Assessment of the influence of fatty acids on indices of insulin sensitivity and myocellular lipid content by use of magnetic resonance spectroscopy in cats

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Objective—To determine whether dietary fatty acids affect indicators of insulin sensitivity, plasma insulin and lipid concentrations, and lipid accumulation in muscle cells in lean and obese cats.

Animals—28 neutered adult cats.

Procedure—IV glucose tolerance tests and magnetic resonance imaging were performed before (lean phase) and after 21 weeks of ad libitum intake of either a diet high in omega-3 polyunsaturated fatty acids (3-PUFAs; n = 14) or high in saturated fatty acids (SFAs; 14).

Results—Compared with the lean phase, ad libitum food intake resulted in increased weight, body mass index, girth, and percentage fat in both groups. Baseline plasma glucose or insulin concentrations and glucose area under the curve (AUC) were unaffected by diet. Insulin AUC values for obese and lean cats fed 3-PUFAs did not differ, but values were higher in obese cats fed SFAs, compared with values for lean cats fed SFAs and obese cats fed 3-PUFAs. Nineteen cats that became glucose intolerant when obese had altered insulin secretion and decreased glucose clearance when lean. Plasma cholesterol, triglyceride, and nonesterified fatty acid concentrations were unaffected by diet. Ad libitum intake of either diet resulted in an increase in both intra- and extramyocellular lipid. Obese cats fed SFAs had higher glycosylated hemoglobin concentration than obese cats fed 3-PUFAs.

Conclusion and Clinical Relevance—In obese cats, a diet high in 3-PUFAs appeared to improve long-term glucose control and decrease plasma insulin concentration. Obesity resulted in intra- and extramyocellular lipid accumulations (regardless of diet) that likely modulate insulin sensitivity. (*Am J Vet Res* 2004;65:1090–1099)

In terms of energy balance, obesity is a result of energy intake exceeding energy expenditure.[1] Concurrent with the increased development of obesity in humans throughout the world, obesity is now the most common nutritional disorder in dogs and cats in the United States. Recent evidence suggests that obesity affects many organ systems, and the role of adipose tissue as an endocrine, autocrine, and paracrine organ is just emerging.[2,3]

As it is in humans, obesity in cats is a risk factor for diabetes mellitus. In obese humans and animals, insulin is not as efficient in its activation of the uptake of glucose in liver, skeletal muscle, and adipose tissue as it is in lean individuals. This diminished effect of insulin is manifest as an impaired ability to suppress hepatic glucose output and promote peripheral tissue glucose uptake and is referred to as insulin resistance.[4,5] Hyperinsulinemia develops as a compensatory response to maintain blood glucose concentration within normal limits.

Results of epidemiologic studies in humans and experimental studies in humans and rats indicate that a high-fat diet results in obesity.[6] However, recent research has revealed that the type of fatty acid in the diet determines insulin sensitivity. **Saturated fatty acids (SFAs)** are characterized by a hydrocarbon chain composed of single bonds, whereas **polyunsaturated fatty acids (PUFAs)** are characterized by a hydrocarbon chain with 2 or more double bonds.[4] Saturated fatty acids are derived from meat and dairy products; in humans and rats, consumption of SFAs results in increased plasma cholesterol, triglyceride, and free fatty acid concentrations and decreased insulin sensitivity.[4-9] In contrast, PUFAs are derived from plant and fish oils; in humans and rats, consumption of PUFAs results in increased insulin sensitivity, decreased concentrations of plasma triglyceride and **low-density lipoprotein (LDL)** cholesterol, and increased concentration of **high-density lipoprotein (HDL)** cholesterol.[7-9] The essential fatty acids (ie, those that the body cannot synthesize) consist of **omega-3** and **omega-6 PUFAs** (**3-PUFAs** and **6-PUFAs**, respectively). In dogs and humans, the essential omega-3 fatty acid is alpha-linolenic acid. Omega-3 fatty acids are derived from plants and fish oils, such as those from salmon and tuna. Fish are the primary source of the long-chain omega-3 eicosapentaenoic acid and docosahexaenoic acid. Omega-6 fatty acids are derived from plant oils, including safflower oil and corn oil.[4] In humans, dogs, and cats, the essential omega-6 fatty acid is linoleic acid; in cats, arachidonic acid is also essential. Omega-3 and omega-6 fatty acids are often assessed in combination in studies to determine the effects of PUFAs on insulin sensitivity and lipid regulation, and conclusions have

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been drawn that PUFAs improve both of those variables. However, from studies in which either omega-3 or omega-6 fatty acid concentration was increased in diets, data indicate that it is actually the omega-3 fatty acids that improve glucose-stimulated insulin secretion and insulin sensitivity,[10-14] although it has also been suggested that there may be a species difference.[15]

In a study[16] involving rats, feeding of SFAs increased skeletal muscle triglyceride concentration and created insulin resistance by decreasing insulin-stimulated glucose disposal and reducing skeletal muscle glycolysis, whereas feeding of fish oil containing primarily PUFAs (predominantly omega-3 fatty acids) did not. Because skeletal muscle is the primary tissue responsible for insulin-stimulated glucose uptake,[17] an alteration in its composition affects whole-body insulin sensitivity. It is believed that obesity is primarily associated with the accumulation of lipids inside the muscle cell (ie, **intramyocellular lipid [IMCL]**), which affects insulin sensitivity by altering signaling pathways and results in altered glucose metabolism.[18-20] The purpose of the study reported here was to determine whether dietary fatty acids affect indicators of insulin sensitivity, plasma insulin and lipid concentrations, and lipid accumulation in muscle cells in lean and obese cats.

Materials and Methods

Animals and diets—Twenty-eight neutered lean adult age-matched domestic shorthair cats (14 males and 14 females[a]) were used for these studies. All cats had been neutered at least 1 year prior to the beginning of the study. Cats were maintained at the College of Veterinary Medicine Animal Care Facility of Georgia University under standard colony conditions. They were housed in individual cages and allowed free access to water. All animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals were determined clinically normal on the basis of results of a physical examination and clinical laboratory data. All cats were accustomed to daily handling and fed a commercially available diet[b] for > 6 months prior to the beginning of the study. The commercial diet contained 17.2% fat. The proportions of omega-3 fatty acids in the diet were as follows: 18:3 n-3, 0.6%; 20:5 n-3, 0.17%; and 22:6 n-3, 0.15%. The proportions of omega-6 fatty acids in the diet were as follows: 20:3 n-6, 0.14% and 20:4 n-6, 0.57%.

The cats were equally and randomly assigned to 1 of 2 diet groups: a group receiving an SFA-diet (7 males and 7 females; SFA group) or a group receiving a PUFA-diet (7 males and 7 females; 3-PUFA group). The compositions of the diets were known to only 1 of the investigators (MW) who was not involved in the execution of the experiments and data analyses (**Table 1**). Food intake was measured each day throughout the study, and the cats were weighed twice weekly. Food intake was adjusted to maintain each cat's body weight within 5% of its weight at the beginning of the study during the lean phase. The cats were maintained in a lean phase for 10 weeks and fed either the SFA or 3-PUFA diet; they were then fed the same diets ad libitum for 21 weeks. Before and after the ad libitum-feeding phase, an **intravenous glucose tolerance test (IVGTT)** was performed. Serum concentrations of glucose, insulin, and **nonesterified fatty acids (NEFAs)** were measured before (time –5) and at 5, 10, 15, 30, 45, 60, 90, and 120 minutes after each glucose injection.

IVGTTs—To allow blood sampling, a catheter was placed in a jugular vein of each cat at 15 to 18 hours before each IVGTT; the IVGTT procedure involved administration of 1 g/kg of 50% dextrose, as described.[14] Catheter patency was maintained by injection of 0.5 mL of 0.38% sterile citrate solution (citric acid [trisodium salt dihydrate])[c] into the catheter every 6 hours. Samples of blood were collected through the catheter. For serum collection, blood was allowed to clot. Serum samples were stored at –20°C until assayed. Food was withheld for 12 to 14 hours before testing.

Assays—Serum insulin concentrations were measured by use of a charcoal method, as described by Hoenig and Ferguson.[21] Serum glucose measurements were performed by use of a colorimetric glucose oxidase method.[c] Serum concentration of NEFAs was measured by use of an enzymatic test kit.[d] Baseline glycosylated hemoglobin concentrations were determined as described.[15,e] Routine blood analyses, including measurements of serum cholesterol and triglyceride concentrations, were performed by the Clinical Pathology Laboratory at the College of Veterinary Medicine of the University of Georgia. Measurement of weight, percentage body fat by **dual energy x-ray absorptiometry (DEXA)**, girth, and body mass index (expressed in kg/m^2) were performed as described.[22,23] To reduce variability, the same investigator (CW) performed all measurements.

Magnetic resonance spectroscopy—The IMCL and **extramyocellular lipid (EMCL)** contents were examined in the same cats before and after the 21-week period of ad libi-

Table 1—Percentage moisture, protein, and ash; percentage calculated carbohydrate; calculated calories per gram; fat; and percentage of fatty acids in the diet containing omega-3 polyunsaturated fatty acids (3-PUFAs) and the diet containing saturated fatty acids (SFAs) fed to cats.

Component	Diet with 3-PUFAs	Diet with SFAs
Moisture	8.41	9.28
Protein	34.40	34.30
Ash	6.45	6.38
Carbohydrate	31.90	32.30
Calories/g	4.33	4.28
Fat	18.40	18.10
Fatty acids		
14:0	3.53	2.01
14:1	0.20	0.39
15:0	0.38	0.33
16:0	21.00	22.50
16:1 n-7	6.33	4.20
17:0	0.55	0.86
18:0	7.33	11.90
18:1 n-9	25.70	34.90
18:1 n-7	2.33	1.75
18:2 n-6	11.40	11.10
18:3 n-3	1.01	0.68
18:3 n-6	0.17	0.11
20:0	0.22	0.18
20:1	0.77	0.32
20:2	0.12	< 0.01
20:3 n-6	0.20	0.13
20:4 n-6	0.66	0.46
20:5 n-3	3.91	0.37
22:2	0.62	< 0.01
22:5 n-3	0.83	0.12
22:6 n-3	4.72	0.46
24:0	< 0.01	< 0.01
24:1	0.17	< 0.01
Unknown fatty acids	5.02	2.48

tum food intake by use of **magnetic resonance spectroscopy** (**^{1}H-MRS**). To obtain water-suppressed, proton-localized spectra of IMCL and EMCL components in cat muscle, a horizontal spectroscopic imaging system[f] operated at 200.56 MHz was used. The actively shielded gradient set had an internal diameter of 22 cm and a maximum gradient strength of 10 G/cm. Each cat was anesthetized with an IM injection of 25 mg tiletamine HCl and zolazepam HCL[g] and placed in dorsal recumbency in a polycarbonate cradle. A specially designed radio frequency transmit-receive surface coil was placed over the quadriceps muscle group of the left hind limb (**Figure 1**). The hind limb was secured in the cradle to orient the muscle fibers and the applied magnetic field in parallel. In this orientation, the signals from IMCL and EMCL components have maximum separation.[24] Selection of the observed voxel was performed with a standard gradient-echo pulse sequence. The muscle group was centered as close as possible to the magnet and gradient center. A transverse image was obtained to locate the muscle center, and a sagittal image was obtained to verify the position along the Z direction, which is parallel to the applied magnetic field. From this image, an oblique image was obtained along the muscle fibers away from any observed fascia. A 10 × 10 × 5-mm voxel with the 5-mm direction perpendicular to the previously defined imaging plane was planned. This procedure allowed reproducible positioning of all the cats in the groups studied. Following this procedure, an optimized version to include a 16-step phase cycle[25] of a localized, non–water-suppressed, **point-resolved spectroscopic (PRESS)** sequence was used to shim the water signal from the selected voxel. The full line width at half height ranged from 12.94 to 23.31 (mean value ± SD, 17.78 ± 2.36).

A water signal was acquired with 16 transients, which were averaged to provide a reference spectrum. Echo time is equal to $2t_1 + 2t_2$, where t_1 is the time from the first 90° selective pulse to the first 180° selective pulse, $t_1 + t_2$ represents the time from the first 180° selective pulse to the second 180° selective pulse, and t_2 is the time from the second 180° selective pulse to the signal echo maximum; in this study, echo time was 33 milliseconds with a t_1 and t_2 equal to 8.5 and 8.0 microseconds, respectively. Water-suppressed spectra were obtained via 3 chemical shift-selective saturation pulses prior to execution of the PRESS section of the sequence. Two sets of spectra were obtained for each cat; these included a short and long echo-time version, each representing the mean of 64 transients. An echo time of 33 milliseconds with a t_1 of 8.5 microseconds and a t_2 of 8.0 microseconds was used for the short echo version. An echo time of 91 milliseconds with a t_1 of 12.5 microseconds and a t_2 of 33 microseconds was used for the long echo version.

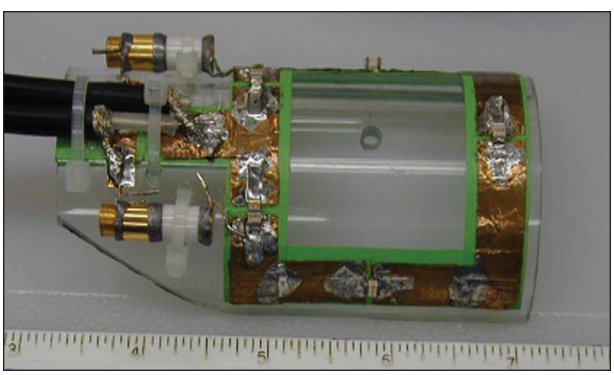

Figure 1—Photograph of a specially designed radio-frequency transmit-receive surface coil that was placed over the quadriceps muscle group of the left hind limb of 28 cats undergoing magnetic resonance spectroscopy.

Calculations—The **glucose disappearance coefficient** (**k**) was calculated by use of computer software.[h] The glucose concentrations versus time data from each IVGTT were fitted to a monoexponential equation by nonlinear regression as follows:

$$Cs = Cs0 \times e^{(-k \times t)}$$

where Cs is the serum glucose concentration at time t (minutes) and Cs0 is the extrapolated initial serum glucose concentration.

The percentage **glucose disappearance per minute** (**K**) was calculated as k × 100. The **area under the curve** (**AUC**) was estimated by the sum of all the trapezoids and triangles bounded by the time versus concentration curve and was mathematically calculated as the integral of the curve. The percentage of the suppression of NEFA concentrations was calculated as follows:

$$\text{Percentage suppression} = 100 - \text{NEFA concentration (t)}/\text{NEFA concentration (t0)} \times 100$$

where t0 represents the data collection point prior to the injection of glucose during the IVGTT.

Spectral data analyses—Spectral data were analyzed by use of the frequency domain analysis package supplied by the manufacturer of the spectroscopic imaging system. Time domain data were apodized with a line broadening of 3.4 Hz. The time domain signal was zero-filled from 8K to 16K data points, Fourier-transformed, and baseline-corrected where necessary. Spectra were fit to a sum of Lorentzian-Gaussian functions (Gaussian fraction of 0.5) to yield the simulated spectra. Areas of each peak were determined from the least squares fit of the data. Areas were normalized to account for instrument factors by scaling to the obtained localized water resonance. The 2 downfield peaks attributable to carnosine were also used to normalize the data.

Statistical analyses—All data were analyzed by use of computer software.[i,j] The data are expressed as means ± SD unless otherwise stated. The significance of differences of means between groups was evaluated by an ANOVA, and differences within a group were evaluated by the Student *t* test for paired analysis. Values of $P < 0.05$ were considered significant.

Results

Cats in both diet groups consumed more kilocalories per body weight at the end of the 21-week period of receiving food ad libitum (68.2 ± 8.3 kcal/kg in the 3-PUFA group and 65.1 ± 5.9 kcal/kg in the SFA group) than they did at the end of the lean phase (50.7 ± 6.7 kcal/kg for the 3-PUFA group and 48.7 ± 6.6 kcal/kg for the SFA group; $P < 0.001$), but there was no difference between diet groups.

Weight, body mass index, girth, and percentage fat were significantly ($P \leq 0.001$) higher in the cats in both diet groups during the ad libitum-feeding phase, compared with values during the lean phase (**Table 2**). In both the 3-PUFA and SFA groups, weight was highly correlated with girth ($r^2 = 0.942$ and 0.940, respectively; $P < 0.001$), body mass index ($r^2 = 0.869$ and 0.873, respectively; $P < 0.001$), and percentage fat ($r^2 = 0.782$ and 0.822, respectively; $P < 0.001$). There was no difference in any of the variables between diet groups.

In both the 3-PUFA and SFA groups, significant differences in plasma glucose, insulin, and NEFA concentrations between the lean and ad libitum-feeding

phases were detected. Significant differences between the 3-PUFA and SFA groups in the ad libitum-feeding phase were detected in insulin AUC (19.9 ± 6.4 nmol/L vs 29.3 ± 9.6 nmol/L per 120 minutes; $P < 0.006$) and glycosylated hemoglobin concentration (1.5 ± 0.34% vs 1.74 ± 0.25%; $P < 0.05$).

The insulin secretory patterns of the cats in the 3-PUFA and SFA groups during IVGTTs were evaluated (**Figure 2**). The baseline insulin concentrations were not significantly different between diet groups or between the lean and ad libitum-feeding phases (**Table 3**); however, the concentrations at 120 minutes after glucose injection were significantly higher in cats during the ad libitum-feeding phase than during the lean phase for both the 3-PUFA and SFA groups, but there was no difference between diet groups. In the SFA group, the insulin AUC was significantly higher during the ad libitum-feeding phase than during the lean phase. In the 3-PUFA group, the insulin AUC was not significantly higher during the ad libitum-feeding phase than during the lean phase. During ad libitum feeding, the insulin AUC was also significantly higher in the SFA group, compared with the value in the 3-PUFA group.

During the ad libitum-feeding phase, 9 of the 28 cats were considered to have normal glucose tolerance as defined by the return of plasma glucose concentrations to within reference range at 120 minutes after glucose injection. These 9 cats had lower insulin concentrations in the lean phase than the 19 cats that became glucose intolerant during the ad libitum-feeding phase. However, the insulin AUC in the lean phase

Table 2—Mean ± SD weight, body mass index, girth, and percentage fat of cats before (lean phase) and after (obese phase) ad libitum intake of a diet high in 3-PUFAs (n = 14) or SFAs (14) for 21 weeks.

Variable	Diet with 3-PUFAs	Diet with SFAs
Weight (kg)		
Lean phase*	3.3 ± 0.5	3.2 ± 0.4
Obese phase	5.4 ± 1.2	5.5 ± 0.7
Body mass index (kg/m^2)		
Lean phase*	34.4 ± 2.7	34.6 ± 2.8
Obese phase	54.5 ± 7.6	57.0 ± 6.7
Girth (cm)		
Lean phase*	27.3 ± 2.9	27.3 ± 2.9
Obese phase	40.1 ± 5.5	42.5 ± 3.1
Fat (%)		
Lean phase*	8.9 ± 1.8	9.5 ± 2.4
Obese phase	30.9 ± 7.5	35.7 ± 4.5

*For weight, body mass index, girth, and percentage fat, values between lean and obese phases were significantly ($P < 0.001$) different for both diets.

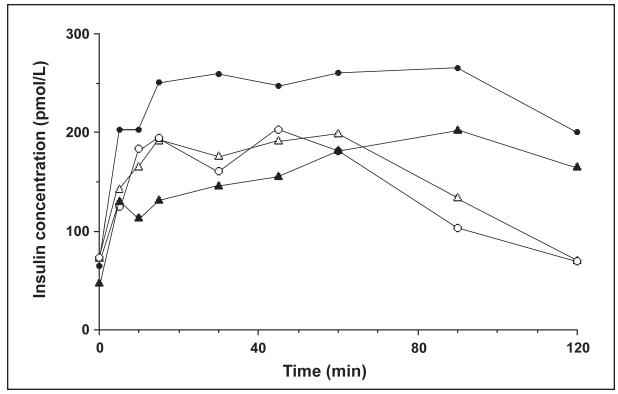

Figure 2—Mean plasma insulin concentration at baseline (time 0) and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after IV administration of 50% dextrose (1 g/kg) in 14 cats fed a diet containing polyunsaturated fatty acids (PUFAs; triangles) and 14 cats fed a diet containing saturated fatty acids (SFAs; circles) before (lean phase; open symbols) and after (closed symbols) receiving food ad libitum for 21 weeks.

Table 3—Results (mean ± SD) of plasma glucose, insulin, and nonesterified fatty acid (NEFA) analyses performed during IV glucose tolerance tests and plasma cholesterol, triglycerides, and glycosylated hemoglobin (GHb) concentrations in cats before (lean phase) and after (obese phase) ad libitum food intake of a diet high in 3-PUFAs (n = 14) or SFAs (14) for 21 weeks.

	Diet with 3-PUFAs			Diet with SFAs		
Variable	**Lean phase**	**Obese phase**	***P* value**	**Lean phase**	**Obese phase**	***P* value**
Glucose (mg/dL)						
Baseline	99 ± 14	98 ± 13	NS	101 ± 18	98 ± 9	NS
120-minute concentration	90 ± 31	148 ± 47	< 0.001	86 ± 15	140 ± 58	0.002
k value	1.71 ± 0.39	1.34 ± 0.31	0.001	1.85 ± 0.45	1.44 ± 0.38	0.002
AUC (g/L)	2.7 ± 0.5	4.2 ± 0.5	< 0.001	2.6 ± 0.4	4.3 ± 0.7	< 0.001
Insulin (pmol/L)						
Baseline	72.7 ± 48.7	49.2 ± 26.6	NS	73.6 ± 64.5	64.6 ± 40.2	NS
120-minute concentration	73.4 ± 42.0	165.0 ± 99.3	0.004	70.2 ± 32.1	200.6 ± 118.2	0.002
AUC (nmol/L)	18.13 ± 8.00	19.88 ± 6.35	NS	18.25 ± 6.40	29.32 ± 9.61	0.001
NEFA (mEq/L)						
Baseline	0.16 ± 0.07	0.34 ± 0.20	0.007	0.20 ± 0.11	0.37 ± 0.14	0.004
120-minute concentration	0.14 ± 0.08	0.11 ± 0.08	NS	0.16 ± 0.08	0.12 ± 0.06	0.02
AUC-%SUPP (mEq/L × 1,000)	7.5 ± 2.3	5.0 ± 1.6	< 0.001	7.2 ± 1.8	4.6 ± 1.7	0.001
Cholesterol (mg/dL)	153 ± 32	148 ± 23	NS	158 ± 29	155 ± 37	NS
Triglyceride (mg/dL)	30 ± 12	33 ± 6	NS	31 ± 12	36 ± 14	NS
GHb (%)	1.46 ± 0.29	1.50 ± 0.34	NS	1.63 ± 0.28	1.74 ± 0.25	NS

Baseline = Plasma concentrations before start of IV glucose tolerance test. 120-minute concentration = Concentration at 120 minutes after start of IV glucose tolerance test. k value = Disappearance coefficient for glucose. AUC = Area under the curve. %SUPP = Percentage suppression = 100 − NEFA concentration (t)/NEFA concentration (t0) × 100. NS = Not significant (value of $P > 0.05$).

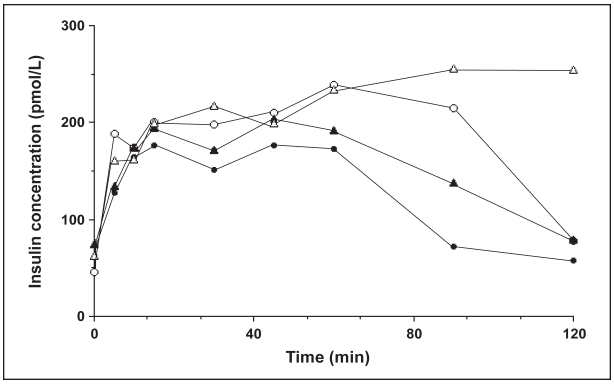

Figure 3—Mean plasma insulin concentration at baseline (time 0) and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after IV administration of 50% dextrose (1 g/kg) in 9 glucose-tolerant cats (circles) and 19 glucose-intolerant cats (triangles) before (closed symbols) and after receiving a high fat diet ad libitum for 21 weeks (open symbols).

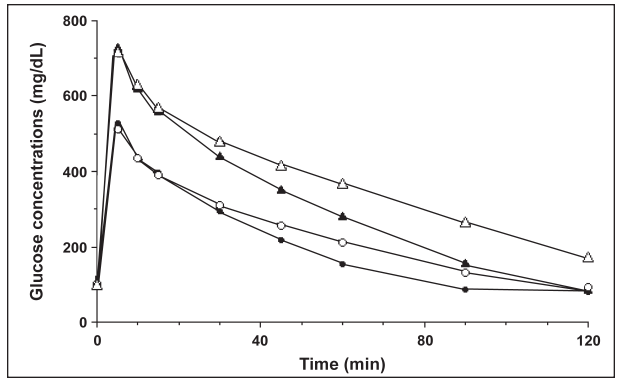

Figure 4—Mean plasma glucose concentration at baseline (time 0) and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after IV administration of 50% dextrose (1 g/kg) in 9 glucose-tolerant cats (closed symbols) and 19 glucose-intolerant cats (open symbols) before (circles) and after (triangles) receiving a high-fat diet ad libitum for 21 weeks.

was significantly ($P = 0.031$) lower only during the last 60 minutes of the IVGTT (5.6 ± 2.0 nmol/L and 8.5 ± 3.4 nmol/L, respectively). In the ad libitum-feeding phase, the 9 glucose-tolerant cats had significantly ($P < 0.001$) lower plasma insulin concentrations detected at 120 minutes during the IVGTT than did the 19 glucose-intolerant cats (78 ± 59 pmol/L and 254 ± 115 pmol/L, respectively); the AUC for insulin was also significantly ($P = 0.032$) lower during the last 30 minutes of the IVGTT (4.7 ± 2.8 nmol/L vs 7.7 ± 3.2 nmol/L, respectively; **Figure 3**).

The plasma glucose concentrations during the IVGTT for the glucose-tolerant and glucose-intolerant cats in the lean and ad libitum-feeding phases were assessed (**Figure 4**). The K value for glucose in the lean phase was significantly ($P = 0.003$) higher for the glucose-tolerant cats than it was for the glucose-intolerant cats (2.11 ± 0.30 and 1.63 ± 0.37, respectively). In the lean phase, the glucose-intolerant cats had a higher glucose AUC during the IVGTT than the glucose-tolerant cats, but this difference was not significant (27.5 ± 5.2 g/dL and 24.1 ± 2.1 g/dL, respectively; $P = 0.068$).

There was no difference in baseline serum triglyceride or cholesterol concentrations between lean and ad libitum-feeding phases in either diet group, and there was no difference between groups (Table 3). In both diet groups, cats had lower baseline plasma NEFA concentrations during the lean phase than during the ad libitum-feeding phase; however, there was no difference between the diet groups (**Figure 5**).

The percentage suppression of plasma NEFA concentrations was significantly greater during the ad libitum-feeding phase than it was in the lean phase in both diet groups, but there was no difference between the diets (Table 3). The AUC of percentage suppression was significantly lower in the ad libitum-feeding phase than the lean phase for males of the 3-PUFA (7.7 ± 2.5 mEq/L and 5.2 ± 1.1 mEq/L × 1,000, respectively; $P = 0.012$) and SFA (7.6 ± 1.5 and 4.3 ± 2.1, respectively; $P = 0.008$) groups; this was also determined for female cats in the 3-PUFA group (7.2 ± 2.2 mEq/L and 4.7 ± 2.1 mEq/L × 1,000, respectively; $P = 0.023$). Between the 2 phases, the AUC of percentage suppression of

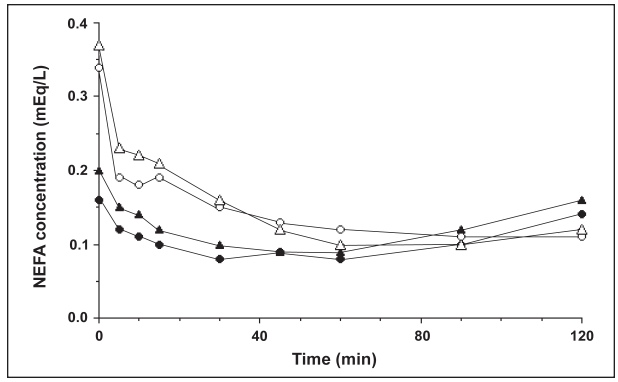

Figure 5—Mean plasma nonesterified fatty acid (NEFA)concentrations at baseline (time 0) and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after IV administration of 50% dextrose (1 g/kg) in 14 cats fed a diet containing PUFAs (circles) and 14 cats fed a diet containing SFAs (triangles) before (closed symbols) and after (open symbols) receiving food ad libitum for 21 weeks.

plasma NEFA concentrations for females of the SFA group did not reach significance (lean phase, 6.8 ± 2.2 × 1,000; ad libitum-feeding phase, 4.9 ± 1.3 × 1,000; $P = 0.059$).

There was no significant difference in the integrated intensity of the peaks of total IMCL and EMCL concentrations between the 3-PUFA and SFA groups in the lean and ad libitum-feeding phases (**Figure 6**). In the 3-PUFA group, the integrated intensities (in **arbitrary units [AUs]**) for the IMCL concentration in the lean and ad libitum-feeding phases were 626 ± 640 AUs and 809 ± 461 AUs; in the SFA group, these values were 771 ± 781 AUs and 919 ± 230 AUs. In the 3-PUFA group, the integrated intensities for the EMCL concentration in the lean and ad libitum-feeding phases were 1,612 ± 1,291 AUs and 3,096 ± 1,686 AUs; in the SFA group, these values were 1,651 ± 819 AUs and 4,037 ± 1,810 AUs. Therefore, the data for both diets were combined, and the integrated intensities of the IMCL and EMCL peak concentrations of all cats (3-PUFA and SFA groups) were compared. In the lean phase, cats (combined data) had lower integrated intensity of the peak IMCL concentration than they did in the ad libitum-feeding phase (652 ± 693 AUs and 869 ± 338 AUs, respectively); also, they had lower integrated

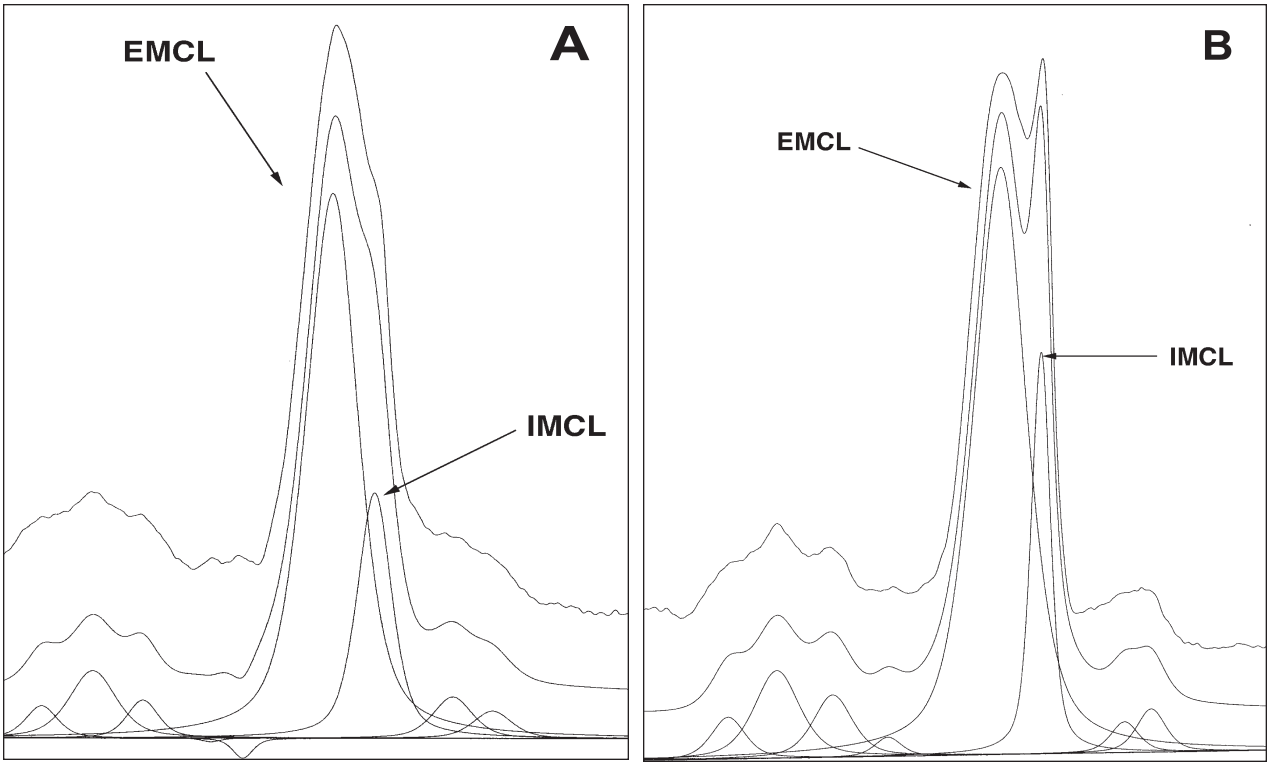

Figure 6—Magnetic resonance spectra of intramyocellular (IMCL) and extramyocellular (EMCL) lipid of the quadriceps muscle group in a lean cat (A) and in the same cat after receiving food ad libitum for 21 weeks (B).

intensity of the peak EMCL concentration (1,612 ± 1,291 AUs and 3,096 ± 1,737 AUs, respectively). However, the difference was only significant for EMCL ($P < 0.002$). The EMCL:IMCL ratio did not change significantly between the lean (6.4 ± 4.2 AUs) and ad libitum-feeding phases (5.3 ± 3.5 AUs). The lipid content in muscle, regardless of location (ie, intra- or extracellular), was significantly different between cats in the lean and ad libitum-feeding phases (1,183 ± 983 AUs and 2,216 ± 1,811 AUs, respectively; $P = 0.018$).

The EMCL content correlated significantly and positively with IMCL content ($P = 0.002$), weight ($r^2 = 0.188$; $P = 0.034$), and girth ($r^2 = 0.281$; $P = 0.008$), as well as with the baseline insulin-to-glucose ratio ($r^2 = 0.271$; $P = 0.011$), serum insulin concentration at 120 minutes after the start of the IVGTT ($r^2 = 0.237$; $P = 0.016$), and insulin AUC ($r^2 = 0.339$; $P = 0.003$). The IMCL content correlated significantly with baseline plasma insulin concentrations ($r^2 = 0.302$; $P = 0.008$) and the pre-IVGTT baseline insulin-to-glucose ratio ($r^2 = 0.261$; $P = 0.013$).

Discussion

During the ad libitum-feeding phase, the cats gained a significant amount of weight regardless of diet. This finding is similar to results obtained in a study in rats by Pellizzon et al.[26] Those investigators detected no difference in weight gain in rats that were fed either a diet containing saturated fat or a diet high in omega-3 fatty acids for 15 weeks. However, in contrast, after short-term feeding (6 weeks), rats fed the diet high in omega-3 fatty acids gained less weight than those fed the diet containing saturated fat. It was speculated that the protective effect of omega-3 fatty acids on weight gain was greatly diminished with long-term feeding. In the cats of the study of this report, there was no evidence of a protective effect of omega-3 fatty acids on comparison of weight gain in the 3-PUFA and SFA groups at 4, 9 (data not shown), or 21 weeks.

Consumption of a diet high in omega-3 PUFAs decreases fat deposition in humans[27] and rats.[25] Deposition of visceral fat, rather than subcutaneous fat, is the main contributor to the increased risk of developing diabetes, coronary heart disease, and hypertriglyceridemia in humans.[28,29] It is believed that the location of fat around the organs (primarily the liver) causes an increased flux of free fatty acids through these tissues.[29] This results in the decreased insulin sensitivity of the liver,[30] which therefore perturbs the ability of insulin to slow hepatic gluconeogenesis and glycogenolysis. Although the cats in our study became obese, it could not be determined from the DEXA scans where this fat was deposited, nor if there was an effect of diet on the distribution of fat.

The amount of omega-3 fatty acids was approximately 10-fold higher in the diet fed to the 3-PUFA group than it was in the diet fed to the SFA group. It might be argued that it is not the concentration of omega-3 fatty acids in the diet but rather the ratio of omega-3 to omega-6 fatty acids that is important for the biological effect. However, a multinational task force has concluded that it is most meaningful to compare absolute amounts of specific PUFAs as a predictor of biological response.[31]

The risk of developing glucose intolerance and insulin resistance is increased with the development of

obesity. Similar to findings of other studies,[32-34] most obese cats (ie, after ad libitum feeding) in the study of this report had a greater glucose AUC, K value, and serum glucose concentration at 120 minutes of the IVGTT, compared with values for lean cats (regardless of diet). However, neither baseline glucose nor baseline insulin concentrations differed as a result of obesity or diet. This finding is consistent with results of the investigation of Summers et al,[9] which indicated that there was no difference in basal concentrations of glucose and insulin between lean and obese humans before and after dietary intervention. It is noteworthy that in our study, the cats of the SFA group during the lean and ad libitum-feeding phases (lean and obese cats, respectively) had higher glycosylated hemoglobin concentrations than the cats of the 3-PUFA group. This difference was only significant between obese cats during the ad libitum-feeding phase in the 2 groups. Nevertheless, it suggests that SFAs exert a negative effect on glucose control. Because these study cats were fed ad libitum for only 21 weeks, one might speculate that long-term consumption of a diet containing SFAs might induce overt glucose intolerance, similar to that detected in rats[8] and an epidemiologic study[7] in humans. The fact that baseline plasma glucose concentrations were not different between cats during the lean and ad libitum-feeding phases but other indices of glucose tolerance (K value, glucose AUC, and plasma glucose concentrations at 120 minutes of the IVGTT) were different between obese and lean cats indicates the importance of challenge tests to identify problems with insulin secretion or action early in their development (problems which would otherwise go unnoticed if only a baseline blood sample were evaluated).

Unlike the cats in the 3-PUFA group, cats fed the SFA diet had a significantly higher insulin AUC in the obese state than in the lean state. This finding is in agreement with both experimental[10,35] and epidemiologic data[4] that have indicated an association between SFAs and hyperinsulinemia in humans and rodents. Hyperinsulinemia has been detected in obese cats,[32-34,36] and in many species, it is believed to contribute to the detrimental effects of obesity on health.[4] Therefore, decreasing plasma insulin concentrations through consumption of 3-PUFAs (as occurred in the cats of our study) or through other therapeutic approaches (eg, administration of pharmacologic agents[37]) might decrease the risk of cats to develop diabetes, similar to findings in humans and other species.[29,38,39] It is theorized that the beneficial effect of unsaturated fatty acids (primarily oleate and linoleate) is caused by an increased expression of islet **peroxisome proliferator-activated receptor-γ (PPAR-γ)**. This leads to upregulation of the expression of uncoupling protein 2 and reduction of glucose-stimulated insulin secretion.[10,40] Similarly, insulin resistance and the abnormal lipid profile associated with obesity can be ameliorated by the omega-3 PUFA eicosapentaenoic acid (eicosapentaenoic acid, 20:5 n-3) through induction of mRNA expression of PPAR-γ in adipocytes.[41] There was a large variation and no difference in plasma insulin concentrations (after withholding of food) between lean and obese cats in the study of this report. Overlapping baseline plasma insulin concentrations have also been detected in other studies,[32,34,36] suggesting that a single baseline plasma insulin measurement is not a good indicator of metabolic alterations.

It is fascinating to note that the 19 cats with glucose intolerance in the ad libitum-feeding phase had detectable alterations in plasma insulin concentration and glucose tolerance during the lean phase. Compared with cats with normal glucose tolerance during the ad libitum-feeding phase, cats with glucose intolerance in that phase had higher insulin AUC during the last 60 minutes of the IVGTT. Those same cats maintained higher insulin AUC values during the ad libitum-feeding phase, compared with values in cats with normal glucose tolerance, although this difference was significant only for the last 30 minutes of the IVGTT. This suggests that cats that later become glucose intolerant have an abnormal insulin secretion pattern when they are lean and apparently glucose tolerant. It is unclear if the abnormal insulin concentrations are caused by a primary beta cell defect or a defect in the cellular response to insulin that creates an insulin-resistant environment. A tendency toward high baseline plasma insulin concentration and insulin AUC has been reported for lean cats that developed glucose intolerance with obesity[36]; however, the differences were not significantly different from values for lean cats that did not develop glucose intolerance with obesity. The obese, glucose-tolerant cats in our study had a higher glucose K value in the lean state than did the obese, glucose-intolerant cats. This suggests that glucose clearance as well as insulin secretion or action were abnormal in the lean cats that would later become glucose intolerant with the development of obesity. Results of another study[42] performed in our laboratory support the findings of the study of this report in that **glucose transporter-4 (GLUT4)** activity in muscle and adipose tissue is altered early in the development of obesity before any changes toward overt glucose tolerance are detected.

The downregulation of GLUT4 in muscle is likely the mechanism causing the abnormal glucose tolerance in most obese cats because muscle represents the major site for insulin-stimulated glucose disposal[43] and GLUT4 is the main insulin-sensitive glucose transporter. This downregulation could be a result of an alteration of the insulin-signaling cascade caused by changes in lipid metabolism and increases in lipid deposition in muscle.[44]

Regardless of diet, obesity resulted in increased baseline plasma NEFA concentrations in the cats of our study. However, whereas other studies[45,46] have revealed a significant increase in the concentration of NEFAs of obese humans, compared with values in lean individuals, this difference is not always evident in obese cats. In a previous study,[23] no significant difference in baseline plasma NEFA concentrations between lean and obese cats fed a commercial maintenance diet was detected, which suggested that an increase in dietary fat (regardless of origin) increases plasma NEFA concentrations in obese cats. In rats[47-49] and humans,[49] no difference in baseline plasma NEFA concentrations was detected between individuals fed a diet high in SFAs

and those fed a diet high in PUFAs. It might be argued that a comparison of serum lipid concentrations between the cats of our study and humans and rats is not valid because the cats were neutered and this might change the concentration or composition of serum lipids and lipoproteins. However, there are data to support that neutering does not significantly affect lipoprotein composition or lipid metabolism in cats.[k] It must be pointed out that there are species differences in the lipid and lipoprotein response to fatty acids; for example, omega-3 fatty acids decrease serum triglyceride concentrations in humans, rats, and pigs, but this effect is not detected in other animal species (eg, other rodents, monkeys, and dogs).[50]

The ability of insulin to suppress the plasma concentration of NEFAs postprandially is impaired in humans with obesity.[51] In contrast to results in humans, the obese cats in the study[37] of this report had more suppression of the IVGTT, which agrees with data from another study in obese cats. It is speculated that the inability of insulin to suppress plasma NEFA concentration in humans with obesity could be attributable to a diminished ability of insulin to reduce hormone-sensitive lipase activity or stimulate lipoprotein lipase activity to take up free fatty acids in adipose tissue.[52-54] In cats, adipose tissue and skeletal muscle appear to become more sensitive to the antilipolytic effects of insulin in the obese state rather than in the lean state. Along with the finding that lipoprotein lipase activity in human skeletal muscle increases with obesity,[55] the lipoprotein lipase activity of muscle of the cats in our study may have similarly increased because there was an increase in the total lipid content of muscle of the obese cats, compared with the total lipid content of muscle of the lean cats.

Obesity has been associated with increased serum concentrations of triglyceride[56] and cholesterol in humans[57] and cats.[23,58] The fact that there was no difference in either variable between lean and obese cats may be attributable to the duration of the ad libitum-feeding phase. Without lipoprotein analysis, it is difficult to compare these findings with those of our previous study[23]; it should also be noted that in that previous investigation, the serum concentrations of triglyceride and cholesterol remained within reference ranges. The results of the study reported here are in accordance with those of Dimski et al[59] who reported no difference in serum triglyceride or cholesterol concentrations between obese and lean cats. Because the cats in each of these studies were fed different diets, this illustrates the influence of diet (especially dietary lipids) on biochemical variables.

Through the use of ^{1}H-MRS, 2 compartments of muscle lipid stores have been identified: IMCL is located around the mitochondria of muscle cells, and EMCL is located between the muscle fibers.[11-13] We were unable to find information about intramuscular lipid stores and changes in lipids in muscle tissue with obesity in cats, perhaps because the invasive means by which muscle biopsy specimens are obtained has limited research efforts. However, it is known that skeletal muscle is a primary site of insulin action, and the deposition of lipids in muscle has been reported to play a key role in the pathogenesis of insulin resistance in several species. A method such as ^{1}H-MRS is important in the investigation of the role of lipids in cats because it is a noninvasive technique and allows for repetitive experiments to be performed on the same individuals. In the study of this report, the method used for spectral data analyses was similar to that used in other investigations[24,60,61] in which IMCL and EMCL integrals were quantified. Placement of the muscle fibers and the field in parallel is a crucial factor in ^{1}H-MRS.[60] In humans, a long muscle group of the leg (soleus muscle) can be used in such investigations, but cats have much smaller limb muscles; the ability to place all muscle fibers and the field in parallel is therefore difficult. In our experience with cats, the quadriceps muscle was the most accessible muscle to investigate with easy placement of the coil, and in this manner, we were able to distinguish IMCL and EMCL deposits. This distinction is important because in other species, partitioning of the fat into the myocellular space is a sensitive indicator of insulin sensitivity.[24,61]

Because placement of the coil and muscle fibers in parallel is crucial to receiving excellent ^{1}H-MRS data, yet difficult to perform in the small muscle groups of cats, we obtained large errors in the integrated intensities, especially in the lean study cats. It might be possible to improve these errors by reducing the size of the coil or choosing a larger muscle group to analyze; however, these options would be difficult to implement in cats.

Obese cats had increases in both IMCL and EMCL with no effect of diet and no change in the EMCL:IMCL ratio. These data are similar to those obtained by Sinha et al[61] and Greco et al,[62] which indicated that obese humans had significantly higher IMCL and EMCL contents than did lean individuals. Sinha et al[61] reported that both IMCL and EMCL were indicators of insulin sensitivity in children, whereas other investigators have determined that glucose intolerance and insulin resistance are primarily associated with an increase in IMCL content.[63,64] In the study of this report, the increase in both IMCL and EMCL contents suggested that lipids are not preferentially partitioned into the intramyocellular space in obese cats, as has been suggested for obese humans.[55] The positive correlations of IMCL content with baseline plasma insulin concentrations and EMCL content with morphometric measures of obesity, as well as with plasma insulin concentrations during the IVGTT, indicated that both lipid components may modulate insulin sensitivity. This is further supported by the fact that there were strong correlations between the insulin-to-glucose ratio (frequently used as a biochemical marker of insulin sensitivity) and IMCL and EMCL contents, respectively; these findings have been reported in humans.[61] It is speculated that conversion of the accumulated long-chain acyl-coenzyme A in muscle to another metabolically active form of lipid, such as diacylglycerol, may alter the activity of enzymes responsible for activation of the insulin signaling cascade[9] and result in impaired translocation of GLUT4 to the cell membrane and subsequent decreased glucose uptake.[64] The detection of decreased GLUT4 expression in obese

cats[42] supports the proposal that such a pathway is also operative in cats. Furthermore, these results suggest that the increase in NEFA clearance during an IVGTT may be partly the result of increased uptake into muscle tissue. It is also possible that the increase in very LDL metabolism detected in obese cats[23] may be linked to stimulation of lipoprotein lipase and uptake of fatty acids into muscle.

[a]Harlan Sprague Dawley, Madison, Wis.
[b]ProPlan, Nestlé Purina Co, St Louis, Mo.
[c]Glucose trinder kit, Sigma Chemical Co, St Louis, Mo.
[d]NEFA-C, Wako Diagnostic, Richmond, Pa.
[e]Glyc-Affin GHb kit, Perkin Elmer Wallac, Norton, Ohio.
[f]Varian/Inova 4.7T, Palo Alto, Calif.
[g]Telazol, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
[h]Passage program for Macintosh computers, Passage Software Inc, Fort Collins, Colo.
[i]Data Desk software, Odesta Corp, Northbrook, Ill.
[j]Prism software, GraphPad Software Inc, San Diego, Calif.
[k]Cook J, Pazak H, Alexander S, et al Gender effects on hormonal and lipid profiles in cats before and after neutering, in *Proceedings*. Purina Res Forum 2000;102.

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