Effects of carnitine and taurine on fatty acid metabolism and lipid accumulation in the liver of cats during weight gain and weight loss

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Objective—To determine the effects of carnitine (Ca) or taurine (Ta) supplementation on prevention of lipid accumulation in the liver of cats.

Animals—24 adult cats.

Procedure—Cats were fed a weight-gaining diet sufficient in n-6 polyunsaturated fatty acids (PUFAs), low in long-chain n-3 PUFAs (n-3 LPUFA), and containing corn gluten for 20 weeks. Cats gained at least 30% in body weight and were assigned to 4 weight-reduction diets (6 cats/diet) for 7 to 10 weeks (control diet, control plus Ca, control plus Ta, and control plus Ca and Ta).

Results—Hepatic lipids accumulated significantly during weight gain and weight loss but were not altered by Ca or Ta after weight loss. Carnitine significantly increased n-3 and n-6 LPUFAs in hepatic triglycerides, decreased incorporation of ^{13}C palmitate into very-low-density lipoprotein and hepatic triglycerides, and increased plasma ketone bodies. Carnitine also significantly increased weight loss but without altering the fat to lean body mass ratio. Taurine did not significantly affect any variables. Diets low in n-3 LPUFAs predisposed cats to hepatic lipidosis during weight gain, which was further exacerbated during weight loss. Mitochondrial numbers decreased during weight gain and weight loss but were not affected by treatment. Carnitine improved fatty acid oxidation and glucose utilization during weight loss without correcting hepatic lipidosis.

Conclusions and Clinical Relevance—The primary mechanism leading to hepatic lipidosis in cats appears to be decreased fatty acid oxidation. Carnitine may improve fatty acid oxidation but will not ameliorate hepatic lipidosis in cats fed a diet low in n-3 fatty acids. (*Am J Vet Res* 2003;64:1265–1277)

Hepatic lipidosis (HL) is a recognized hepatopathy in cats that is characterized by extensive lipid accumulation and other indices of liver failure (eg, increased concentrations of bilirubin). Although diabetes mellitus and acute pancreatitis appear to contribute to the pathogenesis of HL in cats, most cases are believed to result from the nutritional and biochemical peculiarities of cats.[1,2] Many, but not all, cats are obese at the onset of the disease, with anorexia being a common denominator. Stressor events are associated with onset of the disease, and a common stressor appears to be a dietary change to less palatable diets, such as those used for weight reduction.[3] Evaluation of serum biochemical variables usually reveals evidence of cholestatic disease indicated by increases in total serum bilirubin concentration and alkaline phosphatase activity. Although high-protein diets formulated for cats and enteral products and products formulated for humans or cats have been used in the treatment of cats with HL, it is apparent that such treatments are less than ideal, with current mortality rates of 40 to 50%.[2] The ideal diet has yet to be formulated. Better diet formulation is unlikely until the cause of the disease is understood.

Unlike most other pets, cats develop HL during a period when they are not consuming food, which may occur when the diets of cats are changed; in many cases, a cat may simply refuse to eat. On the basis of other studies[4,5] conducted by our laboratory group, as well as another report,[6] the most likely mechanism or mechanisms involved in the development of lipid accumulation in the liver of cats are a decrease in mitochondrial or peroxisomal oxidation of fatty acids or a decrease in both.

Dietary requirements of cats appear to make them more susceptible to HL, compared with other species. Cats have minimal activity of Δ-6 desaturase enzymes[7]; therefore, common sources of vegetable oil, such as corn oil, appear to be inadequate to meet essential fatty acid requirements in cats.[8] Deficiency of essential fatty acids induces fatty livers in cats[9] and other animals.[10,11] There is also an apparent defect in lipoprotein transport from the liver of animals with a deficiency of essential fatty acids,[10] but to our knowledge, this has not been determined for cats. Based on the aforementioned literature and data from our laboratory,[12] the concentrations of 20:4(n-6) and 22:6(n-3) are reduced in the liver of cats undergoing weight loss when fed a corn oil-based diet.

Dietary protein is important in the management of HL in cats, and feeding 25% of the **maintenance energy (ME)** in the form of high-quality protein will attenuate the development of HL, but it does not ameliorate the condition. In 1 study,[13] such dietary manipulation reduced lipid content in the liver by approximately half, compared with results in animals fed a corn oil-supplemented diet.

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In other animal species, methionine deficiency contributes to the development of HL.[14,15] It is speculated that methionine and taurine are involved in the methylation of phospholipids,[16] and taurine is an essential nutrient for cats.[17] Taurine may also contribute to membrane alterations as a free-radical scavenger[18] or membrane stabilizer.[19] Also, taurine can reduce lipid content in the liver of obese children.[20] Little is known about taurine and lipid metabolism or how they may be involved in HL.

Carnitine is required for transporting long-chain fatty acids into mitochondria. It is synthesized in vivo from the amino acids lysine and methionine.[21] When animals are fed a poor-quality source of protein that is low in methionine, lysine, or both, the synthesis of carnitine may also be decreased. Subsequent to decreased carnitine synthesis, transport of fatty acids into the mitochondria and oxidation of fatty acids may be impaired, thereby contributing to the development of HL[22] (eg, accumulation of lipid in the liver).

Hepatic lipid accumulation in overweight cats undergoing weight loss may be caused by an increase in fatty acids available for uptake into the liver, an increase in triglyceride synthesis, a decrease in oxidation of fatty acids, or a decrease in transport of **very-low-density lipoprotein (VLDL)**. As reported elsewhere,[4] the transport of VLDL from the liver is not decreased, and triglyceride synthesis does not appear to be increased in cats during weight loss. Therefore, the primary cause of weight loss-induced hepatic lipid accumulation is likely a result of the inability of mitochondria and peroxisomes to maintain oxidation of fatty acids. The purpose of the study reported here was to test the hypothesis that dietary supplementation with carnitine, taurine, or both will ameliorate lipid accumulation in the liver of overweight cats undergoing weight loss by improving oxidation of fatty acids through an increase in mitochondrial uptake of fatty acids and maintenance of membrane concentrations of **long-chain n-3 polyunsaturated fatty acids (n-3 LPUFAs)**, which are required for membrane function and structure. Specifically, the effects of diets supplemented with carnitine, taurine, or both on ketone body formation; the number and morphologic characteristics of hepatic mitochondria and peroxisomes; and fatty acid composition of hepatic triglycerides and phospholipids were determined. Additionally, incorporation of a stable isotope (ie, labeled palmitate) into hepatic triglycerides and VLDLs, plasma biochemical indices, weight loss, and body composition were also assessed.

Materials and Methods

Animals—Twenty-four ovariohysterectomized domestic short hair cats[a] that were 2 to 5 years old were obtained from a commercial supplier for use in the study. Ovariohysterectomy of each cat was performed by a veterinarian at the commercial supplier. At the commercial supplier, cats were fed a commercial diet,[b] and water was available ad libitum.

After procurement, cats were housed separately in our animal facilities, which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Cats were maintained on a cycle of 12-hours light and 12-hours dark at a mean room temperature of 21°C. All procedures and protocols were performed in accordance with guidelines established in the *Guide for the Care and Use of Laboratory Animals*; all experiments were approved by an institutional animal care and use committee.

Feeding regimen—After a 1-week period for acclimatization, cats were fed a diet intended to induce weight gain (**Appendix 1 and 2**). The diet met **National Research Council (NRC)** recommendations for all nutrients, including n-6 fatty acids (18:2[n-6] and 20:4[n-6]), but was low in n-3 LPUFAs (20:5[n-3] and 22:6[n-3]). The diet was provided ad libitum for 20 weeks. During that time, each cat gained a minimum of 30% over their ideal lean body weight. At that time, they were considered overweight. Gain in body weight ranged from 30 to 118%.

Once cats became overweight, the percentage of body fat was determined by use of dual-energy x-ray absorptiometry. Cats were then assigned to 1 of 4 treatment groups (6 cats/group); each group was balanced on the basis of body fat percentage. Each group was fed 1 of 4 diets intended to induce weight loss (control diet; control diet supplemented with carnitine [0.02%], which is an amount of carnitine in the range used by others[23] to minimize HL; control diet supplemented with taurine [0.5%], an amount selected because of the low content of sulfur-containing amino acids for the diet, which was 5 times the amount of taurine recommended by the Association of American Feed Control Officials; and control diet supplemented with carnitine [0.02%] and taurine [0.5%]; **Appendix 3**). Weight-loss diets were fed to cats at 25% of **ME requirements (MER)**[5] until they attained weight near the initial lean body mass; thus, weight-loss diets were fed for 7 to 10 weeks. These weight-loss diets were similar to low-calorie weight-loss diets for humans that consist of 25% of total energy intake. Body weight of each cat was recorded weekly throughout the study.

Cats were allowed outside of their cages for 30 to 60 minutes daily. Cats were provided with toys and handled on a regular basis.

Collection and preparation of samples—Blood samples were collected after food had been withheld from cats overnight (16-hour period of food withholding) prior to ovariohysterectomy (baseline), after weight gain (overweight), and after termination of weight-reduction diets (final). Cats were sedated by administration of medetomidine hydrochloride prior to blood collection; effects of medetomidine were reversed with atipamezole hydrochloride following blood collection. Each blood sample (10 mL) was collected from a jugular vein, and a portion was allocated into glass tubes containing EDTA for plasma or glass tubes without additives for serum. Serum and plasma samples were stored at –70°C prior to analysis. Plasma samples were used to prepare VLDL fractions and measure ketone bodies. Serum samples were used for biochemical analyses.

Specimens of liver were obtained by use of a wedge-biopsy procedure in anesthetized cats during ovariohysterectomy (baseline), after cats became overweight (overweight), and after termination of weight-reduction diets (final). Liver specimens were used for electron microscopy and measurement of fatty acid content of **triglycerides (TGs)** and **phospholipids (PLs)**. For each surgical procedure (ovariohysterectomy or wedge biopsy), cats were anesthetized by administration of ketamine hydrochloride (15 mg/kg, IM) and acepromazine (1 mg/kg, IM), and anesthesia was maintained by use of isoflurane administered via a small rebreathing circuit. Constant care was provided to the cats during and following surgery.

Assessment of body composition—Whole-body scans of each cat were performed by use of a whole-body x-ray densitometer.[c]

Serum biochemical analyses—Serum biochemical analyses were performed by use of a commercial system.[d] Serum

insulin concentration was measured by use of insulin-coated tubes in a radioimmunoassay[e] performed in accordance with a procedure validated for samples obtained from cats.[24]

Electron microscopy of mitochondria and peroxisomes—Liver specimens were fixed by immersion in a solution of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M Sorenson phosphate buffer (pH, 7.4). Samples were then washed in the same buffer and fixed with 2% osmium tetroxide and dehydrated in a graded series of ethanol solutions. Next, the specimens were embedded in resin and cut into thin sections (60 nm thick) on a microtome.[f] Sections were viewed by use of a transmission electron microscope[g] at 75 kV and photographed at 5,000× magnification. Three cells from each liver sample were photographed for each stage (ie, baseline, overweight, and final). Numbers of mitochondria and peroxisomes per cell were determined by direct count; electron microscopy personnel who performed the counts were not aware of the source of each sample. The mean number of mitochondria and peroxisomes was determined for the 3 cells from each sample.

Infusion of uniformly labeled ^{13}C palmitate into cats—After termination of weight-reduction diets (final), food was withheld overnight from cats (16-hour period), and cats were then anesthetized and infused with 6 mL of a buffered albumin solution that contained 10 mg of **uniformly labeled ^{13}C palmitate (U-^{13}C16:0)**. The palmitate-albumin solution was prepared in accordance with the method of Wolfe et al.[25] The solution was infused via a catheter inserted in a jugular vein; infusion rate was 0.4 mL/min for 15 minutes by use of a calibrated pump. Blood samples were collected from each cat at 0.25, 0.5, 1, 1.5, and 24 hours after infusion. The U-^{13}C16:0, as well as other lipids, was extracted from liver TG and VLDL fractions, derivatized, and measured by use of **gas chromatography-mass spectrometry (GC-MS)**.

Isolation of plasma VLDLs—Plasma VLDLs were isolated as reported elsewhere[4] by use of the method of Brousseau et al.[26] Briefly, 400 µL of plasma was transferred to a polycarbonate tube (11 × 34 mm), overlayed with 600 µL of potassium bromide solution (density, 1.006 g/dL), and submitted to ultracentrifugation (435,680 × g for 2 hours at 15°C). The VLDLs in the top 400 µL of each tube were harvested by aspiration.

Lipid extraction—Lipids were extracted in accordance with the procedure of Hara and Radin.[27] Liver samples were spiked with 100 µL of a solution of phospholipid L-α-dipentadecanoyl lecithin (0.275 mg/mL) and 100 µL of a solution of triglyceride tripentadecanoin (0.208 mg/mL) as internal standards. The VLDL samples were spiked with 100 µL of the triglyceride tripentadecanoin solution. Samples were then extracted with hexane:isopropanol (3:2 [vol:vol]) that contained 0.02% butylated hydroxytoluene.

Separation and derivatization of lipids—Liver TGs and PLs were separated by use of thin-layer chromatographic plates (20 × 20 cm) coated with silica gel G.[h] Each plate was developed in hexane:ethyl ether:glacial acetic acid (80:20:1 [vol:vol:vol]) solvent in a glass tank. After the solvent front reached a point 2.54 cm from the top of the tank, it was removed, dried, and sprayed with rhodamine.[i] The PL and TG fractions were then marked, scraped, and extracted with 2 mL of hexane:isopropanol (3:2 [vol:vol]) or 1.5 mL of methanol:toluene (2:1 [vol:vol]), respectively. Fatty acids in PL and TG fractions were then converted to their methyl esters by use of 14% boron trifluoride in methanol and measured by use of GC or GC-MS.

Analysis of fatty acids—Methylated fatty acids from liver PL, liver TG, and VLDL fractions were injected into a gas chromatograph[j] equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm [inside diameter]; film thickness, 0.25 µm).[k] Nitrogen gas was used as the carrier gas and was maintained at a constant flow of 1.0 mL/min by use of an electronic pressure-control system. Samples were processed by use of oven temperatures that increased from 140 to 240°C at the rate of 4°C/min; final temperature was maintained for 20 minutes. There was a split ratio of 22:1, injection temperature of 250°C, and detector temperature of 260°C. Peaks were identified and quantified on the basis of results for standard fatty acids (external standards) and C15:0 (internal standard).

Measurement of U-^{13}C16:0—After extraction and methylation of U-^{13}C16:0 and other fatty acids in liver TG and VLDL fractions, methylated samples were injected into a gas chromatograph[l] connected to a mass spectrometer[m] that was equipped with a capillary column[n] (30 m × 0.25 mm [inside diameter]; film thickness, 0.25 µm). Helium flow was maintained at 1.0 mL/min by use of an electronic pressure control, and the injection port was set at 280°C. Oven temperature was programmed at an initial temperature of 150°C; it was increased at the rate of 5°C/min to achieve a temperature of 190°C, then it was increased at the rate of 1°C/min to achieve a temperature of 200°C. Oven temperature was maintained at 200°C for 2 minutes and then increased at a rate of 35°C/min to achieve a temperature of 290°C at 35°C/min; it was maintained at 290°C for 4 minutes. Eluting components were ionized by electron ionization (70 eV), and mass spectrometric[m] detection of U-^{13}C16:0 was achieved by use of selected-ion monitoring of specific ion of mass (ie, m/z) 150.

Measurement of ketone bodies—**Acetoacetate (AcAc)** and **β-hydroxybutyrate (BHB)** were measured in accordance with the method of Des Rosiers et al.[28] Briefly, 0.5 mL of 0.1M NaB^2H_4 in 1M NaOH was added to 0.5 mL of plasma and incubated at 4°C for 30 minutes. The NaB^2H_4 converts each AcAc molecule to deuterated BHB, which is necessary because AcAc is not stable during storage but deuterated BHB is. When AcAc is converted to BHB by use of NaB^2H_4, an additional neutron appears at mass +1. After reduction of AcAc, 0.1 mL of 252mM *RS*-[$^2H_6$]BHB (internal standard) was added. Samples then were deproteinated by adding 0.1 mL of saturated sulfosalicylic acid solution and centrifugating at 2,500 × *g* for 15 minutes. The supernatant was adjusted to pH 9 to 11 by adding 1M NaOH, saturating with NaCl, and extracting 3 times with ethyl ether (each ether phase was discarded). The aqueous phase was acidified with 1M HCl, adjusted to pH 1 to 2, and extracted 3 times with ethyl acetate; these 3 ether extracts were combined and the ether evaporated. Dry residue was incubated overnight at room temperature (25°C) with 50 µL of *N*-methyl-*N*-(t-butyldimethylsilyl)-trifluoroacetamide.[o] One microliter of this solution was injected into the GC-MS. A gas chromatograph[l] connected to a mass spectrometer[m] equipped with a capillary column[n] (30 m × 0.25 mm [inside diameter]; film thickness, 0.25 µm) was used. Helium (1 mL/min) was used as the carrier gas. The injection port was maintained at 280°C. The column temperature was initially set at 200°C and programmed to increase at the rate of 5°C/min. The column was baked at 250°C for 5 minutes between samples. The eluting components were ionized by electron ionization (70 eV), and mass spectrometric[m] detection of ketone bodies was achieved by selected ion monitoring of specific ions of m/z 159 (BHB), m/z 160 (AcAc), m/z 161 (3,4-$^{13}C_2$BHB), m/z 162 (3-^{2}H-3,4-$^{13}C_2$BHB), and m/z 163 (*RS*-[$^2H_6$]BHB). Oxidized U-^{13}C16:0 would appear in the ^{13}C-labeled ketone bodies that were analyzed.

Statistical analysis—A 1-way ANOVA was used to test for overall effects. Post-hoc analysis was conducted by use of

the Fisher least-significant difference procedure for pairwise comparisons. Factors included in the model were period (baseline, overweight, and final) and cat (which was included to account for the relationship among observations from the same animal in the repeated-measures design). Data for the 4 groups (control, carnitine, taurine, and carnitine plus taurine) were also analyzed by use of a 1-way ANOVA. Post-hoc analysis was conducted by use of the Fisher least-significant difference procedure for pairwise comparisons. A 2-way ANOVA was performed to determine the effects of carnitine, taurine, and their interactions. To assess relationships among the continuous variables, the Pearson correlation coefficient was calculated. Differences between means were considered significant at $P < 0.05$.

Results

Weight gain—Cats gained between 30 and 118% over their lean body mass. Mean weight gain was 1.15 kg during the 14-week period, at which time further weight gain was not detected. Mean ± SD body weight for the cats was 2.6 ± 0.3 kg at baseline, 3.9 ± 0.6 kg at overweight, and 3.1 ± 0.5 kg at final.

Concentrations of total fatty acids in liver TGs—Overweight cats had significantly higher concentrations of total fatty acids in liver TGs, compared with concentrations for lean cats at baseline (**Fig 1**). These concentrations also increased significantly in cats after weight loss, compared with values for overweight cats. This progressive lipid accumulation was also seen in electron micrographs (**Fig 2**). On the other hand, dietary treatments (carnitine, taurine, or both) did not significantly alter the concentration of total fatty acids in liver TGs during weight loss (data not shown).

Numbers of mitochondria and peroxisomes in mitochondria—Numbers of mitochondria and peroxisomes in hepatocytes were determined (**Fig 3**). The number of mitochondria in hepatocytes of overweight cats was significantly less than the number in hepatocytes of lean cats at baseline and cats after weight loss. The number of peroxisomes in hepatocytes of overweight cats was not significantly different from that in lean cats at baseline; however, it was significantly greater than the number in cats after weight loss. The number of mitochondria in hepatocytes was significantly inversely correlated with the concentration of total fatty acids in liver TGs in overweight cats (r, –0.596; $P = 0.002$) and cats after weight loss (r, –0.502; $P = 0.01$). Numbers of mitochondria or peroxisomes were not significantly affected by carnitine, taurine, or both during weight loss (data not shown).

Serum biochemical analyses—Concentrations of BUN, bilirubin, uric acid, phosphorus, magnesium, potassium, cholesterol, and glucose were significantly higher in overweight cats, compared with concentra-

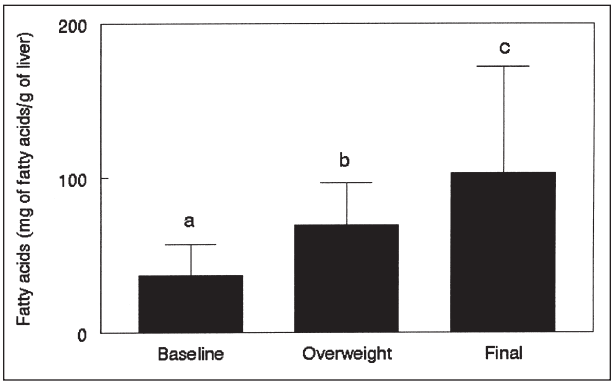

Figure 1—Mean ± SD total liver triglyceride fatty acids for 24 cats prior to weight gain (Baseline), after weight gain of at least 30% (Overweight), and after weight loss (Final). a,b,c—Values with different letters differ significantly ($P < 0.05$).

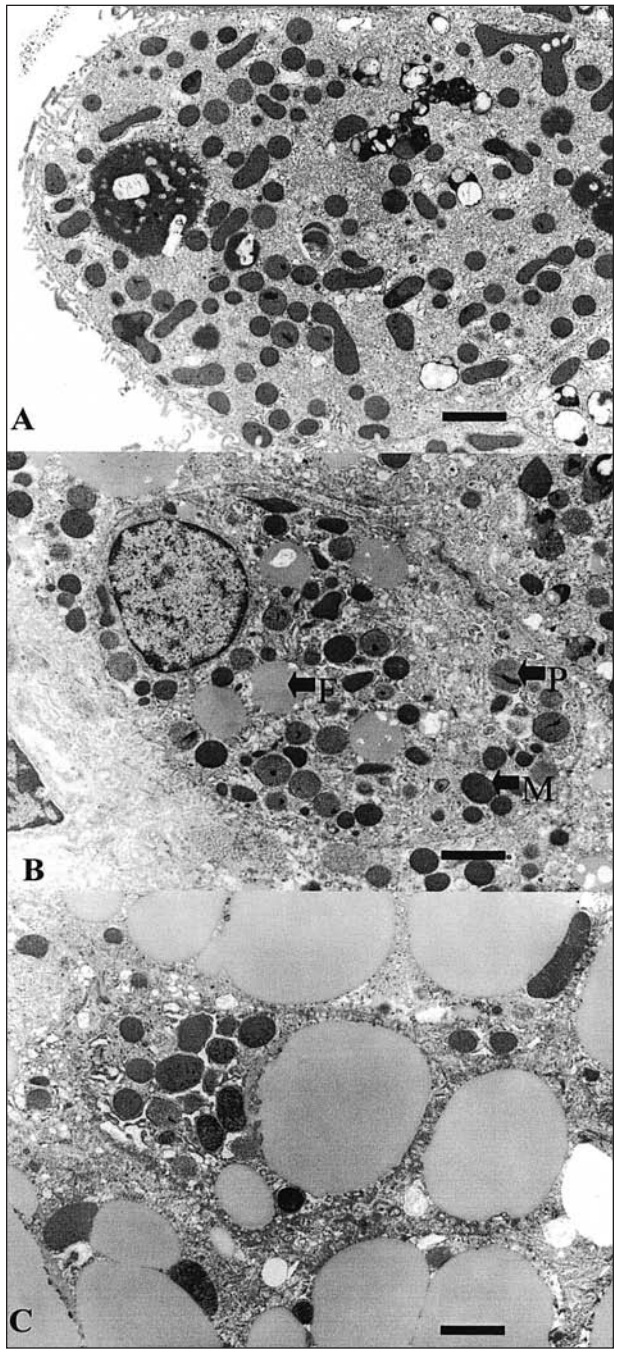

Figure 2—Electron micrograph of a section of liver from a representative cat obtained prior to weight gain (A), after weight gain of at least 30% (B), and after weight loss (C). Notice the changes in the numbers of peroxisomes (P), mitochondria (M), and fat droplets (F). Bars = 2 μm.

tions in lean cats at baseline (**Table 1**). The activity of **lactate dehydrogenase (LD)** was significantly lower in overweight cats, compared with the activity in lean cats at baseline. Activities of **alkaline phosphatase (ALP)**, **alanine transaminase (ALT)**, **aspartate transaminase (AST)**, **creatine kinase (CK)**, and **γ-glutamyltransferase (GGT)** and concentrations of creatinine, globulin, protein, albumin, calcium, sodium, chloride, TGs, and insulin were not significantly affected by weight gain.

Activity of GGT and concentrations of BUN, bilirubin, uric acid, potassium, and glucose were significantly higher in cats after weight loss, compared with values for lean cats at baseline (Table 1). Insulin and TG concentrations and ALP activity were significantly lower in cats after weight loss, compared with values for lean cats at baseline. The rest of the variables did not differ significantly.

Activity of LD and concentrations of bilirubin, uric acid, and glucose were significantly higher in cats after weight loss, compared with values for overweight cats (Table 1). Activity of ALP and concentrations of BUN, calcium, phosphorus, magnesium, TGs, cholesterol, and insulin were significantly lower in cats after weight loss, compared with values for overweight cats. The rest of the variables did not differ significantly.

Activities of ALT, AST, and LD and concentrations of glucose were positively correlated, whereas cholesterol and creatinine concentrations were inversely correlated with the concentrations of total fatty acids in liver TGs of cats after weight loss. Pearson correlation coefficients were 0.714 ($P < 0.001$) for ALT, 0.669 ($P < 0.001$) for AST, 0.582 ($P = 0.003$) for LD, 0.394 ($P < 0.05$) for glucose, –0.522 ($P = 0.009$) for cholesterol, and –0.394 ($P < 0.05$) for creatinine.

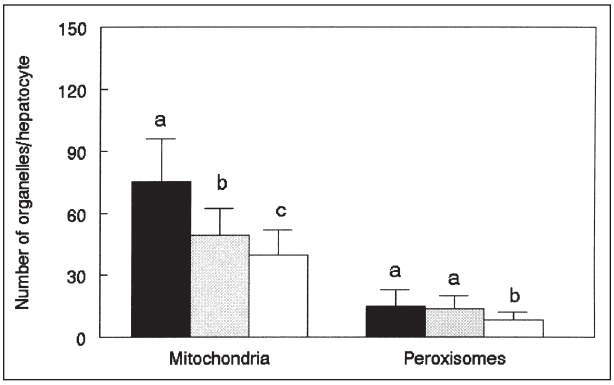

Figure 3—Mean ± SD numbers of mitochondria and peroxisomes in hepatocytes of 24 cats prior to weight gain (black bar), after weight gain of at least 30% (gray bar), and after weight loss (white bar). a,b,c—For each organelle, values with different letters differ significantly ($P < 0.05$).

Table 1—Mean ± SD values for serum biochemical analyses of samples obtained from 24 cats prior to weight gain (Baseline), after weight gain of at least 30% (Overweight), and after weight loss (Final)

Variable	Baseline	Overweight	Final
ALP (U/L)	52.39 ± 18.07[a]	64.88 ± 33.95[a]	46.54 ± 25.03[b]
ALT (U/L)	61.6 ± 68.1	53.6 ± 26.8	99.6 ± 133.8
AST (U/L)	51.3 ± 72.6	32.7 ± 30.8	73.6 ± 104.8
CK (U/L)	71.7 ± 27.7	175.4 ± 317.6	125.9 ± 177.1
GGT (U/L)	0.44 ± 1.25[a]	1.08 ± 1.47[a,b]	1.38 ± 0.71[b]
LD (U/L)	198.1 ± 138.2[a]	81.0 ± 38.3[b]	215.5 ± 211.7[a]
BUN (mg/dL)	17.50 ± 2.12[a]	28.38 ± 8.25[b]	22.79 ± 4.30[c]
Creatinine (mg/dL)	1.57 ± 0.18	1.67 ± 0.49	1.71 ± 0.36
BUN- to-creatinine ratio	11.22 ± 1.13[a]	17.57 ± 4.66[b]	13.47 ± 1.74[a,c]
Bilirubin (mg/dL)	0.04 ± 0.05[a]	0.07 ± 0.06[b]	0.09 ± 0.03[b,c]
Uric acid (mg/dL)	0.00 ± 0.00[a]	0.16 ± 0.07[b]	0.38 ± 0.11[c]
Globulin (g/dL)	2.69 ± 0.31	4.13 ± 6.20	2.78 ± 0.37
Protein (g/dL)	5.67 ± 0.38	6.25 ±1.64	5.80 ± 0.38
Albumin (g/dL)	2.98 ± 0.28	3.25 ± 0.87	3.01 ± 0.32
Calcium (mg/dL)	9.19 ± 0.48[a,b]	10.05 ± 2.70[a]	8.59 ± 1.14[b]
Phosphorus (mg/dL)	4.15 ± 0.57[a]	5.18 ± 1.25[b]	4.30 ± 0.64[a]
Magnesium (mEq/L)	1.97 ± 0.14[a,b]	2.30 ± 0.81[c]	1.85 ± 0.18[a]
Sodium (mmol/L)	148.9 ± 2.5	157.3 ± 42.2	44.8 ± 2.4
Potassium (mmol/L)	3.54 ± 0.29[a]	4.28 ± 1.22[b]	4.07 ± 0.35[b]
Chloride (mmol/L)	120.4 ± 2.3	124.8 ± 33.7	118.0 ± 2.0
Triglycerides (mg/dL)	34.06 ± 6.65[a]	32.38 ± 12.32[a]	27.04 ± 6.94[b]
Cholesterol (mg/dL)	76.5 ± 16.32[a]	97.13 ± 32.18[b]	81.92 ± 16.20[a]
Glucose (mg/dL)	133.2 ± 23.3[a]	166.8 ± 54.8[b]	193.1 ± 37.9[c]
Insulin (mU/mL)	2.98 ± 1.02[a]	3.26 ± 1.51[a]	1.87 ± 0.40[b]

[a,b,c]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

ALP = Alkaline phosphatase. ALT = Alanine transaminase. AST = Aspartate transaminase. CK = Creatine kinase. GGT = γ-Glutamyltransferase. LD = Lactate dehydrogenase.

Effects of carnitine, taurine, or both during weight loss—Cats fed carnitine (alone or in combination with taurine) had significantly lower insulin concentrations and lower, but not significantly so, glucose concentrations, compared with values for cats that were not fed carnitine (**Fig 4**). Cats provided taurine (alone or in combination with carnitine) had significantly lower phosphorus (data not shown) and higher glucose concentrations, compared with concentrations for cats that were not fed taurine.

Effects of carnitine, taurine, or both on body weight and body composition during weight loss—Mean weight loss for all cats was 937 g by the end of the weight-loss period (**Fig 5**); decreases in fat mass and lean body mass contributed to the weight loss. Cats lost more lean body mass (mean, 709 g) than fat mass (mean, 379 g). Cats fed carnitine or carnitine plus taurine lost significantly more weight (approx 200 g), compared with weight loss in the other cats. Cats fed carnitine or carnitine plus taurine lost, on average, 91 g of fat and 94 g of lean body mass. However, the percentage of body fat was not affected by feeding of carnitine (data not shown). Feeding of taurine did not significantly affect weight loss or body composition.

Fatty acid content in liver TGs—Concentrations of C14:0, C18:1(n-9), C18:3(n-6), C18:3(n-3), C20:0, C20:1, C20:2, C20:3(n-6), C22:0, and C22:1 were significantly higher in the liver TGs of overweight cats, compared with concentrations in lean cats at baseline (**Table 2**). On the other hand, concentrations of C14:1, C16:1, C18:2(n-6), C20:4(n-6), C20:5(n-3), C22:5, and C22:6(n-3) were significantly lower in overweight cats, compared with concentrations in lean cats at baseline. Concentrations of C16:0, C18:0, and C22:4(n-6) did not change significantly between cats at baseline and overweight cats.

Concentrations of C18:2(n-6), C20:1, and C20:2

were significantly higher and concentrations of C14:0, C14:1, C16:1, C18:3(n-6), C18:3(n-3), C20:3(n-6), C20:4(n-6), C20:5(n-3), C22:0, C22:1, C22:5, and C22:6(n-3) were significantly lower in liver TGs of cats after weight loss, compared with concentrations in lean cats at baseline (Table 2). Concentrations of C16:0, C18:0, C18:1(n-9), C20:0, and C22:4(n-6) were not significantly different between cats at baseline and cats after weight loss.

Concentrations of C18:2(n-6) were significantly higher and concentrations of C14:0, C14:1, C18:0, C18:1(n-9), C18:3(n-6), C18:3(n-3), C20:0, C20:1, C20:2, C20:3(n-6), C20:5(n-3), C22:0, and C22:1 were significantly lower in liver TGs of cats after weight loss, compared with concentrations in overweight cats (Table 2). Concentrations of C16:0, C16:1, C20:4(n-6), C22:4(n-6), C22:5, and C22:6(n-3) were not significantly different between overweight cats and cats after weight loss.

Effects of carnitine, taurine, or both on fatty acid content in liver TGs during weight loss—Concentrations of C20:2, C20:3(n-6), C20:4(n-6), C20:5(n-3), C22:0, and C22:1 were significantly higher in the liver TGs of cats fed carnitine alone, compared with concentrations in all other treatment groups (**Table 3**). On the other hand, the concentration of C16:0 was significantly lower in the carnitine group, compared with the concentration in the group fed carnitine plus taurine. Also, C16:1 concentration was significantly lower in the carnitine group, compared with concentrations in the taurine group and the carnitine plus taurine group. Concentrations of C16:0 and C16:1 were lower in the carnitine group, compared with concentrations in the control group; however, this difference was not significant. Concentration of C20:4(n-6) was significantly higher in the carnitine plus taurine group, compared with concentrations in the taurine and control groups. Also, concentrations of C20:5(n-3) were significantly lower in the carnitine plus taurine group and the taurine group, compared with the concentration in the control group. Cats fed taurine (alone or in combination with carnitine) had

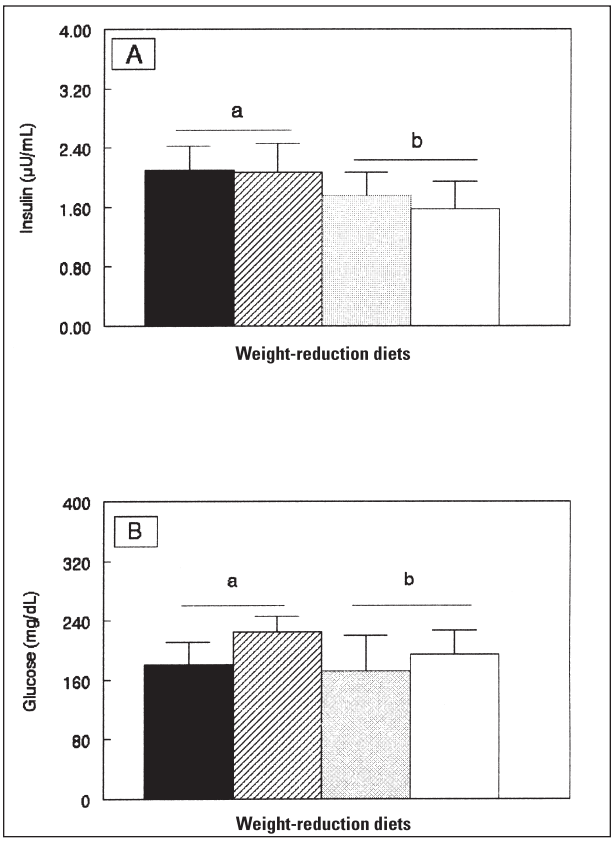

Figure 4—Effects of carnitine, taurine, or both on serum concentrations of insulin (A) and glucose (B) in cats after weight loss. Cats were assigned to 1 of 4 weight-reduction diets (6 cats/group) that consisted of the control diet (Co; black bar), control diet supplemented with carnitine (0.02% carnitine [Ca; diagonal-striped bar]), control diet supplemented with taurine (0.5% taurine [Ta; gray bar]), and control diet supplemented with carnitine (0.02%) and taurine (0.5% [Ca + Ta; white bar]). Cats were maintained on the diets until they attained weight similar to their initial body weight before weight gain. Values represent mean ± SD. a,b—Values with different letters differ significantly ($P < 0.05$).

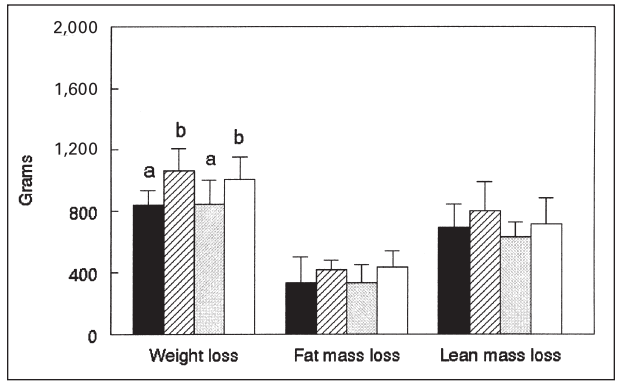

Figure 5—Effects of carnitine, taurine, or both on loss of total body weight, fat mass, and lean mass in cats. Cats were assigned to 1 of 4 weight-reduction diets and maintained on the diets until they attained weight similar to their initial body weight before weight gain. Values represent mean ± SD. *See* Figure 4 for key.

Table 2—Mean ± SD value for fatty acid composition of liver triglycerides in 24 cats prior to weight gain (Baseline), after weight gain of at least 30% (Overweight), and after weight loss (Final)

Fatty acid	Baseline	Overweight	Final
C14:0	6.51 ± 2.17[a]	7.46 ± 1.82[b]	3.82 ± 0.79[c]
C14:1	0.66 ± 0.26[a]	0.44 ± 0.18[b]	0.25 ± 0.14[c]
C16:0	204.0 ± 20.6	208.8 ± 19.1	198.5 ± 16.3
C16:1	16.21 ± 3.42[a]	10.13 ± 1.55[b]	10.19 ± 2.02[b]
C18:0	69.64 ± 13.50[a,b]	73.72 ± 13.08[a]	57.72 ± 8.38[b]
C18:1(n-9)	376.5 ± 34.8[a]	395.1 ± 22.2[b]	376.2 ± 24.6[a]
C18:2(n-6)	231.9 ± 24.6[a]	219.8 ± 15.4[b]	304.6 ± 21.6[c]
C18:3(n-6)	2.07 ± 0.46[a]	2.74 ± 1.20[b]	0.89 ± 0.36[c]
C18:3(n-3)	8.69 ± 2.00[a]	12.38 ± 2.14[b]	5.30 ± 1.51[c]
C20:0	1.35 ± 0.58[a]	3.01 ± 1.67[b]	1.21 ± 0.39[a]
C20:1	4.28 ± 1.77[a]	8.48 ± 1.30[b]	6.09 ± 1.40[c]
C20:2	3.21 ± 1.13[a]	8.91 ± 2.51[b]	4.26 ± 1.16[c]
C20:3(n-6)	4.04 ± 0.77[a]	5.41 ± 2.33[b]	2.21 ± 0.84[c]
C20:4(n-6)	12.38 ± 2.71[a]	9.73 ± 2.03[b]	9.39 ± 3.24[b]
C20:5(n-3)	4.34 ± 1.29[a]	1.53 ± 0.81[b]	0.56 ± 0.44[c]
C22:0	0.31 ± 0.22[a]	0.47 ± 0.32[b]	0.16 ± 0.11[c]
C22:1	0.55 ± 0.41[a]	0.81 ± 0.44[b]	0.09 ± 0.08[c]
C22:4(n-6)	3.62 ± 1.41	4.60 ± 0.94	4.55 ± 1.52
C22:5	7.64 ± 2.29[a]	3.63 ± 0.85[b]	3.63 ± 1.58[b]
C22:6(n-3)	8.69 ± 2.12[a]	1.68 ± 0.93[b]	1.18 ± 0.67[b]

Values reported represent micrograms of fatty acid per milligram of total fatty acids in liver triglyceride fraction. *See* Table 1 for key.

Table 3—Effects of carnitine, taurine, or both on fatty acid composition of liver triglycerides in cats after weight loss*

Fatty acid	Control	Carnitine	Taurine	Carnitine plus taurine
C14:0	4.09 ± 1.05	3.74 ± 0.86	4.00 ± 0.36	3.45 ± 0.80
C14:1	0.24 ± 0.10	0.24 ± 0.18	0.30 ± 0.10	0.24 ± 0.19
C16:0	196.8 ± 13.6[a,b]	186.1 ± 15.2[b]	197.6 ± 11.7[a,b]	213.8 ± 14.2[a]
C16:1	9.99 ± 0.76[a,b]	8.51 ± 2.01[b]	10.70 ± 1.08[a]	11.55 ± 2.65[a]
C18:0	54.93 ± 3.53	65.03 ± 11.20	56.65 ± 6.27	54.27 ± 7.52
C18:1(n-9)	381.3 ± 17.4	382.8 ± 28.9	377.0 ± 7.9	363.7 ± 36.2
C18:2(n-6)	309.2 ± 17.0	298.0 ± 28.7	310.8 ± 19.7	300.3 ± 22.1
C18:3(n-6)	0.91 ± 0.42	0.94 ± 0.56	0.77 ± 0.19	0.93 ± 0.25
C18:3(n-3)	6.05 ± 2.33	4.79 ± 1.74	5.22 ± 0.72	5.13 ± 0.76
C20:0	1.25 ± 0.34	1.30 ± 0.35	1.16 ± 0.48	1.13 ± 0.48
C20:1	6.42 ± 1.83	6.21 ± 1.41	5.95 ± 1.39	5.76 ± 1.22
C20:2	3.83 ± 0.44[a]	5.49 ± 1.66[b]	4.06 ± 0.54[a]	3.66 ± 0.62[a]
C20:3(n-6)	1.80 ± 0.80[a]	3.22 ± 0.81[b]	2.10 ± 0.35[a]	1.73 ± 0.37[a]
C20:4(n-6)	7.20 ± 1.03[a]	14.14 ± 2.10[b]	6.98 ± 0.90[a]	9.25 ± 1.44[c]
C20:5(n-3)	0.47 ± 0.30[a]	1.24 ± 0.18[b]	0.25 ± 0.06[c]	0.27 ± 0.10[c]
C22:0	0.13 ± 0.04[a]	0.32 ± 0.07[b]	0.10 ± 0.04[a]	0.08 ± 0.06[a]
C22:1	0.05 ± 0.02[a]	0.21 ± 0.10[b]	0.06 ± 0.03[a]	0.05 ± 0.03[a]
C22:4(n-6)	3.99 ± 1.22	5.41 ± 1.68	4.55 ± 1.75	4.25 ± 1.36
C22:5	3.31 ± 1.26	4.58 ± 2.52	3.04 ± 0.83	3.60 ± 1.08
C22:6(n-3)	1.12 ± 0.30	1.69 ± 1.13	0.79 ± 0.31	1.14 ± 0.33

Values reported represent mean ± SD micrograms of fatty acid per milligram of total fatty acids in liver triglyceride fraction.
*Cats (6 cats/group) were fed 1 of 4 weight-reduction diets until they attained weight similar to their initial body weight before weight gain. Control = Control diet. Carnitine = Control diet supplemented with carnitine (0.02%). Taurine = Control diet supplemented with taurine (0.5%). Carnitine plus taurine = Control diet supplemented with carnitine (0.02%) and taurine (0.5%). *See* Table 1 for key.

significantly lower concentrations of C20:3(n-6) and C20:4(n-6), compared with concentrations in cats not fed taurine.

Fatty acid content in liver PLs—Concentrations of C14:1, C18:0, C18:1(n-9), C18:3(n-6), C20:0, C20:1, C20:3(n-6), C22:0, and C22:4(n-6) were significantly higher in liver PLs of overweight cats, compared with concentrations in lean cats at baseline (**Table 4**). On the other hand, concentrations of C16:0, C18:3(n-3), C20:5(n-3), C22:1, C22:5, and C22:6(n-3) were significantly lower in liver PLs of overweight cats, compared with concentrations in lean cats at baseline. Concentrations of C14:0, C16:1, C18:2(n-6), C20:2, and C20:4(n-6) in liver PLs did not differ significantly between lean cats at baseline and overweight cats.

Concentrations of C14:1, C18:0, C18:1(n-9), C18:2(n-6), C20:0, C20:1, and C22:4(n-6) were significantly higher in liver PLs of cats after weight loss, compared with concentrations in lean cats at baseline (Table 4). Concentrations of C18:3(n-6), C20:3(n-6), C20:5(n-3), C22:1, C22:5, and C22:6(n-3) were significantly lower in PLs of cats after weight loss, compared with concentrations in cats at baseline. Concentrations of C14:0, C16:0, C16:1, C18:3(n-3), C20:2, C20:4(n-6), and C22:0 in liver PLs did not differ significantly between cats at baseline and cats after weight loss.

Concentrations of C18:1(n-9), C18:3(n-6), C20:3(n-6), and C22:0 were significantly lower in liver PLs of cats after weight loss, compared with concentrations in overweight cats (Table 4). Concentrations of C14:1, C16:0, C18:2(n-6), and C18:3(n-3) were significantly higher in liver PLs of cats after weight loss, compared with concentrations in overweight cats. Concentrations of C14:0, C16:1, C18:0, C20:0, C20:1, C20:2, C20:4(n-6), C20:5(n-3), C22:1, C22:4(n-6), C22:5, and C22:6(n-3) did not differ significantly in liver PLs of overweight cats and cats after weight loss.

Table 4—Mean ± SD value for fatty acid composition of liver phospholipids in 24 cats prior to weight gain (Baseline), after weight gain of at least 30% (Overweight), and after weight loss (Final)

Fatty acid	Baseline	Overweight	Final
C14:0	1.15 ± 0.73	1.21 ± 0.41	1.22 ± 0.54
C14:1	0.27 ± 0.16[a]	0.45 ± 0.38[b]	0.70 ± 0.40[c]
C16:0	110.3 ± 13.4[a]	94.14 ± 13.5[b]	117.8 ± 12.1[c]
C16:1	1 4.36 ± 1.46	16.12 ± 13.23	13.23 ± 5.27
C18:0	236.0 ± 19.0[a]	253.7 ± 25.6[b]	249.0 ± 12.8[b]
C18:1(n-9)	91.4 ± 18.6[a,b]	121.4 ± 25.1[c]	105.9 ± 11.7[a]
C18:2(n-6)	185.4 ± 28.3[a]	192.0 ± 37.5[a]	252.7 ± 24.3[b]
C18:3(n-6)	4.07 ± 1.00[a]	5.50 ± 2.67[b]	2.94 ± 0.60[c]
C18:3(n-3)	2.35 ± 0.67[a]	1.34 ± 0.38[b]	1.90 ± 1.10[a]
C20:0	2.57 ± 0.94[a]	3.84 ± 1.50[b]	3.60 ± 1.18[b]
C20:1	3.06 ± 1.01[a]	6.55 ± 3.25[b]	4.30 ± 1.27[b]
C20:2	6.39 ± 2.73	7.01 ± 1.73	6.46 ± 1.26
C20:3(n-6)	12.89 ± 1.97[a]	16.49 ± 3.89[b]	8.94 ± 2.03[c]
C20:4(n-6)	160.5 ± 23.8	170.7 ± 34.4	167.9 ± 21.4
C20:5(n-3)	16.56 ± 3.54[a]	3.95 ± 1.83[b]	3.06 ± 2.25[b]
C22:0	2.57 ± 1.11[a]	3.46 ± 1.99[b]	2.38 ± 1.28[a]
C22:1	1.78 ± 0.58[a]	0.94 ± 0.74[b]	0.90 ± 0.75[b]
C22:4(n-6)	6.02 ± 2.08[a]	9.97 ± 1.35[b]	10.51 ± 1.79[b]
C22:5	11.08 ± 1.46[a]	7.25 ± 1.06[b]	8.79 ± 1.67[b]
C22:6(n-3)	86.81 ± 35.59[a]	22.97 ± 5.60[b]	17.1 ± 3.63[b]

Values reported represent micrograms of fatty acid per milligram of total fatty acids in liver phospholipid fraction. *See* Table 1 for key.

Effects of carnitine, taurine, or both on fatty acid content in liver PLs during weight loss—Fatty acid content in liver PLs was not significantly altered by any dietary treatments during weight loss (**Table 5**).

Fatty acid content in the VLDL fraction—Concentrations of C16:0, C18:1, and total fatty acids were significantly higher in the VLDL fraction of over-

Table 5—Effects of carnitine, taurine, or both on fatty acid composition of liver phospholipids in cats after weight loss*

Fatty Acid	Control	Carnitine	Taurine	Carnitine plus taurine
C14:0	1.37 ± 0.45	1.06 ± 0.49	1.46 ± 0.73	1.01 ± 0.42
C14:1	0.73 ± 0.48	0.63 ± 0.48	0.89 ± 0.25	0.55 ± 0.40
C16:0	120.4 ± 7.2	107.8 ± 16.8	119.8 ± 6.6	123.4 ± 11.2
C16:1	15.15 ± 2.00	12.86 ± 5.08	14.04 ± 5.82	10.87 ± 7.20
C18:0	242.5 ± 17.1	257.9 ± 12.1	246.2 ± 11.0	249.3 ± 6.2
C18:1(n-9)	111.2 ± 15.6	107.7 ± 13.4	105.6 ± 5.8	99.04 ± 9.2
C18:2(n-6)	246.8 ± 22.7	250.2 ± 38.2	256.9 ± 13.7	256.7 ± 21.5
C18:3(n-6)	2.83 ± 0.50	2.81 ± 0.69	3.21 ± 0.53	2.92 ± 0.70
C18:3(n-3)	1.68 ± 0.76	1.56 ± 0.68	2.66 ± 1.76	1.72 ± 0.67
C20:0	3.32 ± 0.90	3.82 ± 1.53	3.62 ± 1.73	3.63 ± 0.31
C20:1	4.27 ± 1.30	4.35 ± 1.39	4.14 ± 1.38	4.46 ± 1.33
C20:2	7.07 ± 0.79	5.93 ± 0.87	6.52 ± 1.65	6.31 ± 1.55
C20:3(n-6)	9.60 ± 2.46	8.71 ± 1.67	8.67 ± 2.26	8.78 ± 2.06
C20:4(n-6)	165.0 ± 21.1	173.8 ± 36.1	165.5 ± 9.2	167.5 ± 14.6
C20:5(n-3)	3.47 ± 2.04	2.76 ± 2.75	2.77 ± 2.31	3.25 ± 2.39
C22:0	2.99 ± 1.20	2.20 ± 1.48	2.02 ± 1.24	2.33 ± 1.31
C22:1	1.14 ± 0.60	0.52 ± 0.30	1.15 ± 1.13	0.78 ± 0.75
C22:4 (n-6)	9.56 ± 2.69	10.41 ± 1.95	10.45 ± 0.88	11.64 ± 0.57
C22:5	8.06 ± 1.58	8.90 ± 2.54	8.81 ± 1.17	9.41 ± 1.20
C22:6(n-3)	17.36 ± 4.09	17.89 ± 4.78	16.65 ± 3.20	16.64 ± 3.08

Values reported represent mean ± SD micrograms of fatty acid per milligram of total fatty acids in liver phospholipid fraction. *See* Table 3 for key.

Table 6—Mean ± SD values for fatty acid composition of very-low-density lipoprotein (VLDL) in samples obtained from 24 cats prior to weight gain (Baseline), after weight gain of at least 30% (Overweight), and after weight loss (Final)

Fatty acid	Baseline	Overweight	Final
C16:0	0.16 ± 0.09[a]	0.24 ± 0.13[b]	0.14 ± 0.05[a]
C18:0	0.19 ± 0.11[a]	0.22 ± 0.13[a]	0.10 ± 0.03[b]
C18:1	0.15 ± 0.11[a]	0.28 ± 0.18[b]	0.16 ± 0.07[a]
C18:2	0.15 ± 0.18	0.21 ± 0.13	0.16 ± 0.06
Total	**0.66 ± 0.45[a]**	**0.95 ± 0.53[b]**	**0.55 ± 0.19[a]**

Values reported represent micrograms of fatty acid per milliliters of the VLDL fraction.
[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

Table 7—Effects of carnitine, taurine, or both on fatty acid composition of VLDL in cats after weight loss*

Fatty acid	Control	Carnitine	Taurine	Carnitine plus taurine
C16:0	0.12 ± 0.03	0.11 ± 0.04	0.16 ± 0.05	0.15 ± 0.06
C18:0	0.08 ± 0.01	0.09 ± 0.03	0.12 ± 0.04	0.10 ± 0.05
C18:1	0.11 ± 0.02	0.16 ± 0.07	0.21 ± 0.08	0.15 ± 0.06
C18:2	0.12 ± 0.04	0.14 ± 0.04	0.20 ± 0.06	0.18 ± 0.07
Total	**0.43 ± 0.09**	**0.51 ± 0.17**	**0.68 ± 0.21**	**0.58 ± 0.21**

Values reported represent mean ± SD micrograms of fatty acid per milliliter of VLDL fraction. *See* Table 3 for key.

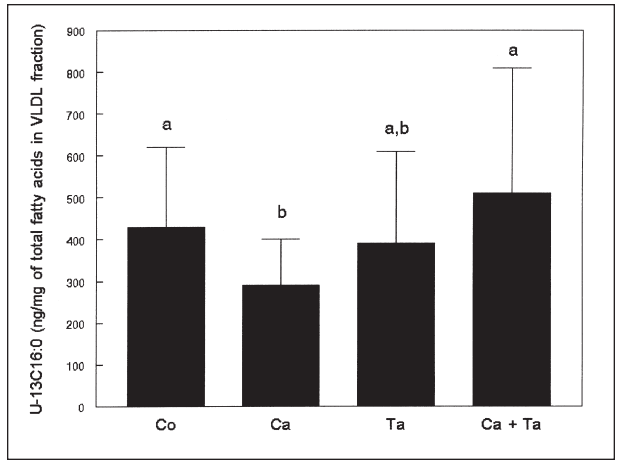

Figure 6—Effects of carnitine, taurine, or both on concentrations of infused uniformly labeled ^{13}C palmitate (U-^{13}C16:0) in very-low-density lipoprotein (VLDL) fractions in cats after weight loss. Cats were assigned to 1 of 4 weight-reduction diets and maintained on the diets until they attained weight similar to their initial body weight before weight gain. After completion of weight reduction, cats were infused with U-^{13}C16:0 and blood was collected 1, 1.5, and 24 hours after infusion. The VLDL U-^{13}C16:0 value for each cat represents the mean ± SD for the 3 measurements. *See* Figure 4 for key.

weight cats, compared with concentrations in lean cats at baseline (**Table 6**). Concentrations of C18:0 and C18:2 did not differ significantly in the VLDL fraction between overweight cats and lean cats at baseline. Furthermore, the concentration of fatty acids in the VLDL fraction did not differ significantly between lean cats at baseline and cats after weight loss.

Fatty acid content of C16:0, C18:0, C18:1, and total fatty acids in the VLDL fraction was significantly lower in cats after weight loss, compared with the content in overweight cats (Table 6). Concentrations of C18:2 in the VLDL fraction did not differ significantly between overweight cats and cats after weight loss.

Effects of carnitine, taurine, or both on fatty acid content in the VLDL fraction during weight loss—Concentration of C18:2 was significantly higher in the VLDL fraction of cats fed taurine (alone or in combination) during weight loss, compared with cats that were not fed taurine (**Table 7**).

Effects of carnitine, taurine, or both on concentrations of U-^{13}C16:0—We did not detect U-^{13}C16:0 in the VLDL fraction 30 minutes after infusion in any of the treatment groups. Sixty minutes after infusion, mean concentration for all treatment groups was 640 ±

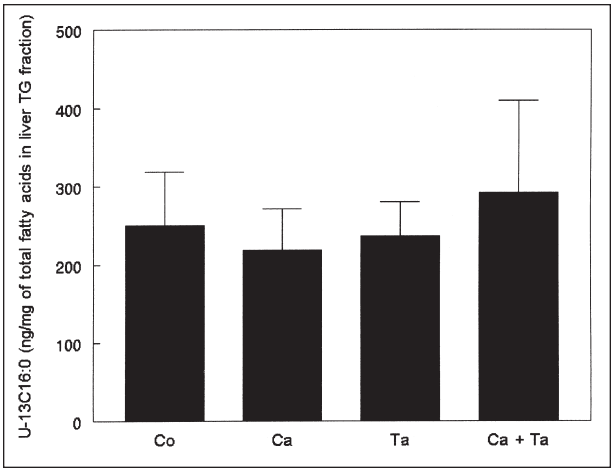

Figure 7—Effects of carnitine, taurine, or both on concentrations of infused U-^{13}C16:0 in liver triglyceride (TG) fractions of cats after weight loss. Cats were assigned to 1 of 4 weight-reduction diets and maintained on the diets until they attained weight similar to their initial body weight before weight gain. Values represent mean ± SD.

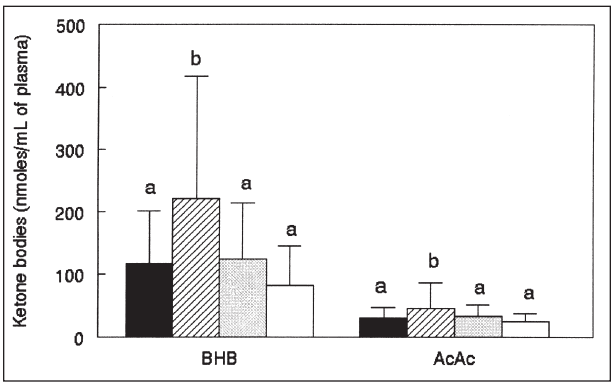

Figure 8—Effects of carnitine, taurine, or both on plasma concentrations of ketone bodies in cats after weight loss. Cats were assigned to 1 of 4 weight-reduction diets and maintained on the diets until they attained weight similar to their initial body weight before weight gain. After completion of weight reduction, cats were deprived of food for 16 hours and then anesthetized; blood was collected 15 and 30 minutes and 1 and 1.5 hours after induction of anesthesia. Concentrations of ketone bodies represent mean ± SD for the 4 measurements. BHB = β-Hydroxybutyrate. AcAc = Acetoacetate. *See* Figure 4 for key.

320 ng/mg of total fatty acids in the VLDL fraction. Mean concentration for all treatment groups decreased to 370 ± 90 ng/mg 90 minutes after infusion and 260 ± 50 ng/mg 24 hours after infusion. Concentration of U-^{13}C16:0 in the VLDL fraction was significantly lower for the carnitine group, compared with concentrations for the control group and carnitine plus taurine group (**Fig 6**).

We detected U-^{13}C16:0 in liver TGs 24 hours after infusion (mean, 248 ± 79 ng/mg of total fatty acids) in all treatment groups. There was not any significant effect for any of the treatment groups, although cats fed carnitine alone had a slightly lower concentration of U-^{13}C16:0 (**Fig 7**).

Effects of carnitine, taurine, or both on plasma concentrations of ketone bodies—Concentrations of BHB and AcAc were significantly higher in the plasma of cats fed carnitine, compared with concentrations for the other treatment groups (**Fig 8**). We did not detect significant differences among the other treatment groups. Also, we did not detect ^{13}C-labeled ketone bodies in any plasma samples.

Discussion

It has been reported[23,29,30] that obese cats do not have increased lipid accumulation in the liver, compared with lean cats. However, weight gain in the study reported here resulted in an increase in lipid accumulation in the liver, compared with baseline values for lean cats. In addition, lipid accumulation in the liver was further increased following weight loss (Fig 1 and 2). The diets fed in this study were designed to limit n-3 LPUFAs. Although n-3 LPUFAs are nutrients not required by the NRC, the limited amounts of n-3 LPUFAs in the weight-gain and weight-loss diets were anticipated to predispose the cats to the development of hepatic lipidosis[5] and, thereby, provide a model for testing the effects of taurine and carnitine on the possible amelioration of hepatic lipidosis. Surprisingly, as the cats gained weight from these diets, they also had decreases in the number of mitochondria, which correlated significantly with lipid accumulation. Therefore, the increased lipid accumulation during weight gain reported here, compared to no changes in liver lipids reported by others,[23,29,30] was most likely attributable to the limiting amount of n-3 LPUFAs in the weight-gain diets, because other macro- and micronutrients were at or above NRC requirements. Because the n-3 LPUFAs and number of mitochondria decreased during weight gain, it could be inferred that the dietary lack of n-3 LPUFAs may have contributed to the decrease in number of mitochondria, because these fatty acids are essential for the integrity of mitochondrial membranes.[31] As suggested by others,[6,7] the decrease in mitochondrial oxidation of fatty acids may contribute to increased lipid accumulation in the liver.

It is evident from the results of our study that fatty acid composition in liver TGs and PLs is markedly altered during weight gain and weight loss in cats. In the overweight cats, there was a significant decrease in n-6 and n-3 LPUFAs in liver TGs, compared with values for lean cats at baseline. In the PL fraction, only the n-3 LPUFAs were decreased, whereas the n-6 LPUFAs were all increased. This decrease in the desaturated, elongated end products of linolenic acids (eg, 22:6[n-3]) has been reported in cats with HL[31] but has not reportedly been associated with obesity. The observed decrease in 22:6 (n-3) is most likely attributable to the low dietary amounts of these n-3 LPUFAs. Also, in another study[32] conducted by our laboratory group, we found that cats fed a complete, balanced, energy-dense diet that contained sufficient amounts of n-3 LPUFAs during weight gain had minimal lipid accumulation in the liver, and the concentration of 22:6(n-3) in storage and membranes was substantially increased over baseline values. Therefore, on the basis of the data obtained for the study reported here, we suggest that a diminished availability of dietary n-3 LPUFAs may contribute to observed increases in lipid accumulation in the liver in obese cats, because this was the only limiting nutrient in the weight-gain diets. These findings corroborate the findings of oth-

ers,[7,8,12] which suggests that cats are unable to synthesize adequate amounts of these LPUFAs. During weight gain, there also was an accumulation of TG-saturated fatty acids as well as monounsaturated fatty acids with a chain length of 18 or more carbons; similar changes were detected in the fatty acid content of liver PLs. Most likely, the increase in saturated and monounsaturated fatty acids is attributable to increases in the synthesis and dietary intake of these fatty acids in the weight-gain diets.

In comparing values for baseline and final hepatic TG content, there was a significant increase in HL and markers of HL (eg, bilirubin concentrations and activities of ALT, AST, and LD). Although these markers of HL were significantly increased, they were still within the reference range. Therefore, it appears that cats consuming 25% of the MER accumulate lipids in the liver but do not develop clinically useful concentrations of markers of hepatic dysfunction. This is further underscored in that after weight loss, all cats were able to consume normal amounts of energy without any adverse effects. After resuming consumption of normal amounts of energy, all of the cats remained healthy.

The increase in lipidosis during weight loss was associated with further decreases in n-3 LPUFAs in storage and membranes. However, increases in the saturated and monounsaturated fatty acids for overweight cats, compared with lean cats at baseline, were not observed after weight loss. This is most likely attributable to the increased catabolic state of cats receiving 25% of MER and increased oxidation of these fatty acids for energy. Also, there was an increase in liver TG and PL 18:2(n-6) contents for the final values, compared with baseline values and values for overweight cats. Because the cats were switched to a corn oil-based diet during the weight-loss phase, increases in 18:2(n-6) content were most likely attributable to the dietary source but may also have been related to lower Δ-6 desaturase activity in cats.[33,34] However, as reported for other species,[35,36] lower protein quality of the diet fed may also be a contributing factor to decreasing overall desaturase activity.

Although the addition of carnitine, taurine, or both did not have significant effects on lipid accumulation in the liver during weight loss, they had, in general, opposing effects on fatty acid composition of TGs. Carnitine significantly increased the concentration of n-3 and n-6 LPUFAs (eg, 20:3[n-6], 20:4[n-6], and 20:5[n-3]), compared with control and taurine groups. Furthermore, there was slightly lower concentrations of 16:0 and 16:1 in the carnitine and carnitine plus taurine groups, suggesting that better oxidation of these fatty acids may be occurring, thus sparing longer-chain essential fatty acids in the TG fraction. Other data supportive of this speculation are the lower amounts of uniformly labeled C16:0 incorporated into the VLDL and liver TG fractions of the groups fed carnitine or carnitine plus taurine (Fig 6 and 7). Changes in TG fatty acids observed in cats fed carnitine alone were reversed when cats were fed carnitine plus taurine. Because plasma and tissue concentrations of carnitine and taurine were not measured, it is difficult to speculate on the mechanism or mechanisms for this opposing effect of taurine, and additional studies are warranted.

Carnitine, taurine, or both did not significantly alter fatty acid composition of PLs; therefore, the hypothesis that carnitine and taurine would maintain membrane n-3 LPUFAs was not supported. Although the amounts of 22:6(n-3) were not significantly increased by feeding of carnitine, it has been hypothesized that carnitine may play a pivotal role in mitochondrial Δ-4 desaturase activity.[37] Because Δ-5 and Δ-6 desaturase activity appears to be decreased or totally lacking in cats,[7,8] and the diets were limiting with regard to precursor fatty acids (ie, 20:5[n-3] and 22:5[n-3]), it is likely that the potential for carnitine to maximize Δ-4 desaturase activity and thereby increase synthesis of 22:6(n-3) may have been compromised.

Another potential mechanism in the development of HL is the inability to transport fatty acids out of the liver in the form of VLDL at a rate compensatory with the increased influx of fatty acids into the liver during weight loss. The VLDL TG content is slightly increased in cats with HL[38]; however, data reported here did not reveal significant differences between baseline and final values (Table 6), although we did detect a significant increase in values for the overweight cats, compared with baseline and final values. Therefore, as suggested by Pazak et al,[38] the inability of the liver to increase VLDL synthesis during weight loss may contribute to the development of HL. However, as reported elsewhere,[4] cats that lost weight when fed diets comparable to those reported here had concentrations of VLDL similar to concentrations in lean cats at baseline did not develop HL. This suggests that although VLDL secretion may contribute to lipid accumulation in the liver, it is likely not the primary cause. Cats with HL have fewer Golgi complexes, endoplasmic reticula, mitochondria, and peroxisomes.[6] In another study,[5] investigators reported a reduction in the number of peroxisomes. Our data revealed that mitochondria are reduced in cats after food deprivation, as well as during weight gain, when n-3 LPUFAs are limited in the diet (Fig 2 and 3). Therefore, it is evident that the organelles involved in lipid export (eg, Golgi complexes and endoplasmic reticula) and oxidation of fatty acids (eg, peroxisomes and mitochondria) are compromised during weight loss and thereby contribute directly to the accumulation of liver lipids. Analysis of our data suggests that there is increased VLDL transport from the liver in overweight cats, as determined on the basis of an increased concentration of plasma VLDL TGs. Although there appears to be an increase in VLDL export from the liver or decreased degradation by lipoprotein lipase, which has been reported for other species after weight gain,[39,40] overweight cats are still accumulating liver lipids. Accumulation of liver TGs during weight gain suggests that VLDL export is not able to compensate for the increased accumulation of fatty acids. Accumulation of liver lipids during weight gain appears to be caused by a reduced number of mitochondria and, possibly, decreased oxidation of fatty acids; these combined mechanisms likely appear to contribute to the observed HL.

Feeding of carnitine during weight loss significantly decreased incorporation of uniformly labeled C16:0 into the VLDL fraction and slightly decreased incorporation into liver TGs. Although incorporation

of the infused palmitate (following partial oxidation) was not detected in ketone bodies, the carnitine group had significantly increased plasma concentrations of BHB and AcAc, suggesting greater oxidation of fatty acids in the cats of this group. Production of ketone bodies is generally enhanced by increased carnitine transport of fatty acids into hepatic mitochondria[41-43] and would explain the findings reported here. It should be mentioned that another study[30] did not find increased production of ketone bodies after the addition of carnitine. As suggested by Calvani et al,[44] carnitine may also be involved with the export of acetyl groups out of the mitochondrial matrix to maximize glucose utilization. In the study reported here, cats in the carnitine group had significantly lower plasma insulin concentrations and slightly lower glucose concentrations, suggesting that carnitine enhances oxidation of fatty acids and promotes better glucose utilization.

Feeding of carnitine also resulted in overall greater weight loss, compared with weight loss for the other treatment groups; this result has been reported in another study.[22] Cats in the carnitine group lost more total fat and lean body mass than did cats in the other treatment groups, but the ratio of fat loss to lean body mass loss was not different among treatment groups. Therefore, it can be concluded that carnitine promoted greater weight loss in cats but did not appear to preserve lean body mass relative to the other treatments. Increased oxidation of fatty acids and improved glucose utilization in the carnitine group was apparently not adequate to rectify the overall imbalance in fatty acid metabolism during weight loss, because this group accumulated liver lipids comparable to amounts detected for the other treatment groups. Perhaps this was related to the postulated role for carnitine in transport of precursor 22:5 (n-3) via carnitine-palmitoyl transferase through the mitochondrial membrane to form 22:6(n-3). As mentioned previously, 22:6(n-3) appears to serve a key function in membrane stability. Although carnitine was added to 2 of the weight-reduction diets, the precursor fatty acid was lacking; therefore, membrane composition of the peroxisomes and mitochondria may have been compromised, leading to a decrease in overall oxidation of fatty acids. The opposing effects of taurine on these variables are difficult to interpret and will require further study.

Exacerbation of lipidosis during weight loss is most likely attributable to increased mobilization of lipid stores, decreased oxidation of fatty acids, altered VLDL transport from the liver, altered TG synthesis, or a combination of these. The marked decrease in PL n-3 LPUFAs likely altered integrity of mitochondrial and peroxisomal membranes, organelles that are involved in oxidation of fatty acids.

Lack of n-3 LPUFAs in the diet during weight gain may predispose cats to develop HL, which appears to be further exacerbated by consumption of a weight-reducing diet that also is limited in n-3 LPUFAs. Based on the decreased numbers of mitochondria and peroxisomes in the study reported here, as well as in another study[45] in which clinical manifestations of HL were observed, the primary mechanism involved in the pathogenesis of HL appears to be decreased oxidation of fatty acids. Feeding supplemental carnitine improved oxidation of fatty acids and glucose utilization but did not correct the accumulation of liver lipids for these experimental conditions.

[a]Hsd Cpb:CaDs, Harlan Sprague Dawley, Indianapolis, Ind.
[b]Harlan Teklad cat diet, Harlan Teklad, Indianapolis, Ind.
[c]Hologic QDR 1000W, Hologic Inc, Bedford, Mass.
[d]Vet panel 1, Roche Cobas Mira, Roche Diagnostic Systems, Somerville, NJ.
[e]Insulin RIA, IVDL Inc, Fishers, Ind.
[f]Reichert Ultracut E, Reichert Inc, Depew, NY.
[g]Hitachi H7000, Hitachi High-Technologies Canada Inc, ON, Canada.
[h]Silica Gel G, Aldrich Chemical Co, Milwaukee, Wis.
[i]Rhodamine, Alltech, Deerfield, Ill.
[j]Shimadzu GC 17A, Shimadzu America, Columbia, Md.
[k]Omega Wax capillary column, Supelco, Bellefonte, Pa.
[l]Varian 3500, Varian Medical Systems Inc, Walnut Creek, Calif.
[m]Finnigan Incos 50, GenTech Scientific Inc, Arcade, NY.
[n]DB5-ms capillary column, J & W Scientific, Folsom, Calif.
[o]TBDMS, Aldrich Chemical Co, Milwaukee, Wis.

Appendix 1

Composition of the diet fed to cats during weight gain

Ingredient	%
Casein	34.94
Animal fat	20.05
Brewers rice	19.00
Ground corn	12.42
Chicken digest	4.00
Beet pulp	3.02
Dried whole egg	3.00
Brewers yeast	1.00
Potassium citrate	0.81
Calcium carbonate	0.52
Sodium chloride	0.40
Mineral mix*	0.31
Vitamin mix†	0.22
Choline chloride	0.26
Taurine	0.04
Preservative	0.01

Values were calculated on a dry-matter basis.
*Provided the following for each kilogram of the diet: iron, 209 mg; zinc, 202 mg; manganese, 61.74 mg; copper, 18.75 mg; iodine, 1.47 mg; selenium, 0.37 mg; and magnesium, 0.06 mg. †Provided the following for each kilogram of the diet: vitamin A, 75,890 IU; vitamin D, 1,706 IU; vitamin E, 150 IU; ascorbic acid, 64.28 mg; thiamin, 18.19 mg; riboflavin, 5.72 mg; pantothenic acid, 23.64 mg; niacin, 83.68 mg; pyridoxine, 15.77 mg; folic acid, 30.65 mg; biotin, 0.44 mg; and vitamin B_{12}, 0.13 mg.

Appendix 2

Fatty acid composition of diets fed to cats during weight gain and weight loss

Fatty acid	Weight gain	Weight loss			
		Control	Carnitine	Taurine	Carnitine plus taurine
C 16:0	25.08	10.79	10.80	10.49	10.36
C 16:1	3.08	0.19	0.12	0.11	0.11
C 18:0	11.37	2.16	2.15	2.12	2.13
C18:1(n-9)	39.07	26.86	26.90	26.93	27.24
C18:2(n-6)	12.47	54.45	54.74	55.04	55.24
C18:3(n-3)	0.57	1.52	1.56	1.54	1.48
C20:4(n-6)	0.31	0.016	0	0	0
C20:5(n-3)	0.008	0.045	0	0	0
C22:6(n-3)	0.056	0	0	0	0

Values reported are g of fatty acid/100 g of fatty acids.

Appendix 3

Composition of diets fed to obese cats for weight reduction*

Ingredient	Control	Carnitine	Taurine	Carnitine plus taurine
Corn gluten meal (%)	66.3	66.3	66.3	66.3
Corn oil (%)	15.8	15.8	15.8	15.8
Corn starch (%)	6.0	6.0	5.5	5.5
Beet pulp (%)	3.0	3.0	3.0	3.0
Calcium carbonate (%)	3.0	3.0	3.0	3.0
Monosodium phosphate (%)	2.1	2.1	2.1	2.1
Minerals* (%)	1.2	1.2	1.2	1.2
Vitamins† (%)	1.2	1.2	1.2	1.2
Potassium citrate (%)	0.7	0.7	0.7	0.7
Choline chloride (%)	0.6	0.6	0.6	0.6
Sodium chloride (%)	0.1	0.1	0.1	0.1
Carnitine (%)	0	0.02	0	0.02
Taurine (%)	0	0	0.5	0.5

Values reported were calculated on a dry-matter basis. Cats were fed 1 of the 4 weight-reduction diets, which were formulated to provide 25% of maintenance energy requirements, until they attained weight similar to their initial body weight before weight gain.

*Provided the following for each kilogram of the diet: zinc, 750 mg; manganese, 250 mg; iron, 164 mg; copper, 60.95 mg; iodine, 5.0 mg; selenium, 0.79 mg; and magnesium, 0.12 mg. †Provided the following for each kilogram of the diet: vitamin A, 145,000 IU; vitamin D, 9,310 IU; vitamin E, 657 IU; niacin, 404.46 mg; ascorbic acid, 336 mg; folic acid, 162.3 mg; pantothenic acid, 105.84 mg; thiamin, 90.54 mg; pyridoxine, 77.12 mg; riboflavin, 25.72 mg; biotin, 2.3 mg; and vitamin B_{12}, 0.76 mg.

Control = Control diet. Carnitine = Control diet supplemented with carnitine (0.02%). Taurine = Control diet supplemented with taurine (0.5%). Carnitine plus taurine = Control diet supplemented with carnitine (0.02%) and taurine (0.5%).

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