Influence of dietary protein and lipid on weight loss in obese ovariohysterectomized cats

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Objective—To determine effects of dietary lipid and protein on development of hepatic lipidosis (HL) and on physical and biochemical indices following 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 per day. Diets contained a low or high quality protein source and a lipid source deficient or sufficient in long chain essential fatty acids (LCEFA). Serum and plasma samples and liver biopsy specimens were obtained for biochemical analyses and determination of hepatic lipid content before and after weight gain and during and after weight loss.

Results—Irrespective of weight-reduction diet fed, all cats lost weight at a comparable rate (4.51 to 5.00 g/d/kg of obese body weight). Three cats developed hepatic lipidosis. Significant changes in plasma insulin, cholesterol, triglyceride, and serum glucose concentrations were detected after weight gain and weight loss in all diet groups, but values for these variables did not differ among groups.

Conclusions and Clinical Relevance—Cats can lose 25 to 30% of their obese body weight over 7 to 9 weeks without developing overt clinical signs of HL, provided that weight-reduction diets are highly palatable, contain a high quality protein, have a source of LCEFA, and are fortified with vitamins and microminerals. However, rapid weight loss may increase risk factors associated with development of diabetes mellitus. (*Am J Vet Res* 2000;61:559–565)

Approximately 10 to 40% of cats receiving veterinary care in the US are reported to be overweight.[1-3] Obese cats are at greater risk for developing certain diseases, and mortality risk for these cats is increased, compared with nonobese cats.[4,5] Factors that contribute to obesity are a sedentary lifestyle, confinement to an indoor environment, and neutering.[5] Neutered cats have a greater tendency toward weight gain than sexually intact cats,[6] which may be attributable to decreased activity and altered metabolic rates in neutered cats[6-8] that exacerbate the imbalance between energy intake and output. Because of the increased health risks associated with obesity,[5] it is critical that cats maintain an optimum body weight.

The most successful approach to weight loss in cats appears to be a regimen that induces rapid weight loss, while at the same time maximizes compliance and minimizes risk of developing **hepatic lipidosis (HL)**.[5] Hepatic lipidosis is an acquired disorder caused by excessive accumulation of triglycerides in hepatocytes that ultimately impair liver function. In approximately half of affected cats, the underlying cause of HL is not known. To minimize the risk of developing HL, safe weight loss plans must consider both diet composition and rate of weight loss.

Safe, rapid weight loss in cats is made more difficult because of special dietary requirements that appear to make them more susceptible to HL than other species. Cats are obligate carnivores and cannot conserve nitrogen. Moreover, common vegetable oil sources of fatty acids, such as corn oil, may not adequately meet essential fatty acid requirements, because activity of Δ6 desaturase is minimal in cats.[9,10] Cats lack the ability to effectively desaturate 18-carbon unsaturated fatty acids. Thus, they also require 20-carbon **long chain essential fatty acids (LCEFA**; arachidonic acid [20:4n6] and, possibly, docosahexaenoic acid [22:6n3]). A deficiency in essential fatty acids is known to induce fatty livers in cats[11] and other animals.[12,13] Essential fatty acid deficiency is also known to affect lipoprotein transport from the liver and activities of lipoprotein lipase, lecithin cholesterol acyltransferase, and fatty acid synthetase.[14,15] These alterations may contribute to the development of HL. Clinical signs of essential fatty acid deficiency generally require many months to develop in cats fed diets deficient in these fatty acids but sufficient in energy and protein. However, to our knowledge, turnover of essential fatty acids during rapid weight loss in cats has not been studied.

Dietary protein quality is also important in the management of HL. Feeding 25% of the calculated energy requirement as high quality protein will attenuate HL but will not ameliorate the condition.[16] Development of HL in cats has been hypothesized to be associated with decreased lipoprotein transport from the liver as **very low density lipoprotein (VLDL)**,

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alterations in fatty acid oxidation, increased flux of fatty acids into the liver, and increased hepatic fatty acid synthesis. However, in studies that tested these hypotheses,[16,17] results did not account for other nutrient deficiencies (eg, vitamins and minerals).

The purpose of the study presented here was to determine effects of diets sufficient in vitamins and microminerals that contained either high or low quality protein and 20- and 22-carbon or 18-carbon essential fatty acids on biochemical and histologic indices related to rapid weight loss in ovariohysterectomized obese cats. Specifically, this study was designed to test the hypothesis that feeding 20- and 22-carbon n3 and n6 fatty acids along with a high quality protein would prevent development of HL during rapid weight loss in such cats.

Materials and Methods

Animals—Twenty-four female cats (Hsd Cpb:CaDs) between 2 and 5 years old were purchased from a commercial dealer.[a] 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 anesthetized with ketamine hydrochloride (15 mg/kg of body weight, IM), acepromazine (1 mg/kg, IM), and isoflurane administered via a small animal rebreathing circuit and were ovariohysterectomized. 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[b] ad libitum until each cat had gained a minimum of 30% over their ideal lean body weight, at which time they were considered obese. Ideal lean body weight was determined from age, growth, body length, and weight data provided by the dealer that supplied the cats. Once cats had become obese (between 11 and 16 weeks), they were assigned randomly to receive 1 of the 4 following diets **(Tables 1 and 2)** 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); low quality protein supplemented with a lipid source sufficient in LCEFA (oil blend; 6); high quality protein (casein) supplemented with a lipid source deficient in LCEFA (n = 6); and high quality protein supplemented with a lipid source sufficient in LCEFA (6). Diets were fed to cats at 25% of **maintenance energy requirements (MER)**, which was determined according to the following formula:

$$\text{kcal/d} = (\{[\text{ideal body weight (kg)} \times 30 \text{ kcal/kg}] + 70\} \times 1.4)/4$$

where 1.4 is the activity factor.[16-19] Weight reduction diets were fed until cats had lost 30 to 40% of their obese body weight (7 to 9 weeks).

Body weight was recorded weekly throughout the study. Thoracic circumference was measured before and after weight loss. Food was withheld from cats for 16 hours, and cats were sedated with medetomidine hydrochloride and atopanezole hydrochloride prior to blood collection. Blood (10 ml) was collected from the jugular vein into glass tubes with EDTA and without additives for plasma and serum, respectively, 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). Serum and 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).

Cats that developed hyperbilirubinemia (serum concentration > 0.4 mg/dl) during the weight-loss period were removed from the study.

Weight-reduction diets—Weight reduction diets were formulated by a commercial company.[b] Vitamins, choline,

Table 1—Composition of weight-reduction diets fed to 22 obese ovariohysterectomized cats

Ingredient	CGCO (n = 6)	CGOB (n = 6)	CACO (n = 5)	CAOB (n = 5)
Corn gluten meal	68.3	68.3	0	0
Casein	0	0	52.0	52.0
Corn oil	13.0	0	18.7	3.9
Poultry fat	0	11.7	0	13.5
Corn starch	5.0	5.0	19.6	19.6
Calcium carbonate	3.7	3.7	0.02	0
Dried beet pulp	3.0	3.0	3.0	3.0
Monosodium phosphate	2.4	2.4	0.49	0.49
Choline chloride	1.5	1.5	1.8	1.8
Mineral mix	1.2	1.2	1.2	1.2
Vitamin mix	1.2	1.2	1.2	1.2
Sodium chloride	0	0	1.0	1.0
DL-methionine	0	0	0.32	0.32
Fish oil	0	0.90	0	0.90
Borage oil	0	0.15	0	0.15
Ground flax	0	0.15	0	0.15
Taurine	0.15	0.15	0.15	0.15
Potassium chloride	0.49	0.49	0.54	0.61
Protein	45.09	44.37	42.97	41.9
Moisture	5.81	7.62	10.28	10.85
Ash	7.87	7.98	7.36	7.06
Fat	17.18	17.29	17.1	18.51
Crude fiber	2.22	2.34	2.28	1.59
Nitrogen-free extract	21.84	20.4	19.79	19.97
Energy (kcal/kg)	4,223	4,146	4,049	4,142

Data (except energy) reported as percentages, calculated on a dry matter basis.
CGCO = Corn gluten meal and corn oil. CGOB = Corn gluten meal and oil blend. CACO = Casein and corn oil. CAOB = Casein and oil blend.

Table 2—Fatty acid composition (% total fatty acids) of weight-reduction diets fed to 22 obese ovariohysterectomized cats

Fatty acid	CGCO (n = 6)	CGOB (n = 6)	CACO (n = 5)	CAOB (n = 5)
C 16:1	0	2.84	0	2.52
C 16:2	4.62	12.96	4.61	12.16
C 18:2n6	59.92	25.25	62.94	29.47
C 18:1	26.87	42.96	27.89	42.64
C 18:3n3	0	3.06	0	2.16
C 18:0	2.17	6.55	2.22	6.24
C 20:4n6	0	0.28	0	0.29
C 20:3	0	0	0	0
C 20:5n3	0	0.82	0	0.80
C 20:0	0.50	0.11	0.58	0.19
C 22:6(n3)	0	1.11	0	0.87
C 22:1	0.18	0.25	0	0.21
C 24:1	0.18	0	0.17	0
C 24:0	0.31	0.16	0.23	0.10
SFA	7.70	19.82	7.64	19.69
PUFA	60.10	30.52	63.27	33.59
MUFA	27.05	46.05	27.89	45.47

C = Carbon. SFA = Saturated fatty acids. PUFA = Polyunsaturated fatty acids. MUFA = Monounsaturated fatty acids.
See Table 1 for key.

taurine, and microminerals were supplemented at 4 times the National Research Council requirements so that cats fed 25% of their maintenance energy requirement would consume adequate amounts of micronutrients. Casein-containing diets were also supplemented with methionine. The **protein efficiency ratios** (**PER**; g of weight gain/g of protein intake) of diets were: **casein-oil blend (CAOB)**, 2.3; **casein-corn oil (CACO)**, 3.0; **corn gluten meal-oil blend (CGOB)**, 1.1; and **corn gluten meal-corn oil (CGCO)**, 1.4.

Determination of hepatic lipid content—A portion of each liver specimen was weighed, extracted with hexane:isopropanol (3:2, vol:vol), and dried under nitrogen. Hepatic lipid concentration was determined by use of gravimetric measurements and expressed on a dry weight basis.

A second portion of each specimen was fixed in phosphate-buffered 10% formalin, embedded in paraffin, sectioned, and stained with H&E and osmium tetroxide for assessment of neutral lipids by use of standard laboratory procedures.[20] Slides were digitized, using a microscope[c] connected to image processing software.[d] Threshold optical density values were set so that nonspecific staining was disregarded. Optical density thus directly correlated with the intensity of neutral-lipid staining, which was expressed as optical density (percentage per millimeter squared). Each specimen was also assigned a liver lipid score (scale, 1 to 6) on the basis of intensity of neutral-lipid staining. A score of 1 to 2 indicated normal or expected intensity of staining; 3 to 4 indicated mildly increased lipid staining; 5 indicated definite accumulation of lipid; and 6 indicated severe lipidosis. Specimens were evaluated by 1 person (GB) who was blinded to specimen origin.

Electron microscopy—Electron microscopy of representative liver specimens was also performed. Specimens (1 mm^3) were immersed in a solution containing 4% paraformaldehyde[e] and 0.5% glutaraldehyde in phosphate buffer (0.14 mol/L; pH 7.0) for several weeks. After thorough washing in the same buffer, specimens were fixed in 2% osmium tetroxide,[f] dehydrated in a series of ethanol solutions, and embedded in resin.[g] Sixty-nanometer (ultrathin) sections were cut with a diamond knife on a ultramicrotome.[h] The ultrathin sections were contrasted with lead citrate[i] and uranyl acetate,[j] and they were viewed and photographed under an electron microscope.[k] Ten cell profiles per section per specimen were counted, and number of lipid inclusions and peroxisomes (small, round structures, approx 250 to 500 nm in diameter, with crystalloid inclusions) were determined for each cell profile. Mean number of lipid-containing particles were determined for each diet group before weight gain (baseline), after weight gain (obese), and after weight loss (final). Changes in mean number of lipid particles were calculated by subtracting obese values from baseline and final values.

Plasma and serum biochemical analyses—Serum and plasma biochemical analyses were performed at a commercial laboratory,[l] using samples collected at baseline, after weight gain, 21 days after starting weight-reduction diets, and after weight loss. Serum insulin concentration was measured by use of an insulin-coated tube radioimmunoassay,[m] according to a procedure validated for cats.[21]

Statistical analyses—Data were analyzed by use of 2-way ANOVA, followed by least square means analysis to determine significant differences between diet groups.[n] Differences were considered significant at $P < 0.05$.

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 (4.51 to 5.00 g/d/kg of obese body weight, averaged over the entire weight loss period). Cats lost 7 to 10% of their obese body weight during the first week on the weight reduction diet, 3 to 5% during the second week, and 2 to 4% each week for the remainder of the weight loss period.

Three cats (1 in the CAOB group, 1 in the CACO group, and 1 in the CGCO group) developed HL during the weight loss period; diagnosis was made on the basis of serum bilirubin concentration > 0.4 mg/dl and a liver lipid score ≥ 5. The cat in the CAOB group consumed only 12% of MER/d, and the cat in the CACO group refused to eat; data from these cats were not included in

Table 3—Results of serum biochemical analyses (mean ± SD) for 22 ovariohysterectomized cats before (baseline) and after (obese) weight gain and during (21 day) and after (final) weight loss

Variable (units)	Baseline	Obese	21 day	Final
ALT (U/L)	78.61 ± 40.61[a]	73.13 ± 33.59[a]	62.82 ± 31.94[a,b]	48.77 ± 25.86[b]
AST (U/L)	32.82 ± 9.94[a]	30.32 ± 14.45[b]	23.72 ± 8.02[b,c]	22.76 ± 11.04[c]
LD (U/L)	131.41 ± 52.29[a]	115.55 ± 49.69[a,b]	94.77 ± 44.35[b]	129.82 ± 72.29[a]
ALP (U/L)	22.21 ± 9.90[a]	32.47 ± 11.01[b]	28.69 ± 9.26[b]	14.09 ± 9.04[c]
CK (U/L)	437.61 ± 281.60[a]	145.17 ± 131.02[b,c]	80.65 ± 30.50[c]	188.18 ± 141.05[b]
Bilirubin (mg/dl)	0.17 ± 0.19[a]	0.10 ± 0.06b	0.069 ± 0.05[b,c]	0.062 ± 0.05[c]
Uric acid (mg/dl)	0.31 ± 0.14[a]	0.09 ± 0.06[b]	0.09 ± 0.07[b]	0.44 ± 0.24[c]
Creatinine (mg/dl)	1.47 ± 0.20[a]	2.00 ± 0.22[b]	1.96 ± 0.20[b]	1.62 ± 0.31[c]
BUN (mg/dl)	20.48 ± 3.64[a]	26.17 ± 4.00[b]	20.61 ± 2.66[a]	25.00 ± 4.35[b]
Protein (g/L)	6.27 ± 0.49[a,b]	6.55 ± 0.48[a]	6.09 ± 0.44[b,c]	5.87 ± 0.73[c]
Albumin (g/L)	3.15 ± 0.40[a]	3.46 ± 0.32[b]	3.42 ± 0.22[b]	3.00 ± 0.24[a]
Globulin (g/L)	3.12 ± 0.37[a]	3.09 ± 0.53[a]	2.67 ± 0.40[b]	2.86 ± 0.68[a,b]
Calcium (mg/dl)	9.53 ± 0.89[a]	9.81 ± 0.57[a]	9.86 ± 0.35[a]	9.12 ± 0.39[b]
Phosphorus (mg/dl)	4.55 ± 0.99[a]	5.24 ± 0.67[b]	4.93 ± 0.51[a,b]	4.61 ± 0.53[a]
Magnesium (mg/dl)	2.13 ± 0.16[a]	2.17 ± 0.25[a]	2.11 ± 0.25[a]	1.96 ± 0.26[b]
Sodium (mEq/L)	154.48 ± 2.64[a]	155.48 ± 5.74[a]	156.22 ± 5.37[a]	153.32 ± 8.42[a]
Potassium (mEq/L)	3.94 ± 0.36[a]	4.26 ± 0.46[b]	3.72 ± 0.62[a]	4.70 ± 0.59[c]
Chloride (mEq/L)	121.26 ± 2.47[a]	121.22 ± 6.25[a]	122.61 ± 6.33[a]	124.27 ± 8.99[a]

ALT = Alanine transaminase. AST = Aspartate transaminase. LD = Lactate dehydrogenase. ALP = Alkaline phosphatase. CK = Creatine kinase.
[a–c]Within a row, values with different letters are significantly ($P < 0.05$) different.

determination of group means. However, the cat in the CACO group that refused to eat lost weight at a greater rate (mean, 7.07 g/d/kg of obese body weight or 5% of body weight/wk) than cats maintained on 25% of MER (mean, 4.26 g/d/kg of obese body weight or 3.3% of body weight/wk). The cat in the CGCO group developed HL while consuming 25% of MER.

Changes in thoracic cavity circumference prior to and after weight loss were similar among groups. However, mean (± SD) thoracic cavity circumference was significantly larger before weight loss than after (32.0 ± 2.2 cm vs 27.8 ± 1.5 cm). Mean body length of all cats was 42.6 ± 1.9 cm.

Serum biochemical analyses—Significant changes in concentration and activities of serum biochemical variables were detected after weight gain (obese values) and weight loss (final values) for all diet groups, although values for all variables remained within reference ranges. Significant differences in final values were not detected among the diet groups. Therefore, final values for all diet groups were combined **(Table 3)**.

Plasma cholesterol and triglyceride concentrations—Mean obese plasma cholesterol concentration was significantly increased, compared with concentrations determined at baseline, 21 days after initiating weight-reduction diets (21-day concentration), and after weight loss **(Fig 1)**. Significant differences between baseline and 21-day concentrations were not detected, whereas final plasma cholesterol concentration was significantly greater than both baseline and 21-day concentrations. Obese triglyceride concentration was also significantly increased, compared with 21-day and final concentrations. However, triglyceride concentration was not significantly increased at the end of the weight loss period, compared with baseline and day-21 concentrations.

Serum glucose and insulin concentrations—Obese and final serum glucose concentrations were significantly increased, compared with baseline and 21-day weight loss concentrations **(Fig 2)**. In addition, final serum insulin concentration was significantly increased (threefold), compared with concentrations determined at all other times.

Hepatic lipid concentration—Hepatic lipid concentration (percentage of dry matter) determined after weight loss was significantly affected by lipid and protein content of diet **(Fig 3)**. Mean lipid concentration was significantly greater in cats in the CGCO group than in cats in the CAOB and CACO groups. However, concentration in the CGCO group was not significantly different from that in the CGOB group.

Electron microscopy—Significant differences in the change in mean number of lipid-containing particles (final–obese values) were not detected among diet groups. However, when values for all cats were combined, there was a 2-fold increase in lipid-containing particles over baseline following weight gain (obese). After weight loss, number of lipid-containing particles were significantly greater in the CGCO group, compared with values for the other 3 groups (percentage increase over baseline: CAOB, 170 ± 24%; CACO, 185 ± 42%; CGOB, 190 ± 58%; CGCO, 350 ± 141%)

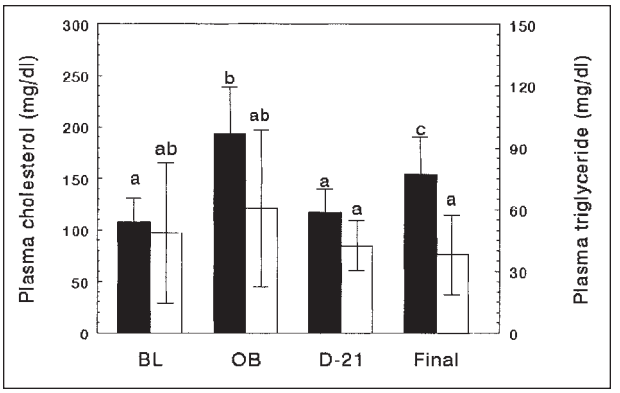

Figure 1—Mean (± SD) plasma cholesterol (O) and triglyceride (Θ) concentrations for 22 ovariohysterectomized cats before (BL) and after (OB) gaining at least 30% of their ideal lean body weight, after 21 days on a weight-reduction diet (D-21), and after losing at least 30% of their obese body weight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

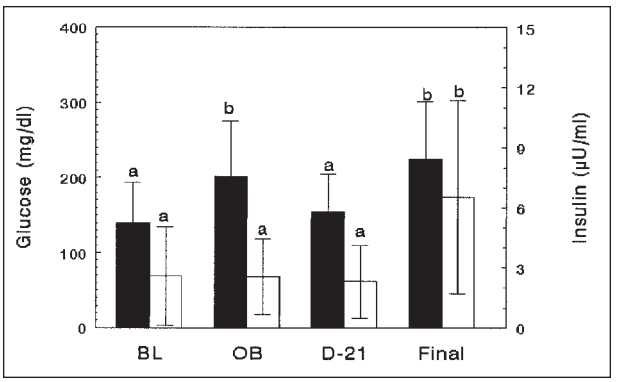

Figure 2—Mean (± SD) serum glucose (O) and insulin (Θ)concentrations for 22 ovariohysterectomized cats before and after gaining at least 30% of their ideal lean body weight, after 21 days on a weight-reduction diet, and after losing at least 30% of their obese body weight. *See* Figure 1 for key.

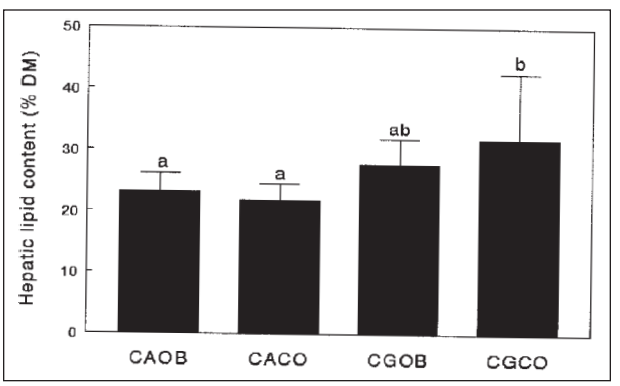

Figure 3—Mean (± SD) hepatic lipid content (percentage of dry matter [DM]) determined from histologic evaluation of biopsy specimens obtained from 22 ovariohysterectomized cats after they had lost at least 30% of their obese body weight as a result of consuming 1 of 4 weight-reduction diets. Values with different letters are significantly ($P < 0.05$) different. CAOB = Casein and oil blend diet. CACO = Casein and corn oil diet. CGOB = Corn gluten meal and oil blend diet. CGCO = Corn gluten meal and corn oil diet.

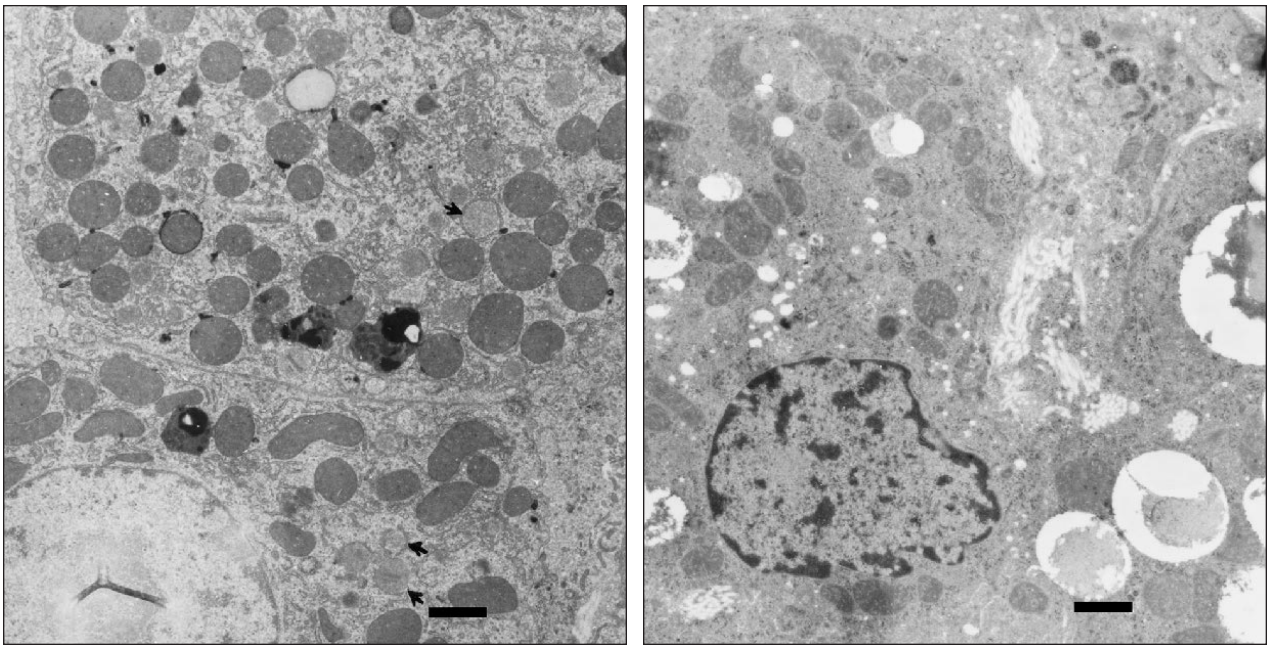

Figure 4—Electron micrographs of liver biopsy specimens obtained from 1 cat in the CAOB group (left) and 1 cat in the CGCO group (right). Specimens were collected after cats had lost at least 30% of their obese body weight. The cat in the CGCO group developed HL during the weight-loss period. Notice the lipid-containing peroxisomes (arrows) in the photomicrograph of the specimen from the cat in the CAOB group; this cat did not have HL. Osmium tetroxide stain contrasted with lead citrate and uranyl acetate; bar = 1 μm.

Histopathologic changes consistent with HL were detected only in the CGCO group.

The 1 cat in the CGCO group that developed HL while eating 25% of MER had pronounced lipid accumulation in hepatocytes and reduction in number of lipid-containing particles (eg, peroxisomes), compared with a cat in the CACO group (**Fig 4**). Furthermore, many of the lipid droplets in the liver specimen from the affected cat were heterogeneous in size and electron density, contained inclusion bodies, and had fine structural changes that suggested an alteration in lipid and protein metabolism.

Discussion

Body weight of the cats in the present study increased consistently for 80 days after ovariohysterectomy. After this time, body weight reached a new plateau (set point), and further weight gain was not observed for up to 108 days after ovariohysterectomy in most cats. Neutering an animal does contribute to weight gain.[4,6] Total weight loss and rate of weight loss while cats were fed the weight-reduction diets did not differ significantly among dietary treatment groups, suggesting that neither low or high quality protein or dietary LCEFA composition markedly altered weight loss patterns. Given the 2- to 3-fold differences in PER among weight-reduction diets, we expected a slower rate of weight loss in cats fed diets with casein. However, we did not detect such differences. Because cats were in a net energy deficit during the weight-loss period, these data suggest that most of the carbohydrate, protein, and fat (oil) fed was catabolized for energy and little, if any, was used for synthesis. Rate of weight loss for these cats that were fed 25% of MER was similar to that reported for cats that were completely anorectic.[16,17] The rate of weight loss reported in the present study (approx 30 to 35% during 8 to 9 weeks) is 3-fold greater than that cited by Center et al[5] as safe weight loss (ie, loss of 10% of initial body weight during approx 8 weeks). The weight-reduction diets in the present study varied in protein quality and provided approximately half the amount of protein required to maintain greater than 80% of lean body mass (2 g of protein/kg of body weight/d).[22,23] Although we did not directly measure lean body mass, cats in the CAOB, CACO, and CGOB groups appeared healthy throughout most of the weight loss period, although plasma insulin and serum glucose concentrations increased after weight loss, compared with baseline concentrations.

Obese cats had a significantly higher plasma cholesterol concentration, compared with baseline concentration. Following 21 days of weight loss, cholesterol concentrations initially decreased in all diet groups and then increased with continued weight loss. Triglyceride concentration after weight loss was also significantly different from concentration before weight loss. These results are in contrast to those reported by Dimski et al,[24] who did not detect significant differences in cholesterol or triglyceride concentrations between obese and nonobese cats prior to weight reduction. These authors reported that, following 28 days of weight loss, serum cholesterol concentration in nonobese cats was greater than in obese cats, but both groups had similar weight loss patterns. Differences between our study and that reported by Dimski et al[24] may be attributable to the percentage of MER fed to cats during the weight loss period; they fed approximately half of the calories that were in the non–weight-reduction diet, whereas we fed 25% of the MER calculated for ideal body weight. Also, cats in the previous study were initially selected as obese or lean, and cats in our study gained at least 30% of their body weight prior to weight loss. Duration of the weight-loss

period also differed between their study (28 days) and ours (63 days). The increase in plasma triglyceride concentration that we detected after weight gain is similar to results reported by Pazak et al.[o] This increase was primarily attributable to an increase in VLDL concentration.

Although the decrease in plasma cholesterol concentration that we detected during the first 21 days of the weight-loss period was expected, it is unclear why concentration was significantly increased after weight loss. Cholesterol concentration also increased during weight loss in lean and obese cats,[24] compared with concentration determined before weight loss. Although triglyceride and cholesterol concentrations in our study and in the study by Dimski et al[24] changed significantly with duration of the weight-loss period, concentrations were within reference ranges reported for cats. Changes in total cholesterol concentration may be related to an increased rate of cholesterol synthesis as a result of an increase in steroid hormone production during the continued catabolic state, decreased clearance of **low density lipoprotein (LDL)** from the circulation because of LDL receptor down regulation, decreased clearance or increased production of **high density lipoprotein (HDL)** associated with an increase in cellular breakdown and reverse cholesterol transport, or a decrease in protein stores resulting in altered apoprotein synthesis. Compared with obese concentration, serum protein concentration decreased significantly during the weight-reduction period. However, although serum protein concentrations were decreased in rats fed protein-deficient diets, LDL and HDL concentrations were also decreased, compared with rats fed protein-sufficient diets.[25] Our data suggest that cats regulate lipoproteins in a manner different to that reported by others.[26,27] Additional data collected from the cats in the present study[28] suggest that increases in HDL concentration, rather than increases in concentrations of the other lipoprotein fractions, account for the increase in total serum cholesterol concentration.

Changes in serum insulin and serum glucose concentrations that we detected differed from those reported by Biourge et al.[29] In the latter study, significant changes in blood glucose concentrations were not detected between cats with a normal body weight and those that gained 30% of body weight. However, insulin concentration was significantly greater in the obese cats. In our study that used a similar weight-gain regimen, a significant increase in serum glucose concentration was detected in obese cats, compared with baseline concentration, but we did not detect a significant alteration in insulin concentration. These differences in results may be attributable to degree of adiposity. In the present study, initial and obese body weights of the cats were less than those reported by Biourge et al.[29] In humans, degree of adiposity has been shown to correlate with increased plasma insulin concentration and other risk factors for development of cardiovascular disease.[30] Therefore, degree of obesity may alter insulin metabolism to varying degrees; profoundly obese cats may have increased plasma insulin concentrations, compared with cats that are less obese. Both Biourge et al[29] and Pazak et al[o] reported that in obese cats, area under the insulin and glucose concentration versus time curves following intravenous glucose tolerance testing were increased in obese cats, compared with nonobese cats. We did not assess these variables in the present study.

During the weight-loss period, serum glucose concentration decreased during the first 21 days, compared with the obese concentration, but significantly increased after weight loss. Serum insulin concentration did not change from obese concentration until the end of the weight-loss period; at this time, it was also significantly increased. At the onset of the study, cats had a slightly higher glucose concentration than the mean reference value (mean, 89 mg/dl). This increase may have been associated with the drugs used to sedate cats for blood collection or the stress of handling during blood collection. Biourge et al[29] found increased insulin concentrations in obese cats and decreased concentrations after the weight-loss period, compared with our final increased values. However, results of the present study should not be directly compared with results of the study by Biourge et al[29]; cats lost weight in the latter study because they refused to eat the weight-reduction diet, whereas in our study, cats lost weight because they consumed 25% of MER and 100% of the NRC recommended intakes of vitamins and microminerals. The glucose intolerance and impaired insulin secretion described by Biourge et al[29] may be caused by anorexia or starvation. Starvation alters insulin- and noninsulin-dependant glucose metabolism pathways. For example, responsiveness of pancreatic β-cells to glucose stimulation is impaired, hepatic gluconeogenesis increases, and insulin resistance develops during starvation states in several mammalian species.[31-33] Abnormalities associated with starvation are adaptations to a decrease in calorie and nutrient intake, because substrate utilization changes during starvation from glucose to fatty acids and ketones. In the present study, cats were not starved, and substrate utilization most likely was not radically altered. However, the increase in insulin and glucose concentrations detected at the end of the weight-reduction period suggests that glucose intolerance may be developing in these cats. Therefore, although clinical signs of HL did not develop in any cat that consumed 25% of MER in the CAOB, CACO, and CGOB groups, the increase in serum glucose and insulin concentrations after weight loss, compared with baseline values, suggests that rate of weight loss may increase risk factors associated with development of diabetes mellitus in cats.

With the exception of 1 cat in the CACO group that refused to eat the weight-reduction diet and 1 cat in each of the CAOB and CGCO groups, none of the cats developed overt clinical, biochemical, or histologic evidence of HL. However, hepatic lipid content was altered depending on the type of weight-reduction diet fed. The greatest degree of lipid accumulation was detected in liver specimens from cats in the CGCO group, which suggests that poor dietary protein quality and lack of LCEFA may be involved in development of HL. However, other markers of HL were not affected by diet.

The data also suggest that greater hepatic accumulation of lipids during a weight-gain period may be associated with or contribute to development of HL during a subsequent rapid weight-loss period, because after gaining weight, degree of lipid accumulation was greatest in

cats that were subsequently assigned to the CGCO group. After weight loss, number of hepatic peroxisomes in the cat in the CGCO group that developed HL was decreased, compared with that in an unaffected cat in the CACO group (Fig 4). A decrease in number of peroxisomes in cats with HL has been reported by others,[34] and this decrease is related to duration of the weight-loss period.[17] Protein and n6 fatty acids are involved in regulating peroxisome proliferation,[35,36] but it is unclear how poor dietary protein quality and lack of LCEFA may result in decreased peroxisome numbers. Perhaps LCEFA are critical to formation of the peroxisomal membrane structure. The reduced number of peroxisomes may also decrease carnitine biosynthesis[37] and thereby diminish mitochondrial β-oxidation of fatty acids.

In summary, our data suggest that cats can lose 25 to 30% of their obese body weight in 7 to 9 weeks without developing overt signs of HL, provided that weight-reduction diets are highly palatable, contain a high quality protein, have a source of LCEFA, and are fortified with vitamins and microminerals. However rapid weight loss may increase risk factors associated with development of diabetes mellitus.

[a]Harlan Sprague Dawley, Indianapolis, Ind.
[b]Eukanuba Veterinary Diets, Nutritional Recovery Formula and Ocean Fish Formula Cat Food, The Iams Co, Dayton, Ohio.
[c]Zeiss, Englewood, Colo.
[d]NIH IMAGE 1.60, Bethesda, Md.
[e]Sigma Chemical Co, St Louis, Mo.
[f]Electron Microscopical Sciences, Fort Washington, Pa.
[g]Durcupan Fluka, Buchs, Switzerland.
[h]Reichert-Jung Ultracut E, Vienna, Austria.
[i]Ted Pella Inc, Redding, Calif.
[j]Merck, Darmstadt, Germany.
[k]JEOL 200CX, Tokyo, Japan.
[l]Vet panel 1, Roche Cobas Mira, Roche Diagnostics Systems, Somerville, NJ.
[m]IVDL Inc, Fishers, Ind.
[n]SYSTAT 7.0, SYSTAT Inc, Evanston, Ill.
[o]Pazak HE, Hoenig ME, Scott MA, et al. Effects of obesity on lipoprotein profiles in the cat. Purina Nutrition Forum, 1997.

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