrations all increased from wk 1 to wk 8, with concentrations at wk 4 similar to both wk 1 and wk 8. Fatty acids 20:5n-3 and 22:0 both showed a time effect that was not elucidated by multiple comparisons. Diet group AD exhibited time differences for 20:3n-6 concentrations, with a decrease from wk 1 to wk 4 that remained at wk 8. A time effect was seen within the DAG group for 18:3n-3 concentrations, which decreased from wk 1 to wk 4 and returned to baseline levels at wk 8. A time effect was also seen for this fatty acid in the high amylose group, with concentrations increasing from wk 1 to wk 4 and decreasing toward baseline so that concentrations at wk 8 were similar to both previous measurements. Arachidonic acid (20:4n-6) changed over time in the DAG groups, but multiple comparisons revealed no significant differences.

Plasma Phospholipid Fatty Acid Composition. As with the plasma EC fatty acid composition, few main effects of starch or oil or starch × oil interactions were seen in the plasma PL fatty acid profiles (Table 2). A main oil effect was seen at wk 1 for 18:3n-3, with higher concentrations in the TAG group than in the DAG group. At wk 8, 18:1n-7 concentrations were higher in the DAG group than in the TAG group. A main starch effect was observed at wk 4 for 16:0, with concentrations higher in the high amylose group than in the waxy corn group. At wk 8, 18:0 and 20:4n-6 concentrations were both higher in the waxy corn group than in the high amylose group. Also at wk 8, 18:1n-9 concentrations were higher in the high amylose group than in the waxy corn

Table 2 Dog study plasma phospholipid fatty acid composition (relative %)

FA	AD			AT			WD			WT		
	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8
14:0	TR[a]	0.1±0.0[b]	TR[ab]	TR	TR	TR	TR[ab]	0.1±0.0[a]	TR[b]	TR	TR	TR
14:1n-5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.0±0.0
16:0	9.8±0.8	12.3±0.7	9.7±0.6	9.9±0.9	10.9±0.5	10.3±1.0	9.9±1.1	10.5±0.9	7.4±1.5	8.7±0.8	9.4±0.7	8.6±0.6
16:1n-7	0.5±0.0	0.6±0.1	0.5±0.1	0.4±0.0	0.5±0.0	0.4±0.0	0.4±0.1[ab]	0.5±0.1[a]	0.3±0.0[b]	0.4±0.1	0.5±0.1	0.4±0.1
17:0	0.5±0.0	0.4±0.1	0.4±0.0	0.4±0.0	0.4±0.1	0.4±0.0	0.4±0.1	0.3±0.1	0.3±0.1	0.4±0.0	0.4±0.1	0.4±0.0
18:0	33.5±1.2	35.0±2.8	30.6±0.3	30.7±1.3	31.9±1.5	29.9±0.8	32.4±0.9	34.2±1.2	30.9±0.4	31.5±0.4	32.4±0.9	32.1±0.8
18:1n-7	3.9±0.3	4.0±0.2	3.9±0.2	3.5±0.2	3.5±0.1	3.3±0.1	3.7±0.2	3.8±0.1	3.3±0.1	3.2±0.1	3.7±0.3	3.2±0.2
18:1n-9	5.6±0.4	5.9±0.6	7.0±0.3	6.4±0.4	6.2±0.7	6.7±0.5	5.6±0.3	5.7±0.3	5.9±0.4	5.8±0.3	7.5±1.2	5.9±0.3
18:2n-6	15.8±1.4	15.9±1.9	17.1±0.9	18.3±0.9	17.6±1.6	18.6±0.8	16.3±0.7	15.6±0.9	16.5±1.2	16.1±0.4	15.8±0.6	15.7±0.4
18:3n-3	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
20:0	0.4±0.1	0.4±0.1	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.0	0.4±0.1	0.4±0.1
20:1n-9	0.2±0.0[ab]	0.2±0.0[a]	0.3±0.0[b]	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
20:2n-6	0.4±0.0[a]	0.2±0.0[b]	0.4±0.0[a]	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.1	0.4±0.0	0.3±0.0	0.4±0.0
20:3n-3	TR	ND	TR	ND	ND	ND	TR	ND	ND	TR	TR	TR
20:3n-6	0.8±0.1	0.8±0.2	0.9±0.1	1.1±0.2	1.0±0.1	1.1±0.2	0.9±0.1	1.0±0.1	1.1±0.1	1.0±0.1	0.9±0.1	1.0±0.1
20:4n-6	21.1±1.5	18.2±2.1	21.4±1.1	21.0±1.2	21.1±1.2	21.0±0.9	22.1±2.2	21.3±1.6	24.7±1.6	23.4±1.1	21.9±1.8	22.9±0.8
20:5n-3	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
22:0	0.6±0.0	0.5±0.1	0.6±0.1	0.5±0.1	0.4±0.1	0.5±0.1	0.5±0.1	0.3±0.1	0.6±0.1	0.5±0.0	0.5±0.1	0.6±0.1
22:1n-9	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR
22:4n-6	0.9±0.1	0.7±0.1	1.0±0.1	1.0±0.2	1.0±0.1	1.0±0.2	0.9±0.2	0.8±0.1	1.2±0.2	1.1±0.1	1.0±0.1	1.2±0.2
22:5n-3	2.6±0.4[a]	1.5±0.2[b]	2.3±0.2[ab]	2.3±0.4	1.9±0.1	2.2±0.4	2.5±0.5	2.2±0.1	3.1±0.5	3.0±0.2	2.3±0.2	3.0±0.3
22:6n-3	0.6±0.1	0.6±0.2	0.6±0.1	0.6±0.1	0.4±0.0	0.6±0.1	0.6±0.1	0.4±0.1	0.6±0.1	0.5±0.1	0.5±0.1	0.6±0.1
24:0	0.3±0.0	0.2±0.0	0.4±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.1	0.5±0.1	0.3±0.0	0.4±0.1
24:1	1.4±0.1	1.3±0.1	1.5±0.3	1.6±0.3	1.3±0.1	1.7±0.3	1.3±0.2	1.2±0.2	1.7±0.2	1.6±0.1	1.3±0.1	1.8±0.3
UI	0.6±0.1	0.7±0.3	0.7±0.3	0.6±0.1	0.5±0.2	0.7±0.3	0.7±0.2	0.7±0.3	0.6±0.3	0.8±0.1	0.4±0.0	0.6±0.2

Values given are mean ± SEM; all diet groups n = 6.
FA = fatty acid; AD = high amylose/DAG; AT = high amylose/TAG; WD = waxy corn/DAG; WT = waxy corn/TAG; ND = not detected; TR = trace (< 0.1%); UI = unidentified.
Superscript letters not in common in a row within a diet group indicate a significant difference between weeks by ANOVA, $p < 0.05$.

group. Overall diet effects were seen for 22:5n-3 and 24:0. Relative concentrations of 22:5n-3 were higher in diet group WT than diet group AT, but both were similar to diet groups AD and WD. Diet group WT had higher concentrations of 24:0 than diet groups AD and AT, but diet group WD was similar to all diet groups. At wk 8, a diet effect was observed for 22:1 but multiple comparisons did not show significant differences. As with oil, starch, and diet effects, time effects were sparse. Relative concentrations of 14:0, 18:0, and 22:5n-3 (docosapentanoic acid, DPA) demonstrated overall time effects. At wk 4, 14:0 concentrations were elevated relative to wks 1 and 8. Concentrations of 18:0 were higher at wk 4 than at wk 8, but measurements at wk 1 were similar to both subsequent measurements. Docosapentanoic acid concentrations decreased from wk 1 to wk 4, then returned to baseline at wk 8.

Plasma Esterified Cholesterol Fatty Acid Type Distribution. Fatty acids were grouped by degree of saturation (saturated [SFA], monounsaturated [MUFA], or polyunsaturated [PUFA]) and by type of unsaturated FA (n-3 or n-6) and the relative percentages detected in the plasma EC fraction were summed and averaged (Table 3). One-way ANOVA revealed an overall time effect for SFA, PUFA, n-3, and n-6 groups. Multiple comparisons showed that SFA decreased from wk 1 to wk 8, with wk 4 values similar to both, and no significant differences between weeks for PUFA. Week 1 n-3 FA relative percentages were higher than weeks 4 and 8, while n-6 FA decreased from wk 1 to wk 8 with wk 4 similar to both. No diet effects or diet×time interactions were observed.

Table 3 Dog study plasma phospholipid and esterified cholesterol fatty acid type distributions (relative %)

FA Type	AD			AT			WD			WT		
	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8	Week 1	Week 4	Week 8
PL												
SFA	45.2±1.0[ab]	49.0±3.3[a]	42.1±0.6[b]	42.3±1.9	44.3±1.6	41.8±1.2	44.1±2.0	46.0±1.6	40.0±1.8	42.0±0.6	43.4±1.3	42.6±1.1
MUFA	11.6±0.4	11.9±0.8	13.1±0.2	12.1±0.3	11.7±0.8	12.2±0.6	11.2±0.5	11.4±0.6	11.4±0.5	11.3±0.3	13.2±1.2	11.7±0.6
PUFA	42.6±1.0	38.4±3.8	44.1±0.5	45.1±1.8	43.6±1.2	45.3±1.0	44.1±2.4	41.9±2.1	48.0±1.7	45.9±0.8	43.0±2.3	45.2±0.8
n-3	3.7±0.5	2.4±0.2	3.2±0.4	3.3±0.6	2.6±0.2	3.2±0.6	3.5±0.5	2.9±0.1	4.1±0.6	4.0±0.2	3.1±0.3	4.1±0.4
n-6	39.0±1.1	36.0±3.8	40.9±0.4	41.8±1.4	40.9±1.2	42.1±0.6	40.6±2.2	39.0±2.1	43.9±1.7	41.9±0.7	39.8±2.1	41.1±0.5
EC												
SFA	13.7±2.3	13.2±1.7	11.2±0.9	14.5±1.1	12.0±0.9	10.3±1.9	11.6±1.7	12.6±2.9	9.3±1.3	14.8±1.8	13.0±2.7	10.4±1.7
MUFA	23.4±1.0	25.0±2.6	21.6±1.4	24.1±1.7	22.6±1.4	26.1±4.0	23.9±1.0	24.9±2.6	24.2±3.4	23.4±3.5	22.1±1.9	20.8±1.2
PUFA	61.7±2.8	60.6±3.7	66.5±1.3	60.7±2.7	64.2±1.8	62.6±3.0	61.9±3.5	61.0±3.4	66.0±2.9	59.2±5.7	62.3±2.7	68.0±1.6
n-3	0.6±0.1	0.3±0.1	0.3±0.1	0.6±0.2	0.3±0.1	0.4±0.1	0.6±0.2	0.3±0.1	0.3±0.1	0.7±0.2	0.5±0.2	0.5±0.2
n-6	61.1±2.7	60.3±3.6	66.1±1.2	60.0±2.5	63.9±1.8	62.2±2.9	61.3±3.5	60.7±3.5	65.7±2.9	58.4±5.6	61.8±2.7	67.5±1.6

Values given are mean ± SEM; all diet groups n = 6.
FA = fatty acid; AD = high amylose/DAG; AT = high amylose/TAG; WD = waxy corn/DAG; WT = waxy corn/TAG; PL = phospholipid; EC = esterified cholesterol; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
Superscript letters not in common in a row within a diet group indicate a significant difference between weeks by ANOVA, $p < 0.05$.

Plasma Phospholipid Fatty Acid Type Distribution. Fatty acids detected in the plasma PL fraction were grouped as described previously and relative percentages were summed and averaged (Table 3). One-way ANOVA identified an overall diet effect for n-3 FA and a diet effect at wk 8 for n-6 FA, but multiple comparisons demonstrated no significant differences in both cases. An overall time effect was shown for SFA and n-3 FA. Saturated FA decreased from wk 4 to wk 8, but relative percentages at wk 1 were similar to both subsequent measurements. Multiple comparisons revealed that n-3 FA concentrations decreased from wk 1 to wk 4, then returned to baseline values at wk 8.

Cat Study

Animals and Diets. Daily monitoring of food intake revealed no aversion to the test diets and all cats that completed the study maintained a healthy weight throughout the experimental period. One of the cats assigned to diet H was found to have oral lesions early on during the feeding period consistent with calici virus and was removed from the study.

Plasma Cholesterol. Total cholesterol concentrations showed no differences due to time, diet, or time × diet interactions (Appendix C, Figure C-1). Both UC and EC exhibited main time effects, with UC concentrations decreased at d 28 relative to days 0 and 14 (Appendix C, Figure C-2) and EC concentrations increased at d 28 relative to days 0 and 14 (Appendix C, Figure C-3). No diet effects or diet × time interactions were seen for UC or EC.

LCAT Activity. No diet, time, or diet × time interactions were seen in LCAT activity (Figure 3).

Plasma Esterified Cholesterol Fatty Acid Composition. No differences in relative percentages of individual fatty acids in the plasma EC fraction were observed between diet groups at baseline (Table 4). As expected, diet effects were widely seen within subsequent time points. As early as d 14, 14:0, 16:0, 16:1n-7, 18:3n-3, 20:4n-6,

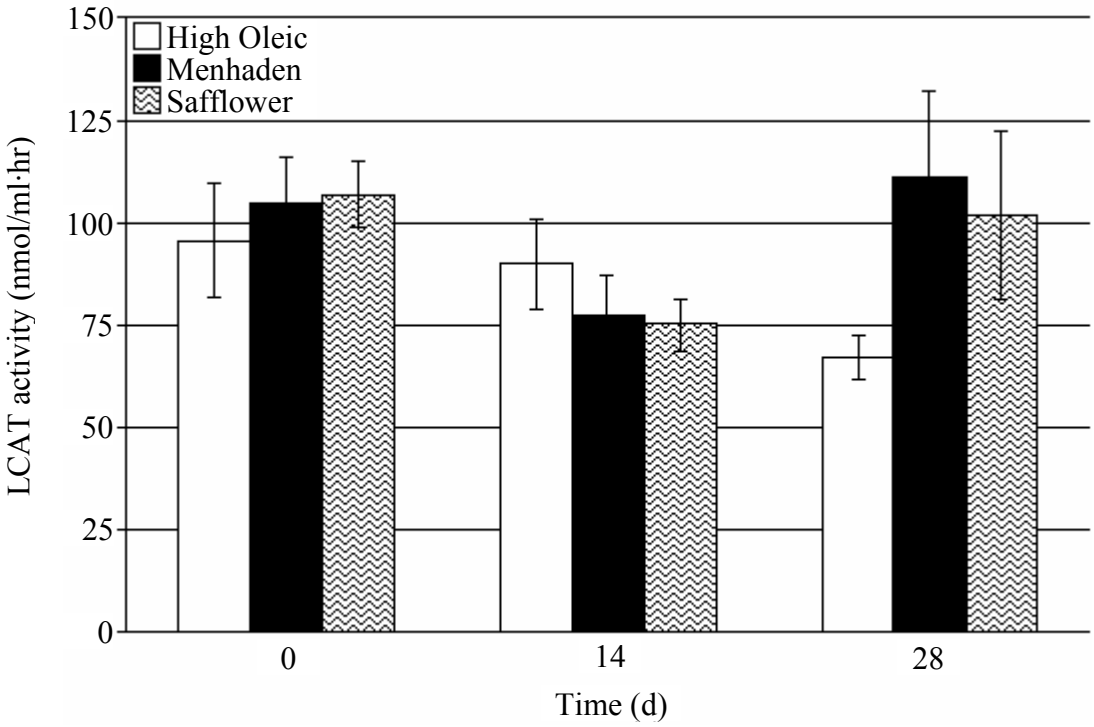

Figure 3 LCAT activities of cats fed diets enriched with high oleic (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil. Values are mean ± SEM.

Table 4 Cat study plasma esterified cholesterol fatty acid composition (relative %)

FA	Diet H (n = 9)			Diet M (n = 10)			Diet S (n = 10)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
14:0	0.19±0.04	0.30±0.05^{1}	0.20±0.02^{1}	0.12±0.04^{a}	0.75±0.09^{b,2}	0.50±0.04^{c,2}	TR	0.26±0.08^{1}	0.17±0.01^{1}
14:1n-5	TR	ND	TR	ND	TR	ND	ND	TR	ND
16:0	9.65±0.50^{ab}	10.12±0.74^{a,1}	7.69±0.24^{b,1}	9.69±0.21^{a}	16.02±1.28^{b,2}	11.74±0.35^{b,2}	9.00±0.50^{a}	10.09±0.65^{a,1}	6.90±0.22^{b,1}
16:1n-7	1.05±0.05^{a}	0.82±0.05^{ab,1}	0.75±0.05^{b,1}	0.87±0.06^{a}	2.04±0.12^{b,2}	1.82±0.11^{b,2}	0.88±0.07^{a}	0.71±0.08^{ab,1}	0.55±0.06^{b,1}
17:0	TR	TR	ND	TR	TR	TR	TR	0.11±0.05	ND
18:0	1.21±0.03	1.50±0.16	1.16±0.09^{1,2}	1.33±0.07^{a}	1.84±0.17^{b}	1.46±0.08^{ab,1}	1.33±0.08^{ab}	1.81±0.23^{a}	1.09±0.09^{b,2}
18:1 iso	ND	2.47±1.22	0.32±0.30	ND	1.19±0.86	ND	ND	ND	ND
18:1n-7	1.96±0.07	3.59±0.92^{1,2}	1.48±0.19^{1,2}	1.98±0.09^{a}	3.17±0.41^{b,1}	2.49±0.07^{b,1}	1.87±0.12^{ab}	2.02±0.21^{a,2}	1.42±0.16^{b,2}
18:1n-9	20.42±0.82^{a}	28.11±1.20^{b,1}	29.40±0.79^{b,1}	19.85±0.70^{a}	17.78±0.64^{b,2}	18.07±0.35^{ab,2}	19.01±0.64^{a}	12.77±0.59^{b,3}	12.88±0.67^{b,2}
18:2n-6	56.72±0.55^{a}	48.02±1.99^{b,1}	53.40±0.91^{ab,1}	55.12±0.91^{a}	38.25±2.03^{b,2}	41.27±0.76^{b,2}	55.99±1.03^{a}	65.71±2.33^{ab,3}	73.19±1.10^{b,3}
18:3n-3	0.41±0.06^{a}	0.22±0.05^{b,1}	0.32±0.02^{ab,1}	0.45±0.02	0.48±0.06^{2}	0.48±0.03^{2}	0.43±0.05^{a}	0.21±0.13^{b,1}	0.19±0.02^{b,3}
20:0	ND	ND	ND	ND	0.11±0.11	ND	ND	0.19±0.19	ND
20:1n-9	ND	ND	ND	ND	TR	ND	ND	0.14±0.14	ND
20:2n-6	ND	ND	ND	ND	TR	ND	ND	0.13±0.13	ND
20:3n-3	ND	ND	ND	ND	TR	ND	ND	0.13±0.13	ND
20:3n-6	0.18±0.04	0.12±0.04	0.23±0.03^{1}	0.30±0.02^{a}	0.19±0.08^{b}	0.20±0.02^{b,1}	0.25±0.06	0.16±0.14	0.10±0.02^{2}
20:4n-6	5.87±0.27^{a}	3.25±0.29^{b,1}	3.95±0.30^{b,1}	6.44±0.47	6.18±0.46^{2}	7.04±0.22^{2}	7.81±0.83^{a}	3.13±0.26^{b,1}	2.77±0.25^{b,1}
20:5n-3	1.53±0.19^{a}	0.70±0.15^{b,1}	0.87±0.07^{b,1}	1.61±0.17^{a}	10.62±0.87^{b,2}	13.98±0.88^{b,2}	1.89±0.24^{a}	0.31±0.14^{b,1}	0.49±0.03^{b,1}
22:0	ND	ND	ND	ND	TR	ND	ND	0.14±0.14	ND
22:1n-9	ND	ND	ND	ND	TR	ND	ND	0.15±0.15	ND
22:4n-6	ND	ND	ND	ND	ND	ND	ND	ND	ND
22:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND
22:6n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND
24:0	ND	ND	ND	ND	ND	ND	ND	ND	ND
24:1	ND	ND	ND	ND	ND	ND	ND	ND	ND
UI	0.69±0.56	0.70±0.44	0.19±0.16	2.18±1.00	0.89±0.59	0.87±0.75	1.38±0.89	1.80±0.84	0.24±0.13

Values given are mean ± SEM.
FA = fatty acid; ND = not detected; TR = trace (< 0.1%); 18:1 iso = 18:1 isomers other than 18:1n-7 and 18:1n-9; UI = unidentified.
Superscript letters not in common in a row within a diet group indicate a significant difference between days by ANOVA, $p < 0.05$.
Superscript numbers not in common in a row within a day indicate a significant difference between diets by ANOVA, $p < 0.05$.

and 20:5n-3 relative percentages were higher in diet group M than in the other two diet groups. Other differences seen at this time point were relative percentages of 18:1n-7, which was higher in diet group M than diet group S with diet group H similar to both; 18:1n-9, which was significantly higher in diet group H than diet group M, which was in turn greater than diet group S; and 18:2n-6, which was greatest in diet group S, followed by diet group H, then diet group M.

By d 28, further differences between diet groups were seen. In addition to the same patterns for 14:0, 16:0, 16:1n-7, 18:1n-7, 18:2n-6, 20:4n-6, and 20:5n-3 as observed for d 14, there were also differences between diet groups in relative percentages of 18:0 and 20:3n-6 as well as differences for 18:1n-9 and 18:3n-3 other than those observed on d 14. Relative percentages of 18:1n-9 were higher for diet group H than the other two diet groups, and 18:3n-3 concentrations were different for all three diet groups, with diet group M being greatest, followed by diet group H, then S. Diet group M exhibited higher 18:0 concentrations than diet group S with diet group H similar to both. Lower 20:3n-6 concentrations were found in cats fed diet S than those fed diets M or H.

Widespread time effects within each diet group were also observed for the plasma EC fatty acid composition. In cats fed diet H, relative percentages of 16:0, 16:1n-7, 18:1 unidentified isomers, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, and 20:5n-3 changed over time. At d 14, relative percentages of 16:0 were elevated relative to d 28, although d 0 was similar to both values. The concentration of 16:1n-7 increased gradually from d 0 to d 28 such that d 14 values were similar to both. Although one-way

ANOVA identified a time effect for 18:1 unidentified isomers, multiple comparisons did not indicate significant differences between time points. Relative percentages of 18:1n-9 increased from d 0 to d 14 and remained elevated at d 28. Both 18:2n-6 and 18:3n-3 relative percentages increased from d 0 to d 14 but dropped at d 28 so that values were similar to both previous measurements. Concentrations of 20:4n-6 and 20:5n-3 were both increased at days 14 and 28 compared to baseline. Diet group M exhibited differences in the relative percentages of 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 20:3n-6, and 20:5n-3 over time. Concentrations of 14:0 increased from baseline to d 14, then decreased at d 28 but remained higher than at d 0. Relative percentages of 16:0, 16:1n-7, 18:1n-7, and 20:5n-3 were elevated at d 14 compared to baseline and remained so at d 28. At d 14, concentrations of 18:0 had increased from baseline values only to decrease at d 28 so that values were similar to both previous measurements. Conversely, 18:1n-9 relative percentages decreased from baseline to d 14, then increased such that values at d 28 were not different than previous measurements. Both 18:2n-6 and 20:3n-6 concentrations declined from d 0 to d 14 and remained so at d 28. Cats fed diet S had altered levels of 16:0, 16:1n-7, 18:0, 18:1n-7, 18:1n-9, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, and 20:5n-3 in plasma EC over time. Relative percentages of 16:0 remained steady from baseline to d 14 and decreased at d 28. Palmitic acid (16:1n-7) levels decreased gradually from d 0 to d 28 such that values at d 14 were similar to both. Concentrations of 18:0 and 18:1n-7 were lower at d 28 than at d 14, although baseline values were similar to both subsequent measurements. A decrease in relative percentages of 18:1n-9, 18:3n-3, 20:4n-6, and 20:5n-3 occurred from baseline to d 14 and remained at d 28.

Linoleic acid (18:2n-6) concentrations were elevated at d 28 compared to d 0 with d 14 values similar to both. Although one-way ANOVA identified a difference in 20:3n-6 relative percentages over time, multiple comparisons indicated no significant differences.

Plasma Esterified Cholesterol Fatty Acid Type Distribution. Fatty acids detected in the plasma EC fraction were grouped as described previously and summarized in Table 5. As with the plasma EC fatty acid profiles, no significant differences between diets were found at baseline but many differences were found between diets at subsequent time points. At d 14, n-3 and saturated FA relative percentages were higher in diet group M than in the other two diet groups. Monounsaturated fatty acid concentrations were different for each diet group, with H having the highest, followed by M, then S. Both n-6 and polyunsaturated FA levels were elevated in diet group S relative to the other two diet groups. Day 28 FA type distributions had identical differences between groups as d 14.

Time effects on fatty acid type distribution within diet groups were also prevalent. Relative percentages of SFA, MUFA, PUFA, and n-3 and n-6 FA varied with time in cats fed diet H. Greater SFA concentrations were seen at d 14 than at d 28, although values at both time points were similar to baseline values. Monounsaturated FA levels were increased relative to baseline at d 14 and remained so at d 28. A decrease in polyunsaturated, n-3, and n-6 FA concentrations was observed at d 14 that was sustained at d 28. Diet group M displayed time effects for SFA, PUFA, and n-3 and n-6 fatty acid relative percentages. Saturated and n-3 FA levels were elevated compared

Table 5 Cat study plasma esterified cholesterol fatty acid type distribution (relative %)

FA Type	Diet H (n = 9)			Diet M (n = 10)			Diet S (n = 10)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
SFA	11.12±0.54^{ab}	12.00±0.97^{a,1}	9.05±0.32^{b,1}	11.20±0.21^{a}	18.86±1.56^{b,2}	13.79±0.35^{b,2}	10.48±0.59^{a}	12.61±1.09^{a,1}	8.16±0.29^{b,1}
MUFA	23.48±0.91^{a}	34.99±1.43^{b,1}	31.98±0.90^{b,1}	22.70±0.73	24.38±1.03^{2}	22.38±0.36^{2}	21.76±0.80^{a}	15.81±0.66^{b,3}	14.85±0.88^{b,3}
PUFA	64.71±0.72^{a}	52.31±2.32^{b,1}	58.77±1.14^{b,1}	63.91±1.15^{a}	55.87±2.91^{b,1}	62.96±0.72^{ab,1}	66.37±1.46^{a}	69.78±2.03^{a,2}	76.75±1.22^{b,2}
n-3	1.94±0.23^{a}	0.92±0.18^{b,1}	1.19±0.09^{b,1}	2.06±0.19^{a}	11.17±0.89^{b,2}	14.45±0.90^{b,2}	2.32±0.28^{a}	0.65±0.30^{b,1}	0.68±0.05^{b,1}
n-6	62.77±0.60^{a}	51.39±2.33^{b,1}	57.58±1.12^{b,1}	61.85±1.07^{a}	44.70±2.42^{b,1}	48.51±0.79^{b,2}	64.04±1.42^{a}	69.13±2.25^{a,2}	76.06±1.21^{b,3}

Values given are mean ± SEM.
FA = fatty acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
Superscript letters not in common in a row within a diet group indicate a significant difference between weeks by ANOVA, $p < 0.05$.
Superscript numbers not in common in a row within a day indicate a significant difference between diets by ANOVA, $p < 0.05$.

to baseline values at both d 14 and d 28. Polyunsaturated fatty acids increased from baseline to d 14, then decreased at d 28 so that values were similar to both previous measurements. Concentrations of n-6 FA were decreased at days 14 and 28 relative to baseline values. Cats fed diet S exhibited differences due to time for saturated, monounsaturated, polyunsaturated, n-3, and n-6 fatty acids. Levels of SFA were stable from baseline to d 14, then dropped at d 28. Monounsaturated and n-3 FA concentrations decreased from baseline to d 14 and remained so at d 28. Relative percentages of polyunsaturated and n-6 fatty acids showed no change from d 0 to d 14, then increased at d 28.

CHAPTER IV

DISCUSSION

Because the dogs were offered the estimated number of calories required to maintain their starting obese body weights, the voluntary diet restriction of the dogs resulted in consistent weight loss in all diet groups. Therefore, in the absence of differences due to diet, differences due to time could be attributed to the effects of the weight loss that occurred. Also, due to the decreased digestibility of the LGI starch diets, greater percentage body weight loss occurred in dogs that received those diets than in dogs fed the HGI starch diets.

Significant differences were identified in plasma cholesterol concentrations. Concentrations of both TC and EC were lower in the LGI starch group than in the HGI starch group. Typically, plasma cholesterol concentrations are associated with dietary fat intake, so a starch effect on TC and EC seems surprising. An overall starch effect indicates that differences were present even at wk 1, before the experimental diets were fed. The presence of differences between starch groups at baseline makes differences at subsequent time points difficult to interpret. Additionally, because certain samples were excluded from the analysis as described previously, the sample size of some groups was reduced which may have contributed to the considerable variability of the remaining results. With these confounding factors in mind, although a statistical difference due to starch type was observed for the TC and EC data, the physiological significance of these findings are uncertain.

On the other hand, UC concentrations exhibited significant differences due to time: an initial increase from wk 1 to wk 4, then a non-significant decrease toward baseline from wk 4 to wk 8. As stated earlier, a time effect in the absence of a diet effect could indicate a physiological effect of weight loss. In this case, weight loss initially occurred in conjunction with a rise in plasma UC concentration, then it seems that adaptation moderated the change in UC so that the subsequent measurement was similar to both baseline and wk 4 values. Interestingly, a similar pattern of change was observed in LCAT activity, which also exhibited significant differences due to time. After a significant increase from wk 1 to wk 4, LCAT activity decreased at wk 8, although not significantly. Again, this time effect might be due to the weight loss that occurred during this interval; however, these changes are more likely due to substrate availability. As concentrations of UC (the substrate for LCAT) increased, so did the enzyme's activity. This result is not surprising considering that enzyme activity is often regulated by substrate availability and that this mechanism is recognized as the major regulator of LCAT activity (14).

The experimental oils were designed to have similar fatty acid contents and as expected, fatty acid profiles of plasma EC and PL fractions demonstrated sparse differences due to oil, starch, diet, or time. The few statistical differences seen in the dog EC and PL fatty acid profiles consist of small numerical differences between groups. The majority of the statistical differences identified resulted from differences between groups of < 0.5 relative percent, and the largest difference identified between groups was only ~2.7 relative percent. Many of the statistical differences occurred in FA present as

< 1 relative percent in the fatty acid profiles. Such small amounts are approaching the detection limit of the methods used in this study, and the slight differences identified statistically may not reflect true differences due to the sensitivity of the methods employed and interassay variation. Regardless of statistical significance, such small differences in fatty acid profiles likely have little physiological significance. As such, the isomeric differences of TAG and DAG oil do not translate into differences in FA composition of the plasma PL or EC fractions.

Linoleic acid constituted the major fatty acid in the plasma EC fraction in all diet groups, with concentrations much higher than those found in plasma PL. This is consistent with reports by others that canine LCAT exhibits substrate specificity for LA (23). Interestingly, this same phenomenon was also observed with 24:1. Concentrations of 24:1 detected in plasma EC were roughly 5 times those found in plasma PL on average, indicating that canine LCAT also shows specificity for this FA, which was present in the diet at 0.57 relative percent, on average. A previous unpublished study in this lab did not find substantial amounts of 24:1 in canine plasma EC; however, the experimental diets in that study did not contain detectable amounts of 24:1 (27).

As with the fatty acid profiles of the dog plasma EC and PL fractions, the fatty acid type distributions of those fractions exhibited few significant differences because of the similar fatty acid profiles of the experimental oils and diets. The differences observed between groups for n-3 FA concentration in both EC and PL FA profiles were all <1 relative percent, and for reasons stated previously are likely of little physiological significance. The other differences seen in the FA type distributions were all modest

time effects, which could either be attributed to interassay variation or differences between the acclimation diet and the experimental diets. In the plasma EC fraction, SFA decreased by about 3 relative percent and n-6 FA concentrations increased by about 5 relative percent from wk 1 to wk 8. This alteration may reflect the slightly higher SFA and lower n-6 FA content of the acclimation diet relative to the experimental diets, on average.

An unexpected finding in the cat study was the significant decrease in plasma UC and increase in plasma EC concentrations at d 28, especially in the absence of any change in plasma LCAT activity or TC concentration. This redistribution of the cholesterol fractions could be related to a decrease in dietary fat intake, which occurred when the cats were switched from the pre-experimental diet, which was 24.3% fat as is, to the experimental diets, which were on average 16.8% fat as is. Because most commercial pet foods contain little cholesterol, most of the plasma cholesterol in these cats was synthesized endogenously from fatty acids provided by the diet, which not only serve as substrate but also as stimulus for cholesterol production. A decrease in the amount of fat available for use in and stimulus of *de novo* cholesterol synthesis could lead to decreased endogenous cholesterol (as UC) production. Plasma TC concentrations depend not only on its absorption from the diet and endogenous synthesis, but also on its rate of uptake and release by tissues. A decrease in cholesterol synthesis could take place in the absence of a decrease in plasma TC concentrations if it occurs concomitantly with a decrease in uptake or an increase in release of cholesterol by tissues. Indeed, stable LCAT activity could indicate that the increase in circulating EC

is due not to an increase in UC esterification, but possibly to decreased uptake of EC by tissues. Thus, a decrease in UC synthesis may have occurred in equal proportion to a decrease in EC uptake by tissues such that TC concentrations remained constant.

Changes in plasma LCAT activity were not expected in the cats. The primary objective of measuring LCAT activity in this species was simply to expand current knowledge of this enzyme in a seldom studied species. No statistical differences were identified between diet groups or time points, and averaging all the data to obtain an LCAT activity in adolescent (7- to 10-month-old) cats yielded a value of 92.4 ± 4.5 nmol of UC esterified/ml of plasma/hr (mean ± SEM). This is quite a bit lower than the value reported for adolescent (8-month-old) cats by Butterwick et al (239 ± 44 nmol of UC esterified/ml of plasma/hr [mean ± SEM]) (21). This disparity has several possible explanations, mainly differences in methodology used to measure LCAT. Butterwick et al employed an artificial proteoliposome substrate method (21), whereas the present study utilized a radiochemical method which relies on endogenous substrate. The former method optimizes the substrate for LCAT, potentially yielding higher activities, while the latter method is more reflective of actual conditions within the animal. Another factor that could have produced differences in LCAT activity between the two studies is the TC concentration. Butterwick et al reported a cholesterol concentration of 6.03 ± 1.22 mmol/l (mean ± SD) for adolescent cats (21), whereas the present study determined a concentration of 3.03 ± .09 mmol/l (mean ± SEM) for total cholesterol. It is possible that the elevated TC concentrations present in the former study compared to

the latter could have lead to increased circulating LCAT due to increased substrate, although the former study did not determine the UC concentration.

Extensive differences between diets and over time were seen in the plasma EC FA profiles due to the different FA profiles of the oils fed. Interestingly, linoleic acid (LA, 18:2n-6) constituted the major FA in plasma EC in all diet groups after 28 days of feeding, even though it was present as only 13.5 and 11.4 relative percent of the fat in diets H and M, respectively. This is consistent with a report by Liu et al that LA is the major FA in feline plasma EC, although diet was not reported in that study (23). Considering the amounts of LA supplied by the diets in the present study, the presence of such high concentrations of LA in the plasma EC FA profile indicates a strong specificity of feline LCAT for LA.

Another FA present in greater percentages in the plasma EC fraction of all diet groups at d 28 than in the diet was AA. Arachidonic acid was present in almost 3 relative percent of plasma EC after 28 days of feeding diet S, which contained no detectable amounts of AA. This represents a decrease from baseline values, which can be attributed to lower content of AA in diet S compared to the pre-experimental diet. Nonetheless, AA was only present as <1 relative percent in the pre-experimental diet FA profile, and baseline values of AA in the plasma EC FA profiles of the cats averaged 6.71 ± 0.36 relative percent (mean ± SEM) overall. The presence of AA in plasma EC at these levels considering the amount supplied by the diet indicates a notable affinity of feline LCAT for AA and poses an interesting question regarding the origin of the AA given the limited Δ-6 desaturase activity in feline species. Some studies have reported

decreased AA content of plasma lipids in animals fed diets supplemented with fish oil (FO) (28, 29). This response was not seen in the plasma EC fraction in the present study. At days 14 and 28, cats fed diet M exhibited greater AA content in plasma EC than cats fed diets H or S. However, in the previously mentioned studies, the diets fed (FO-supplemented and control or alternate experimental diet) provided approximately equal amounts of AA as a percentage of total FA. In the present study, diet M provided more AA than either diet H or S. This pattern of greater plasma lipid AA content in animals fed a FO-supplemented diet containing more AA than the alternate experimental diets is consistent with other studies. Horses fed a FO-supplemented diet vs. a corn oil (CO)-supplemented diet exhibited increased concentrations of AA in plasma lipids, reflecting the greater concentration of AA in the FO compared to the CO diet (30). Thus, the effect of FO feeding on AA content of plasma lipids seems to be related to the AA content of the complete diet.

Cats fed diet M were consuming roughly 5% of their FA as EPA, yet the concentration of this FA in their plasma EC was almost 14% of the total FA after 28 days of feeding. This was an 8-fold increase from baseline values, when their diet provided approximately 1% of FA as EPA. Even in diet groups H and S, which provided either very little or no EPA, this FA could be detected in their plasma EC FA profile at d 28, albeit at <1 relative percent. These findings suggest that, in addition to LA and AA, feline LCAT also shows a favorable affinity for EPA.

Notably absent in the plasma EC FA profile was DHA. As with EPA, DHA was present in diet M as roughly 5% of total FA. However, unlike EPA, DHA was not

detected in plasma EC even after 28 days of feeding. This finding is consistent with results published by Platinga and Beynen reporting that cats fed a diet enriched with DHA had no detectable DHA in plasma EC (22) and contradicts the findings of Liu et al, who reported incorporation of DHA into feline plasma EC, although only at <1 weight percent (23). Fatty acid analysis methodology varied among the studies, present included, to which disparate results might be attributable. Further research into the incorporation of DHA into feline plasma is necessary to elucidate this aspect of fatty acid metabolism. Docosapentanoic acid (DPA, 22:5n-3) was also present in diet M but not detectable in plasma EC even at d 28. One possible explanation is that DPA was present only at approximately 1% of total fatty acids and this was not enough to support incorporation into plasma EC, but other FA comprising 1% or less of total FA, such as α-linolenic acid (ALA, 18:3n-3) and AA, were detectable in plasma EC. Another possibility is that feline LCAT has no measurable affinity for DPA. To our knowledge, this is the first study to mention DPA in relation to cat plasma EC. This is another area which can benefit from further research.

One of the more surprising results obtained in this study was that plasma EC FA profiles showed statistically significant changes in some FA as early as d 14. Other FA concentrations changed gradually from baseline to d 28. There was some concern at the planning stages of this project as to whether 28 days would be enough time to effect changes in feline FA metabolism. Our results indicate that early changes can be detected in as little as 14 days with further effects being apparent at 28 days. A longer study with more sampling periods would be necessary to establish a timeline for achieving a

metabolic steady state, but our data demonstrate that a 28-day study is adequate to measure changes in FA metabolism.

The plasma EC fatty acid type distributions were strongly impacted by the fatty acid type distributions of the diets. Each of the changes seen over time reflect differences between the fatty acid type distributions of the pre-experimental diet and the experimental diets. Notably, PUFAs constitute the major fatty acid type in the plasma EC in each diet group at all sampling times, even though they are not the major fatty acid type in the pre-experimental diet or diets H or M. Similarly, n-6 FA greatly exceed n-3 FA in the plasma EC. Both of these phenomena result from the predominance of LA in the plasma EC. In cats fed diet M, n-3 FA increased markedly due to the incorporation of EPA into plasma EC. Our data clearly show that although diet influences feline plasma EC fatty acid type distributions, the substrate specificity of feline LCAT for specific PUFAs is the major determinant of the plasma EC fatty acid type distribution.

CHAPTER V

SUMMARY

These investigations into diet effects on LCAT activity and substrate specificity have contributed to the current knowledge of cholesterol metabolism in two companion animal species. An increase in LCAT activity and plasma UC concentration was observed in conjunction with weight loss in dogs. No diet effects were seen on these two parameters, indicating that the changes in LCAT activity and UC concentration were indeed due to weight reduction as all diet groups experienced weight loss. Oil type (TAG vs. DAG) did not affect the plasma EC or PL fatty acid composition or fatty acid type distribution. This is likely due to the similar fatty acid composition of the experimental oils and indicates that positional isomers in oil do not affect resultant FA profiles.

No diet or time effects were seen on LCAT activities in cats fed diets differing in fatty acid composition. Thus, the values for all cats at all time points were averaged to obtain a basal LCAT activity for adolescent cats of 92.4 ± 4.5 nmol of UC esterified/ml of plasma/hr (mean ± SEM). This is quite a bit lower than the value for LCAT activity in adolescent cats reported by others, probably due to differences in methodology.

The substrate specificity of feline LCAT for LA was demonstrated as this was the major FA in the plasma EC fraction in all cats even though LA was the major FA in only one of the experimental diets. In cats fed diet M, greater concentrations of EPA were found in the plasma EC fraction than were supplied by the diet, indicating an

affinity of feline LCAT for this fatty acid. Even though similar amounts of EPA and DHA were supplied by diet M and significant amounts of EPA were detected in the plasma EC fraction, no DHA was identified in this fraction, indicating very little or no affinity of feline LCAT for DHA. Diet M supplied DPA as well, but this FA was also absent in the plasma EC fraction, suggesting little or no affinity of feline LCAT for DPA.

Notably, some changes in feline plasma EC fatty acid composition were statistically significant after only 14 days of feeding the experimental diets. This indicates rapid modification of feline FA metabolism by dietary fatty acid composition. By d 28, further significant differences due to time were seen, indicating that while initial differences can be seen in as little as 14 days, a longer study period is necessary to establish a metabolic steady state. Whether 28 days is enough time to accomplish this cannot be determined by the present study, but we did demonstrate that it is a long enough study period to observe alterations in feline FA metabolism.

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APPENDIX A

Table A-1 Fatty acid composition of TAG and DAG oils in dog study (relative %)

Fatty Acid	TAG	DAG
14:0	TR	TR
16:0	4.94	2.76
16:1n-7	0.14	0.11
17:0	TR	TR
18:0	1.89	1.33
18:1	32.09	37.34
18:2n-6	50.31	47.60
18:3n-3	7.49	6.57
20:0	0.36	0.33
20:1	0.63	0.70
20:2n-6	TR	TR
20:3n-6	ND	ND
20:4n-6	ND	ND
22:0	0.26	0.29
22:1	0.14	TR
24:0/22:6n-3	TR	0.10
24:1	0.10	0.10
Unidentified	1.34	2.53

Values are averages of two representative samples.
ND = not detected; TR = trace (< 0.1%).

Table A-2 Ingredients of experimental diets in dog study

Component	Diet			
	WD	WT	AD	AT
	(g/kg)			
Chicken byproduct meal	430	430	430	430
High amylose corn starch	-	-	430	430
Waxy corn starch	430	430	-	-
Vitamin/mineral pre-mix[a]	5	5	5	5
DAG	135	-	135	-
TAG	-	135	-	135
Water	2595	2595	1730	1730

[a] Contains: copper 4000 mg/kg; iodine 560 mg/kg; iron 2.4%; manganese 2000 mg/kg; selenium 120 mg/kg; zinc 4.32%; vitamin A 218 mg/kg; vitamin D_3 2.95 mg/kg; vitamin E 5455 mg/kg; vitamin B_{12} 1.82 mg/kg; riboflavin 272.7 mg/kg; d-pantothenic acid 1364 mg/kg; thiamine 75 mg/kg; niacin 3182 mg/kg; vitamin B_6 90.9 mg/kg; folic acid 143.6 mg/kg; choline chloride 41.2 g/kg; choline 35.8 g/kg; d-biotin 4.5 mg/kg

Table A-3 Fatty acid composition of experimental diets[a] in dog study (relative %)

	Diet			
Fatty Acid	AD	AT	WD	WT
14:0	0.23	0.19	0.25	0.38
14:1n-5	ND	ND	TR	ND
16:0	11.55	12.54	10.30	16.40
16:1n-7	2.15	1.89	2.26	3.52
17:0	TR	0.10	TR	0.12
17:1	0.11	ND	0.11	ND
18:0	4.11	5.36	3.89	5.66
18:1n-7	3.89	10.04	2.89	3.31
18:1n-9	39.67	31.19	38.74	36.57
18:2n-6	30.10	29.86	32.91	27.08
18:3n-3	2.76	3.28	3.33	2.93
20:0	0.41	0.65	0.72	0.42
20:1n-9	0.93	0.94	0.65	0.58
20:2n-6	ND	0.17	ND	ND
20:3n-3	ND	ND	ND	ND
20:3n-6	ND	ND	ND	ND
20:4n-6	0.37	0.28	0.43	0.37
20:5n-3	ND	ND	ND	ND
22:0	TR	0.60	0.39	0.41
22:1n-9	ND	0.19	TR	0.13
22:4n-6	TR	ND	TR	ND
22:5n-3	ND	ND	ND	ND
22:6n-3	0.36	0.78	0.42	0.68
24:0	ND	ND	ND	ND
24:1	0.60	0.55	0.49	0.63
Unidentified	2.53	1.38	1.92	1.20
SFA	16.46	19.44	15.64	23.39
MUFA	47.35	44.80	45.27	44.74
PUFA	33.67	34.37	37.18	31.05
n-3	3.12	4.06	3.75	3.60
n-6	30.55	30.31	33.43	27.45

[a] All diets 19.7% fat (as fed) and 4228 kcal/kg as estimated by calculation from nutrient composition using modified Atwater factors.
Values are averages of two representative samples.
ND = not detected; TR = trace (< 0.1%); AD = high amylose/DAG; AT = high amylose/TAG; WD = waxy corn/DAG; WT = waxy corn/TAG; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table A-4 Fatty acid composition of acclimation diet[a] in dog study

Fatty Acid	Relative %
14:0	0.30
14:1n-5	ND
16:0	13.77
16:1n-7	2.37
17:0	ND
17:1	ND
18:0	5.11
18:1n-7	3.17
18:1n-9	38.15
18:2n-6	28.31
18:3n-3	5.45
20:0	0.56
20:1n-9	0.62
20:2n-6	ND
20:3n-3	ND
20:3n-6	ND
20:4n-6	0.41
20:5n-3	ND
22:0	ND
22:1n-9	ND
22:4n-6	ND
22:5n-3	0.28
22:6n-3	0.41
24:0	0.33
24:1	0.26
Unidentified	0.23
SFA	20.07
MUFA	44.57
PUFA	35.13
n-3	6.14
n-6	28.99

[a] 19.7% fat (as fed) and 4228 kcal/kg as estimated by calculation from nutrient composition using modified Atwater factors.
Values are averages of two representative samples.
ND = not detected; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

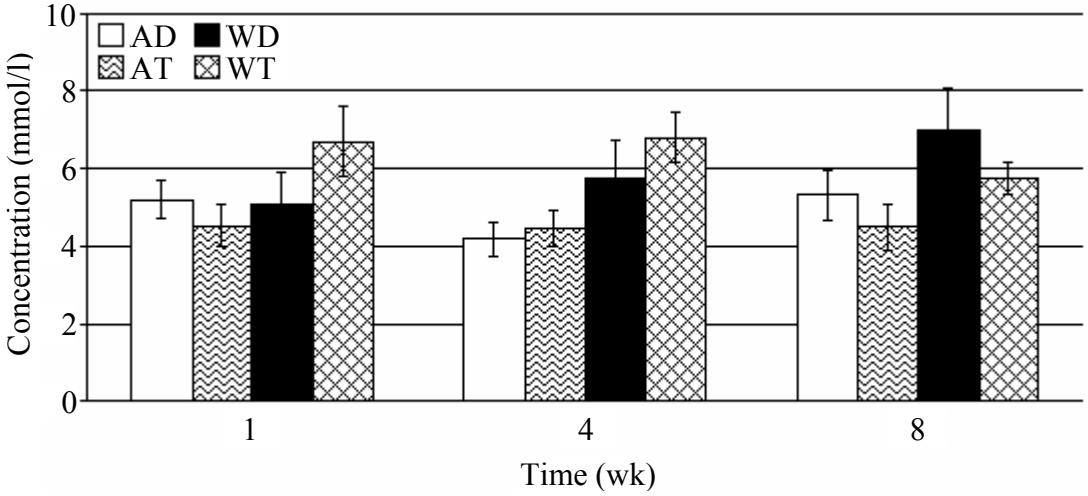

Figure A-1 Total cholesterol concentrations of dogs fed diets differing in starch type (high amylose [A] or waxy corn [W]) and oil type (diacylglycerol [D] or triacylglycerol [T]). Sample sizes varied, see text for description. Values are mean ± SEM.

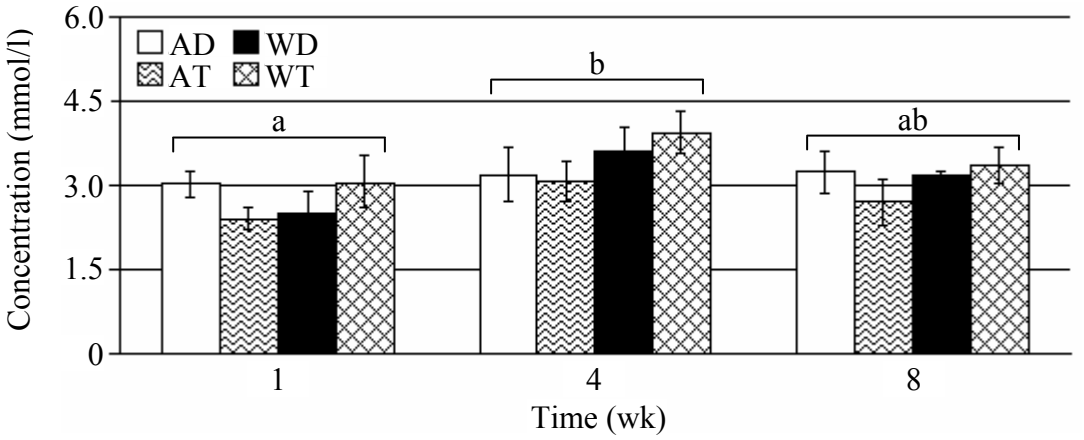

Figure A-2 Unesterified cholesterol of dogs fed diets differing in starch type (high amylose [A] or waxy corn [W]) and oil type (diacylglycerol [D] or triacylglycerol [T]). Sample sizes varied, see text for description. Values are mean ± SEM. Letters not in common indicate a significant difference between weeks by repeated measures ANOVA, $p < 0.05$.

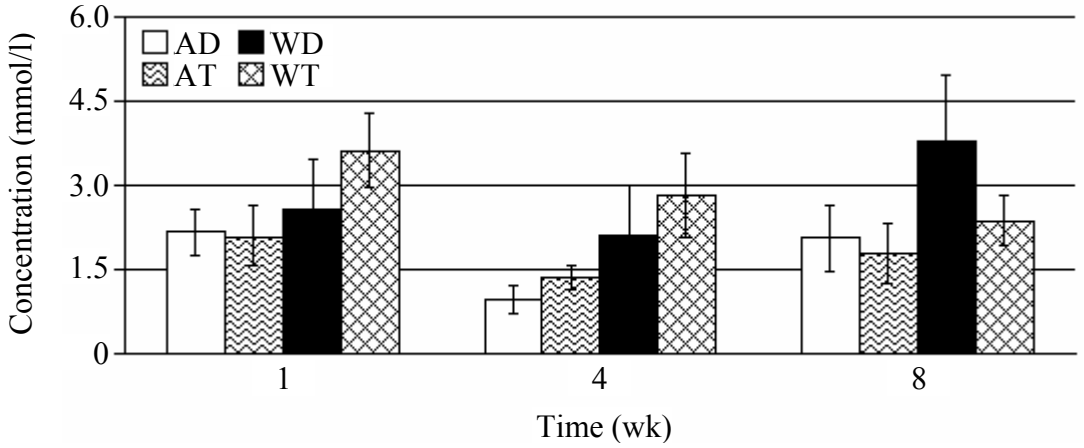

Figure A-3 Esterified cholesterol concentrations of dogs fed diets differing in starch type (high amylose [A] or waxy corn [W]) and oil type (diacylglycerol [D] or triacylglycerol [T]). Sample sizes varied, see text for description. Values are mean ± SEM.

APPENDIX B

Calculation of LCAT activity:

LCAT activity (nmol of UC esterified/ml·hr) = (A – B) × (median of all C[a] ÷ C) × D

A = Sample EC ÷ (Sample EC + Sample UC)[b]

B = Blank EC ÷ (Blank EC + Blank UC)[b]

C = Control EC ÷ (Control EC + Control UC)[b]

D = Plasma UC (mg/dl)[c] × (1 g / 1000 mg) × (1 mol / 386.66 g of UC) × (10^9 nmol / 1 mol) × (1 dl / 100 ml) × 0.2 ml × 5 × 2

[a] Median value of 17 control plasma samples
[b] As determined by LCAT activity assay
[c] As determined by plasma cholesterol analysis

APPENDIX C

Table C-1 Fatty acid composition of pre-experimental period diet[a] in cat study

Fatty Acid	Relative %
14:0	1.46
14:1n-5	TR
16:0	21.86
16:1n-7	3.43
17:0	0.30
18:0	9.80
18:1n-7	3.15
18:1n-9	35.88
18:2n-6	17.25
18:3n-3	1.87
20:0	0.29
20:1n-9	0.73
20:2n-6	0.48
20:3n-3	TR
20:3n-6	0.15
20:4n-6	0.62
20:5n-3	1.03
22:0	ND
22:1n-9	ND
22:5n-3	0.17
22:6n-3	ND
24:0	0.68
24:1	ND
Unidentified	0.71
SFA	34.39
MUFA	43.24
PUFA	21.65
n-3	3.15
n-6	18.50

[a] Hill's Science Diet® Kitten Original; 24.8% fat and 4208 kcal/kg as reported by manufacturer.
Values are averages of two representative samples.
ND = not detected; TR = trace (< 0.1%);
SFA = saturated fatty acids;
MUFA = monounsaturated fatty acids;
PUFA = polyunsaturated fatty acids.

Table C-2 Fatty acid composition and diet characteristics of experimental kibble, oils, and complete diets in cat study (relative %)

		Oil[a]			Diet (kibble + oil)		
Fatty Acid	Kibble[a]	H	M	S	H[a]	M[b]	S[a]
14:0	1.55	TR	7.80	TR	1.38	4.19	1.45
14:1n-5	0.24	ND	ND	ND	0.23	0.28	0.23
16:0	17.05	3.36	15.88	5.98	14.35	19.84	15.39
16:1n-7	1.66	TR	11.36	TR	1.38	5.64	1.36
17:0	ND	ND	ND	ND	0.57	0.83	0.51
18:0	9.43	3.23	2.80	2.35	9.26	10.14	8.50
18:1n-7	3.12	1.26	3.31	0.80	3.06	4.34	3.16
18:1n-9	43.98	82.37	7.02	13.52	53.98	23.57	24.59
18:2n-6	20.87	7.67	1.55	74.78	13.47	11.36	43.52
18:3n-3	1.17	0.22	1.44	0.28	0.66	1.09	0.69
20:0	ND	0.29	0.16	0.46	0.34	0.30	0.37
20:1n-9	ND	0.24	1.42	0.17	0.29	0.78	0.23
20:2n-6	ND	ND	0.26	ND	ND	ND	ND
20:3n-3	ND	ND	0.25	ND	ND	ND	ND
20:3n-6	ND	ND	1.05	ND	ND	0.14	ND
20:4n-6	ND	ND	0.24	ND	0.19	0.57	ND
20:5n-3	ND	ND	14.09	ND	0.12	5.24	ND
22:0	ND	0.68	ND	0.19	ND	0.36	ND
22:1n-9	ND	ND	TR	ND	ND	ND	ND
22:4n-6	ND	ND	0.19	ND	ND	ND	ND
22:5n-3	ND	0.20	2.56	ND	ND	1.02	ND
22:6n-3	ND	ND	12.58	ND	ND	5.13	ND
24:0	ND	0.18	ND	TR	0.17	ND	ND
24:1	ND	ND	0.29	0.11	ND	ND	ND
Unidentified	0.92	0.30	15.74	1.36	0.55	5.17	0.00
SFA	28.04	7.78	26.64	9.14	26.08	35.67	26.23
MUFA	49.00	83.95	23.49	14.69	58.93	34.61	29.57
PUFA	22.04	8.10	37.75	75.07	14.44	24.55	44.21
n-3	1.17	0.42	34.45	0.28	0.78	12.48	0.69
n-6	20.87	7.67	3.30	74.78	13.66	12.07	43.52
% fat (as fed)[c]	11.6	-	-	-	16.6	16.1	17.7
kcal/kg[d]	3442	-	-	-	3816	3816	3816

[a] Values are averages of two representative samples.
[b] Values from one representative sample; according to the manufacturer's specifications, contained significant amounts of fatty acids not present in our standard mixture, such as 3.53% of 18:4n-3.
[c] Determined gravimetrically.
[d] Estimated by calculation from nutrient composition using modified Atwater factors.
ND = not detected; TR = trace (< 0.1%); SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

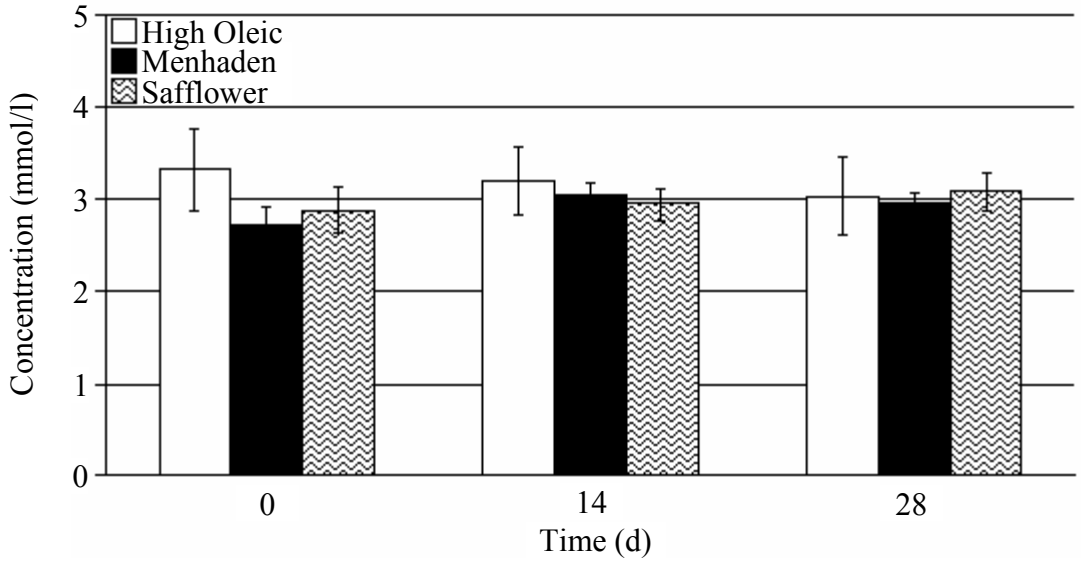

Figure C-1 Total cholesterol of cats fed diets enriched with high oleic (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil. Values are mean ± SEM.

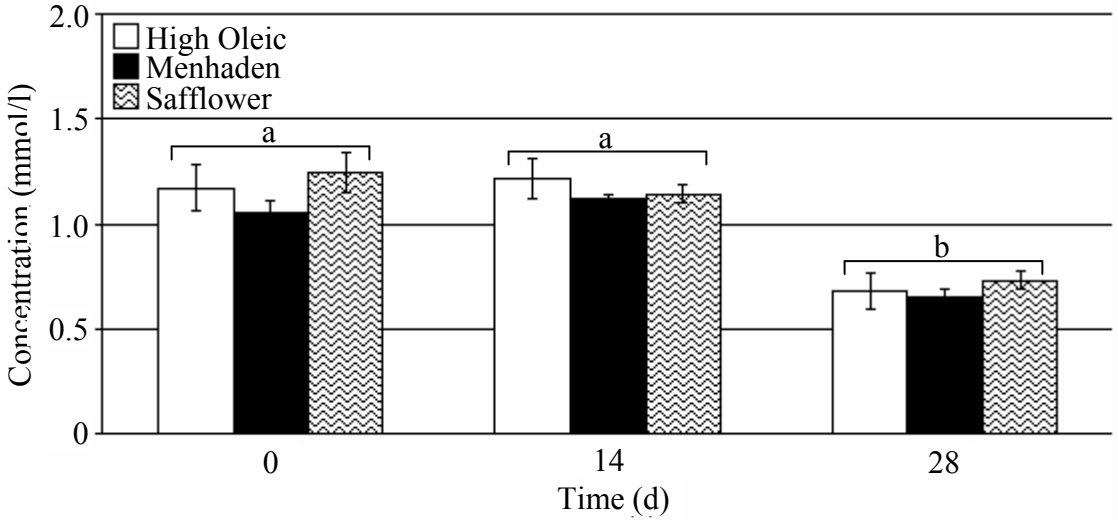

Figure C-2 Unesterified cholesterol of cats fed diets enriched with high oleic (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil. Values are mean ± SEM. Letters not in common indicate a significant difference between weeks by repeated measures ANOVA on transformed data, $p < 0.05$.

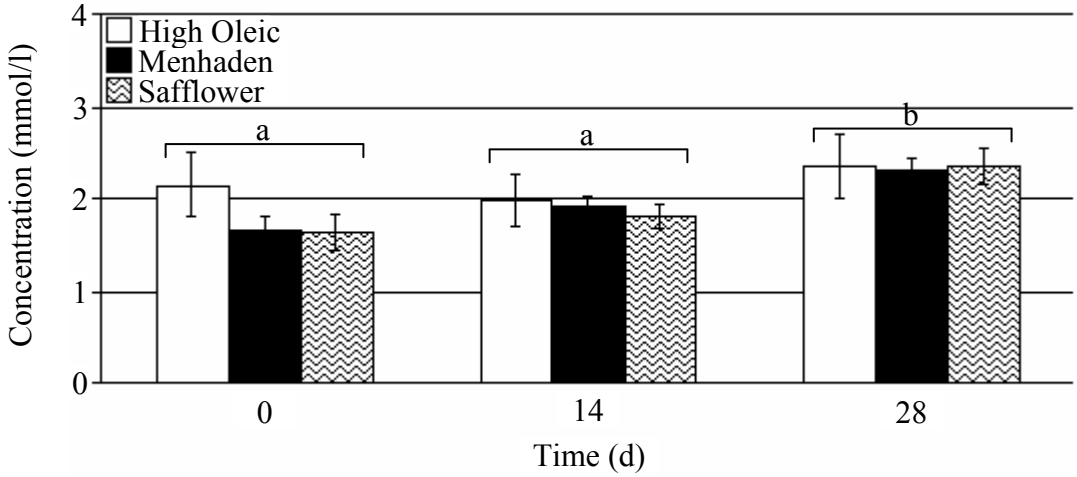

Figure C-3 Esterified cholesterol of cats fed diets enriched with high oleic (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil. Values are mean ± SEM. Letters not in common indicate a significant difference between weeks by repeated measures ANOVA on transformed data, $p < 0.05$.

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