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Dietary diacylglycerol oil has no effect on hypertriacylglycerolaemia in lipoprotein lipase-deficient cats

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A commercially available vegetable oil containing a high concentration (87 %, w/w) of diacylglycerol (DAG) has been investigated in humans and animals for potential beneficial effects in reducing serum TAG concentrations in fasting and postprandial states. Effects of DAG oil as a sole dietary fat source (25 % metabolisable energy) were evaluated in a feline model of hypertriacylglycerolaemia. Eleven adult (1·5 (SEM 0·1) years) male cats deficient of lipoprotein lipase (LPL) catalytic activity from a heritable point mutation of the *LPL* gene were acclimatised to a semi-purified diet containing TAG oil for 21 d. After assignment into two groups, pair-matched by serum TAG concentrations (range 6·1–31·6 mmol/l), the cats were fed the diet with either TAG or DAG oil for 8 d. The dietary fat source was crossed-over and presented for 8 d more. Non-fasting serum concentrations of TAG, cholesterol and NEFA were measured on days 6–8 and days 14–16. Dietary fat source (DAG *v.* TAG) did not significantly affect food intake (491 (SEM 16) *v.* 486 (SEM 14) kJ/kg$^{0·67}$), body weight or serum concentrations (mmol/l) of TAG (37·1 (SEM 4·5) *v.* 33·9 (SEM 3·4)), cholesterol (4·8 (SEM 0·3) *v.* 4·8 (SEM 0·2)) and NEFA (1·4 (SEM 0·2) *v.* 1·4 (SEM 0·2)). The results show that for a feeding trial of 8 d, DAG oil was well accepted and tolerated by cats but did not reduce hypertriacylglycerolaemia resulting from a deficiency of LPL catalytic activity.

Triacylglycerol: Diacylglycerol: Hypertriacylglycerolaemia: Lipoprotein lipase deficiency: Cats

Diacylglycerol (DAG) is a natural component of vegetable oils, ranging from 1·0 % (w/w) in soyabean oil to 9·5 % in cottonseed oil[1,2]. An edible oil rich in DAG (>80 %, w/w) has been synthesised by the reverse reaction of 1,3-specific lipase on vegetable oils such as soyabean and rapeseed which yields 1,3-DAG and 1,2 (2,3)-DAG in a ratio of 7:3 at equilibrium[3,4]. DAG oil has been shown in human and animal studies to reduce fasting concentrations and postprandial elevations in serum TAG concentrations, increase energy expenditure and fat utilisation, reduce body fat accumulation and enhance weight loss[4–7]. The mechanism underlying these effects is hypothesised to be due to the unique molecular structure of DAG oil instead of the fatty acid composition. Unlike dietary TAG, which undergoes hydrolysis by gastric and pancreatic lipases to 2-monoacylglycerol (2-MAG) and NEFA, 1,3-DAG is hydrolysed to 1(3)-MAG and NEFA. The 1(3)-isoform has less affinity for acyl-CoA:monoacylglycerol acyltransferase, the main enzymic pathway involved in re-esterification of TAG in enterocytes, which leads to decreased synthesis of chylomicrons, increased release of NEFA into the portal circulation, and increased β-oxidation in hepatocytes[5].

In human studies, substitution of dietary DAG oil for TAG oil has been shown to reduce hypertriacylglycerolaemia resulting from various lipid disorders[8–13]. Case studies in patients with severe hypertriacylglycerolaemia due to deficiencies of lipoprotein lipase (LPL) or apo C-II have reported lower serum TAG concentrations with DAG oil compared with TAG oil[14,15]. Based on these observations, we hypothesised that substitution of DAG oil for dietary fat in an animal model of LPL deficiency would reduce serum TAG concentrations. The assumption is that fatty acids derived from DAG would be less utilised in synthesis of circulating lipoproteins (chylomicrons, VLDL) than those derived from TAG. However, other human studies using DAG oil have found no effect on hypertriacylglycerolaemia[16–18].

The animals used in the present study were adult domestic cats with LPL deficiency due to a mis-sense mutation in an exon of the LPL gene that leads to a substitution of arginine for glycine at residue 412[19]. Cats homozygous with this mutation are globally devoid of LPL catalytic activity yet survive to adulthood and are able to reproduce[20–23]. The phenotype in these cats is similar to that of familial LPL deficiency in humans, which is a rare autosomal recessive disorder affecting an estimated one in one million of the population[24]. Both LPL-deficient humans and cats have marked hypertriacylglycerolaemia, with reported serum TAG concentrations of 22–325 mmol/l in humans and 2–135 mmol/l in cats (normal fasting values: humans <1·7; cats <0·5). Because these cats are otherwise healthy when maintained

Abbreviations: BW, body weight; DAG, diacylglycerol; LPL, lipoprotein lipase.

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on low-fat diets, they have the potential to serve as a unique model for evaluation of the effects of DAG oil on hypertriacylglycerolaemia resulting from LPL deficiency.

Experimental methods

Animals

Twelve specific pathogen-free domestic shorthair male cats aged 1·5 (SEM 0·1) years, body weight (BW) 4·6 (SEM 0·2) kg were obtained from and individually housed at the Feline Nutrition and Pet Care Center (University of California, Davis, CA, USA). Daily exercise and socialisation were provided in a group setting. Each cat was determined to be homozygous for the Gly412Arg LPL mutation by PCR-based mismatch analysis[19]. Cats were given fresh food and water each morning, and remaining food from the previous day was collected and frozen for later DM determination. Diets and water were offered for *ad libitum* consumption. Food intakes, BW, faecal quality and general health observations were recorded daily. Body condition scores were assigned weekly according to a nine-point integer system in which 5 is ideal, 1 is leanest and 9 is the heaviest possible body condition[25].

Test oils

The evaluated DAG-rich oil was a commercially available product (Enova® oil; Kao Health and Nutrition LLC, Itasca, IL, USA). A TAG oil was prepared by mixing rapeseed oil, soyabean oil (Crisco® Pure Rapeseed and Crisco® Pure Vegetable Oil; J. M. Smucker Company, Orrville, OH, USA) and safflower-seed oil (LouAna Pure Safflower Oil; Ventura Foods, Brea, CA, USA) so that the fatty acid profile was similar to that of the DAG oil (Table 1). The fatty acid compositions of the TAG and DAG oils were analysed by extraction-methylation and GC[26].

Diets

Two semi-purified diets were formulated to meet or exceed the recommended nutrient allowances for maintenance of adult cats[27]. The diets differed only in oil source (Table 2). Diets were mixed and extruded through a meat grinder die (Hobart M-802, Hobart 4812, 1 cm die; Troy, OH, USA) to form pellets. The diets were stored frozen and thawed immediately before use.

Table 1. Acylglycerol and fatty acid compositions of the test oils

	DAG oil	TAG oil
Acylglycerol species (g/100 g)*		
Monoacylglycerol	0·6	0
DAG	87·4	0·1
TAG	11·0	>98·5
Fatty acid composition (g/100 g)†		
16 : 0	3·3	6·6
18 : 0	0·5	1·5
18 : 1	40·4	40·5
18 : 2	47·1	43·5
18 : 3	7·9	6·5

DAG, diacylglycerol.
* Analysed at Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO, USA.
† As measured from lipid extracts followed by methylation and GC analyses as described in Experimental methods (*n* 3).

Design

Two identical dietary cross-over studies were conducted approximately 6 months apart. For each study, six cats were gradually adapted from the standard colony diet, a commercial dry-type extruded diet (34 % metabolisable energy from fat, Whiskas® for kittens; Mars, Brentwood, TN, USA) to the semi-purified TAG diet for 21 d. At 2 d before the start of the study, a non-fasting 1 ml blood sample was collected from each cat by jugular venepuncture and submitted to a commercial laboratory (IDEXX, West Sacramento, CA, USA) for determination of serum TAG concentration. Cats were pair-matched based on serum TAG concentrations (range 6·1–31·6 mmol/l) and assigned to two groups of three cats each so that the range in serum TAG concentrations was similar between the groups. One group continued to receive the TAG diet after the adaptation period while the other group was switched to the DAG diet. After 8 d, the diets were crossed-over and presented for 8 d more with no

Table 2. Diet composition (g/kg, as-fed basis) and macronutrient content*

Treatment diet...	DAG	TAG
Ingredients (g/kg as-fed)		
Casein, high-N†	300	300
Soya protein isolate‡	250	250
Maize starch†	200	200
Sucrose†	91	91
DAG oil§	100	0
TAG oil‖	0	100
Mineral mix¶	40	40
Vitamin mix**	10	10
Choline††	4	4
Arachidonic acid‡‡	3	3
Taurine§§	2	2
Water added (g/kg diet)	400	400
Macronutrient content		
ME (MJ/kg DM)	17·1	17·1
Protein (% ME)	47	47
Fat (% ME)	25	25
Carbohydrate (% ME)	28	28

DAG, diacylglycerol; ME, metabolisable energy.
* Calculated assuming protein, carbohydrate, and fat contain 16·7, 16·7 and 37·7 MJ/kg, respectively.
† Dyets, Inc. (Bethlehem, PA, USA).
‡ Supro 661 (Dyets, Inc., Bethlehem, PA, USA).
§ Enova® oil (Kao Health and Nutrition LLC, Itasca, IL, USA).
‖ Crisco® Pure Rapeseed and Crisco® Pure Vegetable Oil (J. M. Smucker Co., Orrville, OH, USA), LouAna Pure Safflower Oil (Ventura Foods LLC, Brea, CA, USA).
¶ NRC Cat Salt Mix (Dyets, Inc., Bethlehem, PA, USA) provided (g/kg diet): calcium phosphate tribasic, 570; potassium phosphate, 250; sodium chloride, 35; potassium chloride, 80; magnesium oxide, 19; manganous carbonate, 0·35; ferric citrate, 13; zinc carbonate, 2·8; cupric carbonate, 0·35; potassium iodate, 0·02; sodium selenite, 0·01; chromium potassium sulfate, 0·55; sucrose, 28·92.
** NRC Cat Vitamin Mixture (Dyets, Inc., Bethlehem, PA, USA) provided (g/kg diet): thiamin HCl, 0·7; riboflavin, 0·5; pyridoxine HCl, 0·6; niacin, 4·5; calcium pantothenate, 0·7; folic acid, 0·09; biotin, 0·01; vitamin B_{12} (0·1 %), 2·2; vitamin A palmitate (500 000 U/g), 1·3; vitamin D_3 (400 000 U/g), 0·15; vitamin E acetate (500 U/g), 10·0; menadione sodium bisulfite, 0·02; myo-inositol, 20; sucrose, 959·23.
†† Choline chloride (Dyets, Inc., Bethlehem, PA, USA).
‡‡ VEVODAR Crude Arachidonic Oil (DSM Food Specialties, Delft, the Netherlands).
§§ Sigma Chemical Co. (St Louis, MO, USA).

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washout period. The 8 d test period was selected to allow sufficient time for lipoprotein turnover[20].

Samples and analyses

Non-fasting blood samples were collected by jugular venepuncture from each cat on days 6–8 and again on days 14–16. The samples were allowed to clot, centrifuged at about 1200 ***g*** for 10 min, and serum separated and frozen at −20°C for later analysis.

Serum TAG. A commercial enzymic-colorimetric kit (Infinity™; Thermo Electron, Pittsburgh, PA, USA) that measures glycerol after hydrolysis of acylglycerol was used to determine serum TAG concentrations. Due to the unusually high triacylglycerolaemia, serum was diluted from 1:5 to 1:15 with normal saline (0·9 % NaCl) so that assayed TAG concentrations were in the linear range of the test kit.

Serum cholesterol. Serum turbidity even after serial dilutions precluded use of colorimetric kits for cholesterol determinations. Cholesterol was determined in an organic-phase extract of serum by HPLC[28] with minor modifications. For each assay, 50 μl serum was mixed with 2 ml alcoholic potassium hydroxide solution (56·1 g potassium hydroxide in 1 litre ethanol) in a loosely capped glass tube and incubated in a 75°C water-bath for 30 min. After cooling, 2 ml deionised water was added and mixed. The tube was agitated with 5 ml hexanes for 15 min in a horizontal position then centrifuged at 200 ***g*** for 5 min. A 3 ml sample of hexanes (upper layer) was dried under N_2 gas at 40°C. The residue was dissolved in 0·85 ml isopropanol and 50 μl reconstitute was injected onto an HPLC column (Microsorb 100-5 C18, 250 × 4·6 mm; Varian, Lake Forest, CA, USA). Cholesterol was eluted with an isocratic mobile phase (1:1 acetonitrile–isopropanol, 1·0 ml/min) in a peak monitored at 205 nm approximately 8·5 min after injection.

Serum NEFA. A commercial enzymic-colorimetric kit (Wako Diagnostics, Richmond, VA, USA) was used to determine serum NEFA concentration. Before analysis, samples were centrifuged at 13 000 ***g*** for 10 min at 4°C. The infranatant fraction (serum separated from the lipid layer) was used in the assay to avoid interference from sample turbidity.

Diet samples. The food intake of each cat was determined from the daily difference in DM mass of food offered and food remaining. For this, frozen (−15°C) samples of presented diet and uneaten diet samples were collected each day. Samples were dried in a convection oven at 110°C for 48 h and weighed to determine the DM. Each batch of diet was assayed for fatty acid composition by extraction-methylation and GC[26].

Calculations and statistical analysis

Results are expressed as means with their standard errors. Paired *t* tests and one-way repeated-measures ANOVA with Bonferroni adjustment for multiple comparisons were used to evaluate the effect of diet type and diet presentation sequence on mean serum concentrations of TAG, cholesterol and NEFA. The relationship between food intake and serum biochemical variables was evaluated by calculating Pearson correlation coefficients. Differences were considered significant at $P<0·05$. Statistical analysis was performed with SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) and SigmaStat (version 3.5; Systat Inc., San Jose, CA, USA).

The care and housing of the cats and the experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of California, Davis, CA, USA. All institutional, state and federal guidelines were followed during the study.

Results

One cat was found to have normal serum TAG concentrations (pooled mean 0·52 (SEM 0·17) mmol/l). Repeat analysis for occurrence of the Gly412Arg mutation in this cat revealed normal LPL alleles. Therefore, all observations from this cat were excluded from further analysis.

Body weights and general health

All cats accepted the semi-purified diet during the acclimatision period and the study periods. BW and body condition scores were maintained throughout the trial. Soft stool but not diarrhoea was noted in three cats on nine occasions; six while eating the DAG diet and three while eating the TAG diet.

Serum TAG, cholesterol and NEFA

Serum TAG concentrations varied by more than 400 % among the cats (Fig. 1). In contrast, within each cat, serum TAG concentrations were substantially less variable. The mean CV (SD/mean × 100) of TAG concentrations within cats during the TAG diet periods was only 23 ± 20 % and during the DAG diet periods was only 19 ± 13 %.

There was no significant effect of sampling day on serum TAG, cholesterol and NEFA concentrations. Therefore, mean concentrations were determined for each dietary period for each cat and these means were used for evaluation of the effect of diet. Serum TAG concentrations were not significantly different when cats received the TAG diet compared with the DAG diet ($P=0·47$) (Table 3). The diets

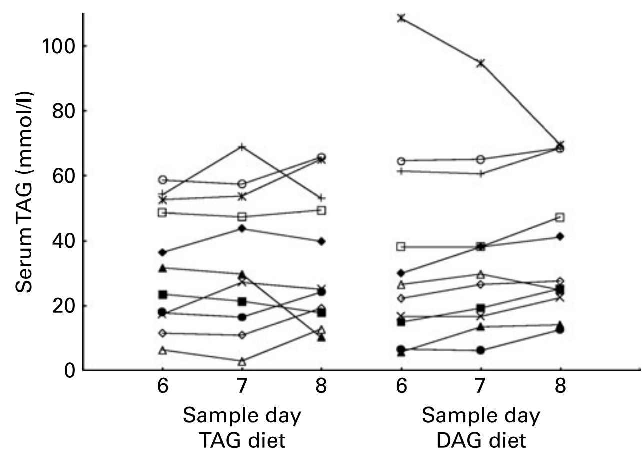

Fig. 1. Serum TAG concentration in lipoprotein lipase-deficient cats given diets with diacylglycerol (DAG) and TAG vegetable oils as the fat sources. Each symbol and connecting lines indicate observations common to each cat.

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Table 3. Serum TAG, cholesterol and NEFA concentrations in lipoprotein lipase-deficient cats when given diets with TAG or diacylglycerol (DAG) vegetable oils as fat sources

(Mean values with their standard errors for eleven cats)

Treatment diet...	DAG		TAG		
	Mean	SEM	Mean	SEM	P
TAG (mmol/l)	37·09	4·52	33·91	3·41	0·47
Cholesterol (mmol/l)	4·82	0·29	4·77	0·24	0·81
NEFA (mmol/l)	1·36	0·18	1·39	0·18	0·62

had no significant effect on serum cholesterol and NEFA concentrations ($P=0{\cdot}81$, 0·62). The order of diet presentation had no significant effect on serum TAG, cholesterol and NEFA concentrations (Table 4).

To evaluate the effect of amount of food intake on the serum measurements, the significance of correlations between food intake and serum concentrations of TAG, cholesterol and NEFA were determined. For this analysis, food intake was calculated as the mean daily DM intake over the 8 d period of diet presentation and corresponding serum concentrations were calculated as the means of TAG, cholesterol and NEFA from the three sampling days of each diet period. No significant correlations were found between food intake and serum concentrations of TAG, cholesterol and NEFA when either diet was fed ($P>0{\cdot}33$).

Discussion

The semi-purified diets presently used contained either TAG or DAG oil as the sole fat source. The diets were well accepted by all cats as indicated by food intake observations. Intake of the diets on a metabolisable energy basis (Table 5) was higher than the reported requirement for lean cats at maintenance, 418·4 kJ/kg $BW^{0{\cdot}67}$[27]. The DAG oil appeared well tolerated because BW, faecal quality and overall health when the DAG oil diet was presented were not different from observations when the TAG oil diet was presented. Based on these findings, we conclude that DAG oil is palatable for cats and is suitable for inclusion in a complete and balanced feline diet. Long-term feeding studies of DAG-enriched diets are needed to verify nutritional adequacy.

There is normally a wide variation in circulating TAG concentrations among homozygous LPL-deficient cats[20,23,29]. This was confirmed by a more than 400 % range in serum TAG concentrations observed in the present study (Fig. 1). Because of the great between-individual variance, we elected

Table 4. Serum concentrations of TAG, cholesterol and NEFA by diet sequence (six cats for diacylglycerol (DAG)/TAG column; five cats for TAG/DAG column)

(Mean values with their standard errors)

Diet sequence...	DAG/TAG		TAG/DAG		
	Mean	SEM	Mean	SEM	P
TAG (mmol/l)	35·77	3·29	35·27	4·42	0·53
Cholesterol (mmol/l)	4·83	0·23	4·76	0·28	0·16
NEFA (mmol/l)	1·42	0·18	1·33	0·17	0·67

Table 5. Daily food intakes by diet and intakes normalised to metabolic body weight (BW)

(Mean values with their standard errors for eleven cats)

Treatment diet...	DAG		TAG		
	Mean	SEM	Mean	SEM	P
Food intake (g)*	79·8	2·5	78·5	2·4	0·71
Food intake (kJ/kg $BW^{0{\cdot}67}$)†	491	15·9	486	14·1	0·82

DAG, diacylglycerol.
* DM basis (per d).
† Metabolisable energy, metabolic body weight basis (per d).

to evaluate dietary treatment effects with a cross-over design and balance grouping for order of diet presentation by preliminary serum TAG concentrations. We observed that TAG concentrations were relatively stable within individuals even with sampling during *ad libitum* food intake. The stability in serum TAG concentrations probably reflects inconsequential impact of meals. Serum TAG is very slowly cleared in LPL-deficient cats[20], and TAG formed from the eleven to twenty meals per d typically consumed by cats[30] probably contributes only a small fraction to the large pool of circulating TAG in LPL-deficient cats.

The cause for the large and consistent between-individual differences in serum TAG concentrations was not apparent. Serum TAG concentrations did not appear to reflect the amount of dietary fat consumed because the TAG concentrations were not significantly correlated with food intake. Activity of secondary mechanisms of TAG removal, perhaps involving hepatic lipase[31], endothelial lipase[32] or receptor-mediated transport[33], might underlie individual differences in serum TAG concentrations. These secondary mechanisms would appear especially effective in cats. Normalisation of triacylglycerolaemia in LPL deficiency after an oral fat challenge occurs more rapidly in cats (about 24 h)[20] than in humans (>40 h)[34].

We did not identify lower mean serum TAG concentrations in cats receiving the DAG diet compared with the TAG diet (Table 3). Therefore, our observations do not support the hypothesis that substitution of DAG oil as the sole source of dietary fat reduces serum TAG concentrations in this animal model. Although dietary DAG oil did not appear to be harmful in LPL-deficient cats, we did not find evidence of a benefit. It is noteworthy that our findings may not reflect responses of humans with LPL deficiency. Metabolism of DAG oil in cats may importantly deviate from that in humans.

A simple explanation for a lacking effect of DAG oil is that the ability of the oil to lower TAG may depend on the presence of functional LPL. Chylomicrons generated by the metabolism and absorption of DAG were reported to be more efficiently hydrolysed compared with those generated by dietary TAG oil in mice[35]. The LPL-deficient cats studied are devoid of LPL catalytic activity and do not produce an immunoreactive mutant protein[19]. Other enzymes such as hepatic lipase and endothelial lipase may be principally responsible for chylomicron hydrolysis in LPL-deficient cats[29]. Activities of these enzymes in removal of chylomicrons may be little affected by dietary fat source. A non-catalytic function of LPL may mediate the beneficial effect of dietary DAG oil. Dietary fat substitution with DAG oil was recently reported to reduce

triacylglycerolaemia in a human patient with apo C-II deficiency[15]. Though not catalytically active, LPL was present in the patient. However, a beneficial effect of DAG oil in reducing triacylglycerolaemia was reported in a patient with no functional LPL mass or LPL catalytic activity[14].

Cats have many similarities to humans with respect to lipid metabolism including abundant and separable HDL2 and HDL3 subfractions[31,36]. Serum cholesterol was presently measured as an indicator of the potential effects of DAG oil on cholesterol-rich lipoproteins. Serum cholesterol concentrations were not significantly affected by the dietary fat source (Table 3). This observation is consistent with other human and animal studies in that total cholesterol concentrations remain unchanged by dietary DAG oil, with few exceptions in which serum cholesterol was lowered[37–40].

Although DAG oil fatty acids are reputedly absorbed principally as NEFA[6], the jugular venous serum NEFA concentrations presently observed were unaffected by dietary DAG (Table 3). This observation is also consistent with previous reports[11,37,41]. However, in some studies serum NEFA concentrations were higher with DAG oil compared with TAG oil[42,43]. LPL-deficient compared with normal cats tend to have higher NEFA concentrations when food is continuously present, while in normal cats NEFA levels are higher when food is withheld[29]. In the present study, diets were not withheld at any time and NEFA fluctuated within a narrow range of concentrations.

The study was designed to evaluate short-term effects of DAG oil on serum TAG concentrations. Food was continuously presented, and there was no attempt to collect fasting or postprandial blood samples. In a previous report of LPL-deficient cats, plasma TAG concentrations after an oral fat load increased 10-fold (from 0·97 to 9·36 mmol/l) and peaked at 7 h compared with a 2-fold increase and 3 h peak in normal cats[20]. Further studies are needed to determine if DAG oil has the potential to reduce postprandial TAG concentrations or increase the rate of clearance in LPL-deficient cats.

In summary, the present results show that cats voluntarily consume and tolerate diets prepared with DAG oil at 10 % DM weight and 25 % metabolisable energy for short time periods. The present results also show that the marked hypertriacylglycerolaemia seen with LPL deficiency is unchanged by substitution of TAG with DAG oil. Our finding of a lack of effect of DAG oil on triacylglycerolaemia is not without precedent. Lowering of triacylglycerolaemia in normal animals is not consistently reported. Nevertheless, the lack of effect on triacylglycerolaemia that we observed may be unique to the LPL deficiency model studied, or it may be a consequence of a unique attribute of the fatty acid metabolism of cats. Future studies of dietary applications of DAG oil in LPL-deficient and normal cats are warranted. These should include evaluation of postprandial effects on lipid profiles and long-term studies on DAG effects on energy metabolism, obesity prevention and treatment, and disorders such as insulin resistance and diabetes mellitus, which are observed in cats.

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C. A. D. designed the study, carried out most of the experimental procedures and prepared the manuscript. R. C. B. assisted with study design, experimental procedures and the manuscript. He served as the mentor for C. A. D.'s graduate school training. K. L. F. assisted with experimental procedures and provided laboratory support.

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