

# Effect of dietary protein quality and fatty acid composition on plasma lipoprotein concentrations and hepatic triglyceride fatty acid synthesis in obese cats undergoing rapid weight loss

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**Objective**—To determine effects of dietary lipid and protein on plasma lipoprotein and free fatty acid concentrations and hepatic fatty acid synthesis during weight gain and rapid weight loss in cats.

**Animals**—24 ovariohysterectomized cats.

**Procedure**—Cats were fed a high energy diet until they gained 30% of their ideal body weight and then randomly assigned to receive 1 of 4 weight reduction diets (6 cats/diet) at 25% of maintenance energy requirements. Diets contained a low or high quality protein source and a lipid source deficient or sufficient in long chain essential fatty acids. Plasma samples and liver biopsy specimens were obtained before and after weight gain and during and after weight loss for determination of free fatty acid, triglyceride, and lipoprotein concentrations. Synthesis of these substances was measured by use of isotope enrichment.

**Results**—Plasma total cholesterol concentration and concentration of lipoprotein fractions increased after weight gain, compared with baseline values. Weight loss resulted in a significant decrease in concentrations of all lipoprotein fractions except high density lipoprotein. High density lipoprotein concentration was significantly greater in cats fed diets containing an oil blend, compared with cats fed diets containing corn oil. Fatty acid synthesis after weight loss was below the detection limit of the measurement technique.

**Conclusions and Clinical Relevance**—In cats undergoing rapid weight loss there is neither increased triglyceride synthesis nor decreased transport of very low density lipoproteins from the liver, suggesting that their involvement in the development of hepatic lipidosis may be minimal. (*Am J Vet Res* 2000;61:566–572)

Obese cats are reported to be at greater risk for development of certain diseases, such as osteoarthritis, ligament injuries, perineal dermatitis, diabetes mellitus, lower urinary tract disease, and

cardiomyopathy.<sup>1-3</sup> Mortality risk is also increased. Therefore, it is critical that cats maintain an optimum body weight to minimize disease risk. Safe weight loss plans must consider diet composition and rate of weight loss to minimize the risk of developing, among other pathologic conditions, hepatic lipidosis (HL).

Safe, rapid weight loss in cats can be difficult, because cats have special dietary requirements that appear to make them more susceptible than other species to HL. Hepatic lipidosis in cats is an acquired disorder caused by excessive accumulation of triglycerides in hepatocytes that ultimately impair liver function. This disorder is often referred to as idiopathic HL, because the underlying cause is not known in approximately half of affected cats. Cats are obligate carnivores; they cannot conserve nitrogen, because feline liver enzymes are unable to adapt when nitrogen intake decreases below a minimal level. Cats may also require a dietary source of long chain essential fatty acids (LCEFA; arachidonic acid [22:4n6] and docosahexaenoic acid [22:6n3]), because  $\Delta 6$  desaturase is only minimally active.<sup>4,5</sup> Therefore, common vegetable oil sources of fatty acids, such as corn oil, appear to be inadequate for meeting LCEFA requirements. Deficiency of n6 fatty acids is known to induce fatty livers in cats<sup>6</sup> and other animals.<sup>7,8</sup> Long chain essential fatty acid deficiencies also affect lipoprotein transport from the liver and activities of lipoprotein lipase, lecithin cholesterol acyltransferase, and fatty acid synthetase.<sup>9,10</sup> Alterations of any of these variables may contribute to development of HL. Thus, it is plausible that cats are more susceptible to LCEFA deficiency and the associated changes in hepatic lipid metabolism than are other species. Diets that have been used in recent studies to induce HL in cats are most likely LCEFA-deficient, because they were only supplemented with corn oil.<sup>11</sup>

Triglycerides accumulate in the liver when rate of removal from the liver is less than rate of synthesis. The majority of lipids are removed from the liver as triglyceride packaged into very low density lipoproteins (VLDL) or oxidized through peroxisomal or mitochondrial  $\beta$ -oxidation. In feline plasma, VLDL cholesterol concentrations are low, compared with high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations.<sup>12,13</sup> Cats with HL have 30 times more triglyceride and 20 times more cholesterol ester stores in hepatocytes than

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unaffected cats.<sup>14</sup> Patterns of hepatic triglyceride fatty acid composition in cats with HL are suggestive of LCEFA deficiency.<sup>14</sup> Both hepatic and adipose tissue arachidonic and docosahexaenoic acid concentrations are profoundly reduced in cats with HL, compared with healthy cats. As speculated by others,<sup>4,6</sup> LCEFA status of cats may be compromised, because  $\Delta 6$  desaturase activity is minimal. During an anorectic period in cats, LCEFA may not be synthesized from precursor fatty acids in an amount adequate to meet needs, because the majority of lipids stored in adipose tissue are saturated and monounsaturated fatty acids, and the predominant PUFA is linoleic acid. This lack of LCEFA may contribute to the pathogenesis of HL.

We hypothesized that LCEFA deficiency (which may develop during rapid weight loss in cats) and dietary protein quality contribute to the pathogenesis of HL. Because LCEFA and adequate concentrations of amino acids are needed for synthesis of specific phospholipids and proteins that form components of cell membranes (eg, VLDL, peroxisomes, and mitochondria), deficiency of either may result in inadequate synthesis of VLDL or altered oxidation of fatty acids. Triglyceride transport from the liver would thus decrease, and instead, triglycerides may accumulate. In rats that are deficient in essential fatty acids, serum concentration of VLDL is less than in rats sufficient in essential fatty acids.<sup>15</sup>

The etiopathogenesis of HL appears to be multifactorial, with an interplay between factors that regulate composition and deposition of fatty acids into membrane and storage lipids, mobilization of fatty acids from lipid stores, peroxisomal and mitochondrial oxidation of fatty acids, re-esterification of fatty acids into hepatic triglycerides, and transport of hepatic triglycerides via VLDL. To date, researchers who have induced HL in cats did so using primarily starvation diets<sup>16-18</sup> or diets with only single nutrients (protein, carbohydrate, or fat),<sup>11</sup> which may have resulted in multinutrient (minerals, vitamins, etc) deficiencies. Also, to our knowledge, there have been no studies that address interactions of dietary protein and lipid on plasma lipoprotein and plasma free fatty acid (FFA) concentrations or hepatic triglyceride synthesis in cats. The purpose of the study presented here was to assess hepatic triglyceride synthesis and interactions of dietary lipid and protein on plasma lipoprotein and FFA concentrations during weight gain and weight loss in cats. Specifically, the study was designed to test the hypothesis that a diet containing high quality protein and LCEFA would prevent HL in obese cats undergoing rapid weight loss by increasing transport of lipids from the liver as VLDL, decreasing hepatic triglyceride synthesis, or decreasing plasma FFA concentration.

## Materials and Methods

**Animals**—Twenty-four female cats (Hsd Cpb:CaDs) between 2 to 5 years old were purchased from a commercial dealer.<sup>a</sup> Cats were individually housed in an American Association for Laboratory Animal Sciences accredited facility, maintained on a 12 hour light-dark cycle at an average

room temperature of 21 C, and provided with a commercial diet and water ad libitum. After 1 week of acclimatization, cats were ovariohysterectomized.<sup>19</sup> Surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

**Experimental protocol**—Following surgery, cats were fed a high quality energy dense diet<sup>b</sup> ad libitum until each cat had gained a minimum of 30% over their ideal lean body weight, at which time they were considered obese. Once cats had become obese, they were assigned randomly to receive 1 of the 4 following diets<sup>19</sup> in staggered intervals (4 cats/wk; 1 cat/diet/wk): low quality protein (corn gluten meal) supplemented with a lipid source deficient in LCEFA (corn oil; n = 6; CGCO diet), low quality protein supplemented with a lipid source sufficient in LCEFA (oil blend; n = 6; CGOB diet), high quality protein (casein) supplemented with a lipid source deficient in LCEFA (n = 6; CACO diet), and high quality protein supplemented with a lipid source sufficient in LCEFA (n = 6; CAOB diet). Diets were fed to cats at 25% of maintenance energy requirements (MER)<sup>19</sup> for 7 to 8 weeks, or until cats had lost 30 to 40% of their obese body weight.

Body weight was recorded weekly throughout the study. Blood (10 ml) was collected after food had been withheld from cats for 16 hours 2 days prior to ovariohysterectomy (baseline), after weight gain (obese), 21 days after initiation of weight-reduction diets (day-21 weight loss), and after termination of weight-reduction diets (final). Blood was collected into glass tubes with EDTA for plasma. Plasma samples were stored at -70 C prior to analysis.

Wedge biopsy specimens of the liver were obtained from anesthetized cats during ovariohysterectomy (baseline), after cats attained a minimum 30% weight gain (obese), and after loss of at least 30% of obese body weight (final; obtained during isotope enrichment protocol).

**Weight-reduction diets**—Weight reduction diets were formulated by a commercial company<sup>c</sup> and are described in detail elsewhere.<sup>19</sup>

**Plasma lipoprotein analysis**—Plasma lipoproteins were fractionated by use of the modified method of Brousseau et al.<sup>20</sup> Cholesterol and triglyceride concentrations in VLDL and cholesterol concentrations in intermediate density lipoproteins (IDL), LDL, and HDL were measured enzymatically, using commercially available cholesterol and triglyceride kits.<sup>d</sup>

To prepare VLDL, 400  $\mu$ l of plasma was transferred to a polycarbonate tube (11  $\times$  34 mm), overlaid with 600  $\mu$ l of a potassium bromide (KBr) solution with a density of 1.006, and ultracentrifuged at 435,680  $\times$  g for 2 hours at 15 C. The upper fraction (400  $\mu$ l) containing VLDL was harvested by aspiration. To prepare IDL, density of the remaining lower fraction was adjusted to 1.019 by addition of 24.3  $\mu$ l of a KBr solution with a density of 1.34. Volume was adjusted to 1 ml by adding the appropriate volume of KBr solution with a density of 1.019. The sample was ultracentrifuged again, and the upper fraction that contained IDL was harvested by aspiration. To prepare LDL, 600  $\mu$ l of the remaining lower fraction was mixed with 95.3  $\mu$ l of a KBr solution with a density of 1.34 and 304.7  $\mu$ l of a KBr solution with a density of 1.063. This sample was ultracentrifuged at 435,680  $\times$  g for 2.5 hours at 15 C, and the upper fraction (400  $\mu$ l) that contained LDL was harvested by aspiration. Finally, to prepare HDL, 600  $\mu$ l of the remaining lower fraction was adjusted to a density of 1.21 by addition of 678.5  $\mu$ l of a KBr solution with a density of 1.34. Volume was adjusted to 1.3 with 21.5  $\mu$ l of a KBr solution with a density of 1.21. One milli-

liter of this sample was ultracentrifuged at  $435,680 \times g$  for 3 hours at 15 C, and the upper fraction (400  $\mu$ l) that contained HDL was harvested by aspiration. To determine cholesterol concentration in the HDL fraction, we used a correction factor of 1/3.

**Determination of plasma FFA concentration—**Plasma FFA (nonesterified) concentrations were determined, using a commercially available enzymatic colorimetric kit.<sup>c</sup>

**Determination of fatty acid, triglyceride, and cholesterol synthesis—**To measure synthesis of hepatic and VLDL fatty acids and triglycerides after rapid weight loss, we used a modification of the isotopic enrichment of total body water, using the deuterated water ( $D_2O$ ) method described by Diraison et al<sup>21</sup> and Lee et al,<sup>22</sup> as well as isotopomer analysis.<sup>23,24</sup> Synthesis of hepatic cholesterol was also determined by use of these methods. Briefly, after cats had lost weight, they were given a loading dose of  $D_2O^f$  (35 ml/kg of body weight, SC). For the next 7 days, cats were given free access to water that contained 7%  $D_2O$ . Blood samples were obtained prior to and 72 and 168 hours after administration of the loading dose of  $D_2O$ ; a wedge biopsy specimen of the liver was obtained at 168 hours. Lipids were extracted from plasma and biopsy specimens with hexane:isopropanol (3:2, vol/vol) containing 0.05% butylated hydroxy toluene.<sup>25</sup> Total plasma lipids were used to determine concentration of VLDL. We assumed that after a 12-hour withdrawal of food, chylomicron would not contribute to the circulating triglyceride pool. Extracted hepatic lipids were separated, using silica gel H plates<sup>g</sup> and a solvent system consisting of hexane:ethyl ether:glacial acetic acid (80:20:1, vol/vol), into triglyceride, cholesterol, and phospholipid fractions on the basis of concomitant fractionation of standards.<sup>4</sup> Bands containing each lipid fraction were scraped from the plates, and lipids were eluted with hexane:isopropanol (2:1, vol/vol). Triglycerides were transmethylated with 14% boron trifluoride-methanol, and cholesterol was converted to the trimethylsilyl (TMS) derivative.<sup>26,d</sup> Samples were analyzed for deuterium enrichment by use of gas chromatography-mass spectrometry (GC-MS). Methylated samples were injected into the GC-MS<sup>h</sup> connected to a mass selective detector<sup>i</sup> and equipped with a DB-5ms<sup>l</sup> capillary column (30 m  $\times$  0.32 mm [outside diameter]  $\times$  0.25 mm [inside diameter]). Helium flow was maintained at 0.8 ml/min with an electronic pressure control, and injection port and transfer line temperatures were set at 280 C. The initial temperature for the column was 130 C for 3 minutes; temperature was then increased in 20 C/min increments to 280 C and held there for 5 minutes. Fatty acid methyl ester data were collected on the molecular ions (myristate, 242 MW; palmitate, 270 MW; stearate, 298 MW), and an additional 15 to 20 ions were collected for these fatty acid methyl esters. For cholesterol GC-MS analysis, the injection port temperature was 340 C, and the transfer line temperature was 310 C. The initial temperature for the column was 200 C for 2 minutes; temperature was then increased in 25 C/min increments to 300 C and held there for 14 minutes. Cholesterol data were collected on a major fragment of the TMS-cholesterol ester (mass +0, 368) by use of selected ion monitoring. Twenty ions (mass -1 through +18) were monitored.

**Statistical analyses—**Data were analyzed by use of 2-way ANCOVA, with obesity as a covariant, followed by least square means analysis to determine significant differences between treatment groups. Differences were considered significant when  $P \leq 0.05$ .

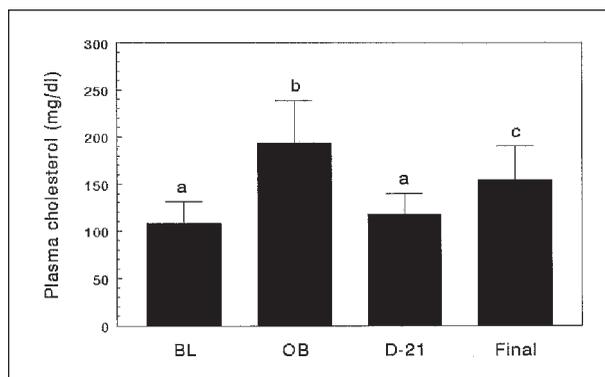


Figure 1—Mean ( $\pm$  SD) plasma cholesterol concentration determined in 22 ovariohysterectomized cats before (BL) and after (OB) gaining at least 30% of their ideal lean body weight, after 21 days on a weight-reduction diet (D-21), and after losing at least 30% of their obese body weight (final). Values with different letters are significantly ( $P < 0.05$ ) different.

## Results

**Weight gain and weight loss—**After ovariohysterectomy, cats gained a mean of 4.7 g/d until 80 days after surgery, at which time further weight gain was not detected. Irrespective of the weight reduction diet fed, cats in the different treatment groups lost weight at a comparable rate.<sup>19</sup> Three cats developed HL during the weight loss period.<sup>19</sup> One cat in the CAO group and 1 cat in the CACO group consumed less than 25% of MER; data from these cats were not included during calculation of group means.

**Plasma total cholesterol and triglyceride concentrations—**Mean plasma cholesterol concentration increased significantly after weight gain (obese concentration), compared with baseline concentration (Fig 1). Regardless of weight-reduction diet fed, mean cholesterol concentration after cats had been fed diets for 21 days (21-day weight loss concentration) was significantly less than the obese concentration; however, concentration at this time was not significantly different from baseline. At the end of the weight loss period, total plasma cholesterol concentration was significantly greater than baseline and 21-day weight loss concentrations, and was also significantly different from the obese concentration.

Mean obese plasma triglyceride concentration was significantly greater than concentration determined after weight loss (final concentration). However, obese concentration was not significantly different from baseline concentration (mean [ $\pm$  SD] baseline,  $48.61 \pm 37.2$  mg/dl; obese,  $60.65 \pm 38.2$  mg/dl; 21-day weight loss,  $42.52 \pm 14.03$  mg/dl; final,  $38.18 \pm 19.56$  mg/dl). Significant differences in plasma total triglyceride or plasma total cholesterol concentrations were not detected among the dietary treatment groups.

**Plasma VLDL cholesterol and triglyceride concentrations—**Mean plasma VLDL cholesterol concentration in obese cats was significantly greater than baseline concentration. In addition, obese concentration was greater than final concentration in all dietary treatment groups (Fig 2). At the end of the weight

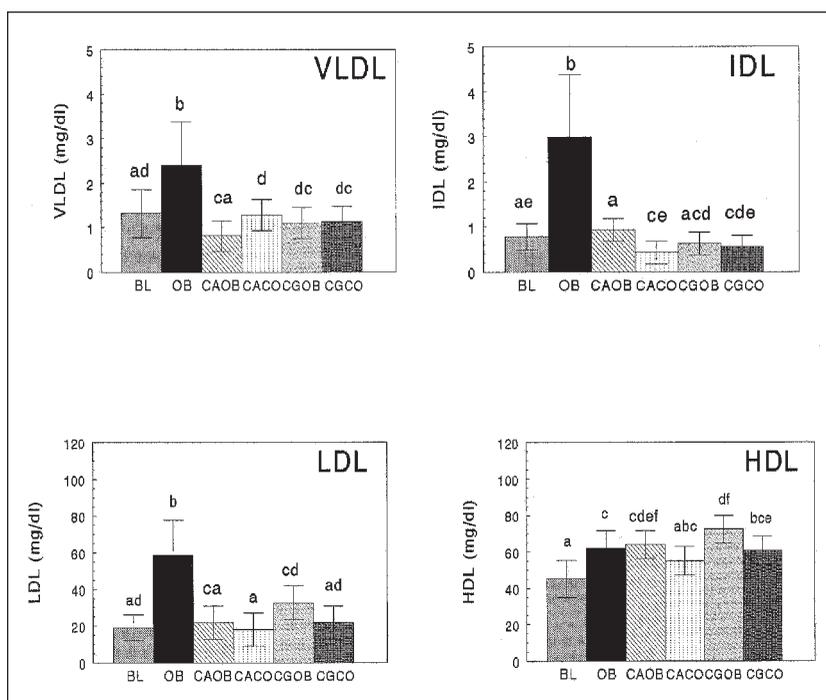


Figure 2—Mean ( $\pm$  SD) plasma lipoprotein cholesterol concentrations determined in ovariectomized cats before (BL) and after (OB) gaining at least 30% of their ideal lean body weight and after losing at least 30% of their obese body weight (CAOB, CACO, CGOB, CGCO). After gaining weight, cats were randomly assigned to 1 of the 4 following weight-reduction diets: casein with oil blend (CAOB;  $n = 5$ ); casein with corn oil (CACO;  $n = 5$ ); corn gluten meal with oil blend (CGOB;  $n = 6$ ); and corn gluten meal with corn oil (CGCO;  $n = 6$ ). VLDL = Very low density lipoprotein. IDL = Intermediate density lipoprotein. LDL = Low density lipoprotein. HDL = High density lipoprotein. See Figure 1 for key.

loss period, mean final VLDL cholesterol concentration for cats in the CAOB group was significantly less than that for cats in the CACO group. Changes in VLDL triglyceride concentration were similar to those detected for cholesterol, except that triglyceride concentration for cats in the CAOB group was significantly greater than for cats in the CGCO group (baseline,  $6.98 \pm 2.02$  mg/dl; obese,  $7.64 \pm 1.92$  mg/dl; CAOB [final],  $5.59 \pm 1.195$  mg/dl; CACO [final],  $4.91 \pm 0.946$  mg/dl; CGOB [final],  $5.05 \pm 0.86$  mg/dl; CGCO [final],  $4.25 \pm 1.24$  mg/dl). Furthermore, in the 3 cats that developed HL, mean final plasma VLDL triglyceride concentration was slightly, but not significantly, greater than obese concentration (obese,  $7.86$  mg/dl; final,  $8.97$  mg/dl). We did not detect significant effects of dietary oil or protein or oil-protein interactions on plasma VLDL cholesterol or triglyceride concentrations.

**Plasma IDL cholesterol concentration—** Significant effects of oil on IDL cholesterol concentration were detected. Plasma IDL cholesterol concentration was approximately 4-fold greater in obese cats, compared with baseline and final concentrations (Fig 2). Furthermore, final IDL cholesterol concentration in the CAOB group was significantly greater than in the CACO and CGCO groups.

**Plasma LDL cholesterol concentration—** Significant oil or protein effects or oil-protein interac-

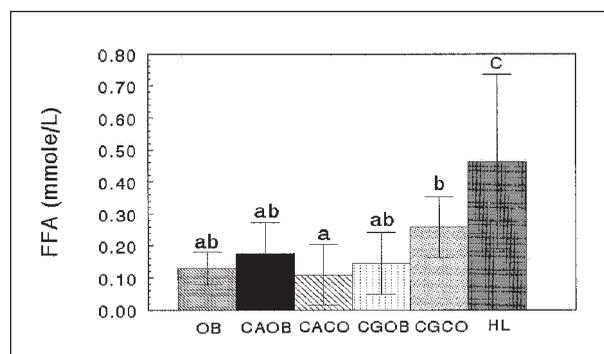


Figure 3—Mean ( $\pm$  SD) plasma free fatty acid (FFA) concentration determined in 22 obese ovariectomized cats before (OB) and after (OB) gaining at least 30% of their obese body weight (CAOB, CACO, CGOB, CGCO). Obese cats were randomly assigned to 1 of 4 weight-reduction diets. Final FFA concentration was also determined in 3 cats that developed hepatic lipodosis (HL). See Figures 1 and 2 for key.

tions on LDL cholesterol concentrations were not detected. However, obese LDL cholesterol concentrations were significantly greater than baseline or final concentrations in all dietary treatment groups (Fig 2). Final concentration for the CGOB group was significantly different from that for the CACO group.

**Plasma HDL cholesterol concentration—** Composition of dietary lipid had a significant effect on plasma HDL cholesterol concentration; however, effect

of dietary protein was not significant ( $P = 0.502$ ). We did not detect a significant effect of oil-protein interaction on HDL cholesterol concentration. Obese HDL cholesterol concentration was significantly greater than baseline concentration but did not differ from final concentration for any dietary treatment group. Final concentration of HDL cholesterol in the CGOB group was significantly greater than in the CACO and CGCO groups.

**Plasma FFA concentration**—Significant effects of dietary oil or protein on FFA concentration were not detected. However, protein-oil interaction did have a significant effect. Plasma FFA concentration in the CGCO group was significantly greater than in the CAOB group (Fig 3). Final mean FFA concentration in cats that developed HL was significantly increased, compared with concentrations before and after rapid weight loss in all other cats.

**Triglyceride, fatty acid, and cholesterol synthesis**—Following  $D_2O$  administration, fractional abundance of the isolated hepatic triglyceride palmitate was not significantly different from enrichment of standard palmitate. Furthermore, we did not detect significant incorporation of deuterium into hepatic myristate or stearate, VLDL palmitate, or plasma or hepatic cholesterol. After weight loss, synthesis of fatty acids incorporated into triglycerides or VLDL and cholesterol was less than the detection limit of the GC-MS (incorporation of deuterium less than 2% of each anaylate).

## Discussion

Cats, unlike most other animals, may develop HL during periods of rapid weight loss or anorexia. The 4 most likely mechanisms involved in HL are increased triglyceride synthesis, decreased fatty acid oxidation, increased transport of fatty acids to the liver, and decreased transport of VLDL from hepatocytes. Our data suggest that synthesis of hepatic and plasma fatty acids and cholesterol is negligible in cats after a period of rapid weight loss. There are 3 main paths by which deuterium can be incorporated into newly synthesized lipids. Deuterium may be directly incorporated from water or indirectly through exchange with NADH or NADPH. Alternatively, deuterium may be derived from the methyl group deuterium of acetyl-CoA.<sup>21-23</sup> Percentage of hepatic palmitate that contained deuterium (ie, percentage of newly synthesized palmitate) determined by use of isotope enrichment and isotopomer analysis is between 39 and 50% in rats fed ad libitum.<sup>21</sup> It is not surprising that in cats with an energy deficit, percentage of newly synthesized hepatic or VLDL fatty acids was less than the detection limit of GC-MS (2%). These results suggest that HL in cats may not be caused by increased synthesis of acetate units into myristate, palmitate, or stearate, because we did not detect deuterium incorporation into these fatty acids. Chain elongation of mobilized myristate or palmitate to stearate is unlikely to contribute to development of HL, because we also did not detect deuterium incorporation into palmitate or stearate. Only 3

cats in the present study developed HL during the rapid weight-loss period; however, we did not detect deuterium incorporation into triglyceride fatty acids in these 3 cats.

High concentrations of hepatic lipids in cats with HL may be a result of decreased transport of VLDL from the liver. We did detect a significant decrease in plasma VLDL cholesterol after weight loss in all dietary treatment groups, compared with concentrations before weight loss. However, the only significant difference in final concentration was between the CACO and CAOB groups. Cats that developed HL had plasma VLDL cholesterol concentrations similar to concentrations in cats without HL, suggesting that plasma VLDL concentrations are not altered in affected cats. Furthermore, because VLDL synthesis was negligible, it appears that VLDL transport is not limiting, and therefore, does not contribute to the development of HL. Although decreases in plasma cholesterol concentrations during the first 21 days of the weight-loss period were expected, it is unclear why cholesterol concentration significantly increased after weight loss, particularly because we did not detect synthesis of hepatic or plasma VLDL cholesterol. Although we detected significant differences in concentrations of IDL and LDL cholesterol among dietary treatment groups, these differences did not correlate with development of HL. We did detect a significant effect of dietary oil on concentration of HDL cholesterol. Cats that received diets supplemented with the oil blend had plasma HDL cholesterol concentrations that were greater than cats that received diets with corn oil. The significant difference in obese and final total plasma cholesterol concentrations, compared with baseline concentration, was primarily a result of changes in concentration of HDL cholesterol. Concentrations of VLDL, IDL, and LDL cholesterol returned to baseline values following rapid weight loss, but HDL cholesterol concentration increased further after weight loss. In the 3 cats that developed HL, final VLDL triglyceride concentration remained at or slightly greater than mean obese concentration, suggesting that VLDL triglyceride concentrations may remain high to transport more lipids from the liver. There was negligible synthesis of fatty acids for incorporation into VLDL triglyceride in these cats. Although we cannot rule out that decreased VLDL synthesis contributes to HL, our data and data reported by Pazak et al<sup>27</sup> suggest that other mechanisms may play a more important role. Others have reported that serum cholesterol concentration increased during a briefer weight loss period (28 days) in lean and obese cats.<sup>12</sup> It should be pointed out although cholesterol concentrations changed significantly in our study and the study by Dimski et al,<sup>12</sup> these concentrations were within reference ranges. These changes in total cholesterol concentration were most likely not related to increased hepatic cholesterol synthesis, because deuterium was found to be incorporated into < 2% of total hepatic cholesterol synthesized at the end of the weight reduction period with the cats in a catabolic state. Decreased HDL clearance or increased HDL production may be associated with greater cellular breakdown during a catabolic state. It appears that a weight-reduction diet containing an oil

blend as the lipid source may be more beneficial than a diet containing corn oil for maintaining HDL concentrations, because HDL cholesterol concentration in the oil blend groups was significantly greater than in the corn oil groups. Feeding diets that contain corn oil as the sole lipid source to humans and other animals will result in a decrease in HDL concentration.<sup>28</sup>

Our data suggest that neither increased triglyceride synthesis or decreased VLDL transport from the liver is the primary mechanism involved in the development of HL during rapid weight loss in cats. Therefore, the mechanisms involved in development of HL in cats are not clear but may be associated with increased mobilization of lipid stores, as has been suggested by others.<sup>14,29</sup> We did detect significantly greater final plasma FFA concentrations in cats that developed HL, compared with final concentrations for unaffected cats. Moreover, final FFA concentration in the CGCO group was somewhat higher than in the other treatment groups. Biourge et al<sup>11</sup> also detected significant increases in FFA concentrations in cats undergoing partial fasts. This increase was most pronounced in cats that received either protein or lipid as the sole energy source; the lowest concentration of FFA was found in the carbohydrate supplemented group. During periods of decreased food intake, the glucagon-to-insulin concentration ratio increases, which stimulates the release of hormone-sensitive lipase. This, in turn, results in the rapid release of fatty acids from adipose triglyceride stores.<sup>14</sup> These fatty acids are transported to the liver and other tissues via albumin, where they are primarily oxidized for energy. In the liver, peroxisomes and mitochondria are the sites of oxidation. In cats with HL, number of hepatic peroxisomes are profoundly less than in unaffected cats.<sup>30,31</sup> Number of peroxisomes is related to duration of decreased food intake.<sup>17</sup> Furthermore, Center et al<sup>30</sup> have shown that cats with HL develop mitochondrial alterations. Protein and n6 fatty acids appear to be involved in the regulation of peroxisome proliferation,<sup>31,32</sup> but it is unclear how poor dietary protein quality and lack of LCEFA may result in a decreased number of peroxisomes or altered mitochondria. Perhaps LCEFA are critical for formation of peroxisomal or mitochondrial membrane structure. The reduced number of peroxisomes may also decrease carnitine biosynthesis<sup>33</sup> and thereby diminish fatty acid mitochondrial  $\beta$ -oxidation.

<sup>a</sup>Harlan Sprague Dawley, Indianapolis, Ind.

<sup>b</sup>Eukanuba Veterinary Diets, Nutritional Recovery Formula and

Ocean Fish Formula Cat Food, The Iams Co, Dayton, Ohio.

<sup>c</sup>The Iams Co, Dayton, Ohio.

<sup>d</sup>Sigma Chemical Co, St Louis, Mo.

<sup>e</sup>Boehringer Mannheim, Indianapolis, Ind.

<sup>f</sup>99.8% D, Cambridge Isotope Inc, Andover, Mass.

<sup>g</sup>Alltech, Deerfield, Ill.

<sup>h</sup>Hewlett-Packard model 5890 (series II) GC, Palo Alto, Calif.

<sup>i</sup>MSD, Hewlett-Packard model 5971, Palo Alto, Calif.

<sup>j</sup>Alltech, Deerfield, Ill.

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