Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss

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1Department of Physiology and Pharmacology, University of Georgia College of Veterinary Medicine, Athens, Georgia; 2Institute of Biomedical Engineering of the Italian National Research Council, Padua, Italy; and 3Nestle Purina Research, St. Louis, Missouri

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Hoenig M, Thomaseth K, Waldron M, Ferguson DC. Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss. Am J Physiol Regul Integr Comp Physiol 292: R227–R234, 2007. First published August 10, 2006; doi:10.1152/ajpregu.00313.2006.—Obesity is a major health problem in cats and a risk factor for diabetes. It has become clear that cats are always gluconeogenic and that the rise in obesity might be related to high dietary carbohydrates. We examined the effect of a high-carbohydrate/low-protein (HC) and a high-protein/low-carbohydrate (HP) diet on glucose and fat metabolism during euglycemic hyperinsulinemic clamp, adipocytokines, and fat distribution in 12 lean and 16 obese cats before and after weight loss. Feeding diet HP led to greater heat production in lean but not in obese cats. Regardless of diet, obese cats had markedly decreased glucose effectiveness and insulin resistance, but greater suppression of nonesterified fatty acids during the euglycemic hyperinsulinemic clamp was seen in obese cats on diet HC compared with lean cats on either diet or obese cats on diet HP. In contrast to humans, obese cats had abdominal fat equally distributed subcutaneously and intra-abdominally. Weight loss normalized insulin sensitivity; however, increased nonesterified fatty acid suppression was maintained and fat loss was less in cats on diet HP. Adiponectin was negatively and leptin positively correlated with fat mass. Lean cats and cats during weight loss, but not obese cats, adapted to the varying dietary carbohydrate/protein content with changes in substrate oxidation. We conclude that diet HP is beneficial through maintenance of normal insulin sensitivity of fat metabolism in obese cats, facilitating the loss of fat during weight loss, and increasing heat production in lean cats. These data also show that insulin sensitivity of glucose and fat metabolism can be differentially regulated in cats.

euglycemic hyperinsulinemic clamp; indirect calorimetry; obesity; diabetes; leptin; adiponectin; magnetic resonance imaging

Obesity is increasing at a rapid rate in cats. It is a major health problem and is associated with several diseases, among them diabetes mellitus. To be able to develop treatment regimens for obesity, it is important to understand the pathophysiologic mechanisms that lead to its manifestation. With the euglycemic hyperinsulinemic clamp (EHC), obese cats have been shown to have decreased glucose effectiveness (i.e., efficiency of insulin-independent glucose removal) and insulin sensitivity (19). Prolonged insulin resistance is one of the factors contributing to beta cell exhaustion (12). It has become clear that lipid distribution is a major factor in the development of insulin resistance (3, 15). We recently showed (49) that obese cats preferentially partition fatty acids to muscle tissue, resulting in increased muscular lipid content. Increased lipid deposition in muscle tissue can be associated with insulin resistance (47). It also has been shown that the abdominal distribution of fat is important, not only regarding insulin resistance but also regarding the development of the comorbidities of obesity (29, 34, 38, 42, 46). In humans, it has long been known that the abdominal fat distribution of males called “android” is associated with diabetes mellitus, coronary artery disease, and gout. This association is not found in women, because they have a gynoid fat accumulation pattern called “gynoid” that is not associated with similar comorbidities.

Obesity and insulin resistance have been linked to alterations in adipocytokines and hormones (2, 3, 12, 22, 32), among them leptin and adiponectin (2, 3, 12, 22, 32). Leptin is an important regulator of fat mass, and its concentration in serum is positively correlated with fat mass (11). It acts by stimulating energy expenditure and decreasing food intake (9). Similar to insulin resistance, there is leptin resistance in obese subjects (21). In contrast to leptin, adiponectin serum concentrations are negatively correlated with obesity and positively with insulin sensitivity (13). Adiponectin is expressed and differentiated in adipocytes. It is not known whether macronutrients have an effect on these hormones.

It has been suggested that a high-protein diet is beneficial for diabetic cats and leads to improvement of insulin requirements (10). The reasons are not understood, although it is well known that cats are obligatory carnivores, and therefore a high-protein diet appears to be efficacious with respect to glucose parameters in both obese and diabetic cats. There is also some evidence that cats might not be able to adapt to different macronutrients because in one study persistently upregulated gluconeogenic enzymes were found in cats fed either a commercial kibble (likely a high-carbohydrate diet) or a diet containing 17.5% or 70% protein (37).

The main goal of this study was to elucidate whether cats are able to adapt to high and low dietary protein and carbohydrate levels. In particular, we were interested in the dietary effect on insulin sensitivity, substrate oxidation, fat distribution, and leptin and adiponectin levels. We compared these parameters between lean and obese cats and between obese cats before and after weight loss.

MATERIALS AND METHODS

Animals. Twelve lean (6 females and 6 males) and 16 obese (8 females and 8 males) adult neutered purpose-bred cats (Harlan

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Sprague Dawley, Madison, WI) were used. The age of the lean cats was 4.3 ± 0.4 yr, and that of the obese cats was 5.1 ± 2.2 yr. The obese cats had been obese for >1 yr. Obesity had been originally induced in the cats by allowing ad libitum food intake, whereas lean cats were fed the amount needed to maintain their body condition.

The cats were maintained at the University of Georgia College of Veterinary Medicine Animal Care Facility, under standard colony conditions. Cats were housed separately in cages and were provided unlimited access to water. 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. It was determined that the cats were healthy on the basis of results of physical examination and clinical laboratory tests. All cats were used to being handled daily. Cats were fed once daily with one of two diets, high-carbohydrate/low-protein (HC) and high-protein/low-carbohydrate (HP) (Table 1), and food intake was recorded at each feeding. Their weight was monitored once weekly. Food intake and weight are shown in Table 2.

The study design was as follows: The cats were evenly and randomly allocated to the two diets. Each diet was fed for a period of 4 mo, and then the diet was switched, i.e., cats that had been fed diet HC were now fed diet HP and vice versa. During these 8 mo, the cats were fed the amount necessary to maintain their weight within a narrow range (Table 2). After a total of 8 mo, the obese cats were maintained on the same diet that had been fed during the preceding 4 mo; however, food intake was decreased to obtain an ~1.5% decrease in body weight (BW) per week. As shown in Table 2, the food intake during that time was similar for the groups. Insulin sensitivity was tested with the EHC after 4 mo (T1), after 8 mo (T2), and 6 mo later when the obese cats returned to their original lean weight (T4). Magnetic resonance imaging (MRI) of abdominal and subcutaneous fat was performed after 4 mo (T1), after 8 mo (T2), and after the obese cats had lost ~50% of their excess weight (T3).

### Table 1. Composition and metabolizable energy of diets HC and HP

<table>
<thead>
<tr>
<th>Nutrient composition, g/100 g</th>
<th>Diet HC</th>
<th>Diet HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>27.5</td>
<td>45.2</td>
</tr>
<tr>
<td>Fat</td>
<td>15.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>38.1</td>
<td>24.7</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Ash</td>
<td>5.7</td>
<td>7.6</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/g&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.01</td>
<td>4.04</td>
</tr>
</tbody>
</table>

**Components, %**

- Rice, brewers 33 16
- Chicken, whole 20.5 26
- Corn gluten meal 6 25
- Poultry by-product meal 17 25
- Soybean meal 10 14
- Corn, whole 10 2
- Corn, bran 1 1
- Beef tallow 5.5 6
- Palatent 2 2
- Potassium chloride 0.5 0.5
- Sodium chloride 0.25 0.25
- Phosphoric acid 0.75 0.75
- Mineral PMX 0.25 0.25
- Choline chloride 0.15 0.15
- Vitamin E 0.2 0.2
- Vitamin PMX 0.08 0.08

HC, high carbohydrate/low protein; HP, high protein/low carbohydrate; PMX, premix. *Determined by bomb calorimetry.

**Blood sampling.** To allow blood sampling, catheters were placed in the jugular vein of cats 36–40 h before each of the EHC and in the cephalic vein before the clamp. Catheter patency was maintained by injection of 0.5 ml of 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate; Sigma) every 8 h. Whole blood was taken through the jugular catheter and allowed to clot for serum collection; for the collection of plasma, blood was placed into tubes containing cold potassium oxalate (1 mg/ml), sodium fluoride (1 mg/ml), and benznidamide (1 M) and the samples were centrifuged immediately. Serum and plasma samples were stored at −20°C until assay. Mixed venous samples were drawn for glucose and insulin determinations. Infusions were administered via cephalic catheter. Whole blood glucose was measured with a glucometer (Elite XL, Bayer, Mishawaka, IN) at frequent intervals. Plasma glucose and insulin concentrations were determined at −10, 0, 30, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min as described previously (19).

**Testing of insulin sensitivity and modeling of data.** Insulin sensitivity was examined with the EHC, using the minimal model of glucose disappearance (4a) modified to account for exogenous glucose administration, as described in detail previously (19). Tracer glucose was omitted. Briefly, saline was infused for 120 min. The clamp was started at time 120 min and lasted for 120 min, according to the protocol described by Petrus and coworkers (33) with the following modifications: at time 120 min an initial insulin bolus of 180 pmol/kg BW recombinant human regular insulin (Eli Lilly, Indianapolis, IN) was administered intravenously, and a solution of saline with added glucose (20% wt/vol) was infused for 120 min at a rate necessary to maintain the cats’ fasting blood glucose concentrations.

The analysis was carried out on data between t = 0 (start of insulin and glucose infusion) and t = 120 min. The minimal model of glucose disappearance used to fit plasma glucose concentrations is described by the differential equations

\[
dG(t)/dt = -(S_G + X(t))G(t) + S_CG_b + u_G(t)/V_G
\]

\[
X(t)/dt = -P(t)X(t) - (S_I(t) - I_b)
\]

where \(G(t)\) and \(I(t)\) represent dynamic plasma glucose and insulin concentrations, respectively; \(X(t)\) represents insulin action in a remote compartment that lags behind insulin variations from basal according to the rate constant \(P\); \(G_b\) and \(I_b\) are the basal concentrations of glucose and insulin, respectively; \(S_C\) and \(S_I\) are the main parameters called glucose effectiveness and insulin sensitivity, respectively, which characterize, in turn, insulin-independent fractional glucose disappearance rate at basal insulin and insulin-dependent increase of fractional glucose disappearance rate per unitary increase of insulin concentration from basal; \(u_G(t)\) represents exogenous glucose administer.
istered during EHC to maintain euglycemia; and $V_G$ is the glucose distribution volume.

The minimal model was fitted to plasma glucose measurements by estimating the minimal model parameters $S_G$ and $S_I$ using as model inputs the plasma insulin concentration profile, $I(t)$, reconstructed individually by linear interpolation of available measurements, and the piecewise constant glucose infusion pattern, $u_G(t)$. The other minimal model parameters, i.e., $V_G$ and $P_2$, were assigned values estimated from a previous study with a frequently sampled intravenous glucose tolerance test (1); the basal concentrations $G_0$ and $I_0$ were assigned the measurements taken at $t = 0$.

Parameter estimates (log-transformed values) of each individual experiment (viewed as independent from the others) were obtained by fitting a nonlinear mixed effects (NLME) population model simultaneously to all available data. The fixed effects (population averages) were found to be significantly dependent on body size (lean vs. obese). Individual parameter estimates were obtained from the population estimates of fixed effects and individual estimates of random effects by the so-called best linear unbiased prediction. The evaluation of possible dependencies of individual glucose kinetics parameters from the diet (intraindividual variability) was subsequently assessed by linear mixed effects (LME) modeling. NLME and LME analysis were performed within the open-source statistical software R (R Development Core team: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria) combined with model equations generated with the modeling tool Pansym (44).

Blood measurements. Baseline adiponectin concentrations at time $-10$ min before the EHC were measured with an ELISA kit from B-Bridge International (Sunnyvale, CA). The assay has been validated for use in cats in our laboratory. All samples were tested in duplicate. The standard curve for serial dilutions of serum from clinically normal lean and obese cats was observed to be parallel to the standard curve for mouse adiponectin standards. Addition of 5 concentrations of mouse standard to feline serum resulted in mean $\pm$ SD recovery of $77.4 \pm 10.5\%$. The assay had a working range of 0.25–8 ng/ml.

Baseline leptin concentrations at time $-10$ min before the EHC were measured with a kit from Linco (St. Charles, MO). This assay has been validated for use in cats in our laboratory (17).

Insulin concentrations were measured as previously described (16). Glucose measurements were performed with a colorimetric glucose oxidase method (glucose trinder kit; Diagnostic Chemicals, Charlottetown, PE, Canada).

Serum concentrations of nonesterified fatty acids (NEFA) were measured with an enzymatic test kit (NEFA C; Wako Diagnostic, Richmond, PA). The percentage of suppression of NEFA concentrations was calculated as follows:

\[
\text{percentage of suppression} = 100 - \frac{\text{NEFA concentration (t)}}{\text{NEFA concentration (t$_0$)}} \times 100
\]

where $t_0$ represents the data collection point before the injection of insulin during the EHC.

**Indirect calorimetry.** All indirect calorimetry measurements were performed in the laboratory of Dr. William P. Flatt (Dept. of Food and Nutrition, University of Georgia, Athens, GA). The calorimetry system and calculations of respiratory exchange ratio (RER) and heat production were described previously (20).

All cats had been accustomed to the calorimetry chamber over a 5-day period. Baseline measurements were taken for 2 h. The cats were then fed, and measurements were continued for another 22 h. Lean cats ate their food within a 15-min period. Obese cats did not eat their food within a certain time period. The 24-h period consisted of a 12:12-h light-dark cycle. The data were extrapolated to 24 h to allow comparison between different time points, among groups, and with data from other studies (20).

**Magnetic resonance imaging.** MRI measurements were performed in all cats after injection of tiletamine HCl and zolazepam HCl (11 mg/kg im; Fort Dodge Animal Health, Fort Dodge, IA). All cats were investigated on a Varian/Inova 4.7T horizontal spectroscopy and imaging system as described previously (49) with a $16 \times 16$-cm field of view and a standard spin-echo sequence. The slice thickness was 3 mm with 256 $\times$ 256 points. We obtained 7 slices centered on the abdomen, with the number of averages equal to 4. Repetition time (Tr) was 0.4 s, and echo time (TE) was 20 ms. The data of the subcutaneous and abdominal fat mass were analyzed with the Image J program from the National Institutes of Health (Bethesda, MD).

**Statistical analysis.** Except for the modeling analysis, performed as described above, all other data were analyzed by use of computer software (Prism software, GraphPad Software Inc, San Diego, CA). The data are expressed as means $\pm$ SE unless otherwise stated. The significance of differences of means between groups was evaluated by ANOVA and within a group by paired $t$-test. Values of $P < 0.05$ were considered significant.

**RESULTS**

A significant difference in food intake and BW was found between lean and obese cats at T1, T2, and T4 (Table 2). There was no difference between the weight of lean cats at T1 and T2 or between the weight of cats at T4 and lean cats in T1 and T2. At T3, the weight of the cats on diets HP and HC was 4.6 $\pm$ 0.2 and 4.8 $\pm$ 0.4 kg, respectively.

**Glucose modeling.** The estimates of the parameter values of glucose effectiveness $S_G$ and insulin sensitivity $S_I$ (fixed effects) were significantly lower in obese compared with lean cats, i.e., $S_G$(lean) $= 0.0494(28\%)$ min$^{-1}$ [mean(CV%)] and $S_G$(obese) $= 0.0181(37\%)$ min$^{-1}$, or only 36.6% that of lean cats ($P = 0.03$). Similarly, $S_I$(lean) $= 10.71 \times 10^{-5}(23\%)$ min$^{-1}$pmol$^{-1}$·1 and $S_I$(obese) $= 3.73 \times 10^{-5}(25\%)$ min$^{-1}$pmol$^{-1}$·1, or 34.8% of that of lean cats ($P = 0.002$). There was no diet effect in lean or obese cats on either $S_G$ or $S_I$ (data not shown).

The estimated effect of BW on log $S_G$ and log $S_I$ is shown in Table 3. The intercept represents the reference value of log $S_G$ at BW = 0 kg. Since BW is a continuous variable, Table 3 predicts that log $S_G$ $= -7.8567 - 0.3694 BW$, i.e., that for each kilogram increase $S_G$ decreases to 69% ($= e^{-0.369458}$), or by 31%. Similarly, for each kilogram increase $S_I$ decreases to 70%.

**Indirect calorimetry.** The baseline RER was significantly higher in lean cats on diet HC than in obese cats on either diet before and after weight loss (Table 4). It trended to be higher than the baseline of lean cats on diet HP ($P = 0.075$). Baseline RER was lowest in cats that had lost weight regardless of diet; however, in those cats, the RER increased significantly after eating ($P < 0.001$). The 24-h RER was significantly higher in lean cats on diet HC (T2) than on diet HP, and lean (T4) and

| Table 3. Effects of body weight on individual estimates of glucose effectiveness and insulin sensitivity during euglycemic hyperinsulinemic clamp |
|---|---|---|
| **Log $S_G$ (intercept)** | $-1.7807$ | $0.4956$ |
| **$\Delta$ Log $S_G$ (BW)** | $-0.3528$ | $0.0990$ |
| **Log $S_I$ (intercept)** | $-7.8567$ | $0.4386$ |
| **$\Delta$ Log $S_I$ (BW)** | $-0.3694$ | $0.0916$ |

$n = 24$ cats. $S_G$, glucose effectiveness; $S_I$, insulin sensitivity.
obese (T2) cats on diet HC. There was no difference in 24-h RER values of obese cats. Dark cycle values were not different than 24-h values (not shown).

The baseline heat production per metabolic body size (MBS; kg$^{0.75}$) per 24 h was not significantly different among the groups and ranged from 49.1 ± 0.02 to 54.8 ± 0.02 in obese cats on diet HP at T2 to 50.2 ± 0.01 in lean cats on diet HC at T2. Heat production increased significantly during the first hour after food was offered but returned to baseline levels in lean cats fed diet HC, whereas it remained higher than baseline in lean cats fed diet HP at T2 (Fig. 1). The difference between diets HC and HP was significant during the 22-h evaluation period ($P < 0.002$).

Cats that had lost weight (T4) also increased heat production during food intake; however, this was not significant. Obese cats did not increase their heat production per MBS after food intake (49.1 ± 0.02 and 50.2 ± 0.01 during baseline and 50.3 ± 0.01 after food intake in HC- and HP-fed cats, respectively).

**Fat distribution.** There was no difference between abdominal subcutaneous and intra-abdominal fat depot size in either lean or obese cats. Figure 2 shows the fat distribution of an obese cat. There was also no effect of diet on the distribution of fat between those two sites. However, the fat mass was significantly larger in obese cats than in lean cats (T1 and T2). When obese cats lost weight (T4), cats on diet HP lost significantly more total fat than cats on diet HC, although the cumulative or total weight loss was the same (Table 5). Abdominal subcutaneous ($P < 0.04$), intra-abdominal ($P < 0.02$), and total ($P < 0.004$) fat correlated significantly with insulin sensitivity ($P < 0.04$, $P < 0.02$, and $P < 0.004$, respectively).

**Adipocytokines.** Diet had no effect on adiponectin concentrations (data not shown). Therefore, the data for both diets within the lean and obese cat groups were combined (Fig. 3). Adiponectin concentrations were significantly lower in obese cats than in lean cats (T1 and T2). The levels increased significantly with weight loss (T4) and were not different from the concentrations in lean cats (T1 and T2).

In contrast to adiponectin levels, leptin concentrations were significantly higher in obese cats than in lean cats (T1 and T2) and decreased with weight loss (T4) to the concentrations seen in lean cats (Fig. 4). There was no effect of diet (data not shown), and the diet groups were therefore combined.

**Nonesterified fatty acids.** The suppression of NEFA concentrations during the EHC is shown in Table 6. There was no diet effect in lean cats at T1 and T2. However, obese cats on diet HC showed a significantly greater suppression during the EHC than obese cats on diet HP. Weight loss of obese cats did not cause a change in the degree of suppression (comparison of T2 and T4).

**DISCUSSION**

Obesity, but not dietary protein or carbohydrate content, led to severe insulin resistance in cats and a marked decrease of
an indication that abdominal fat was the primary site of fat accumulation in cats because we had previously shown (18) that girth correlates well with dual-emission X-ray absorptiometry (DEXA) scans. However, DEXA scans are not sensitive enough to distinguish intra-abdominal fat from abdominal subcutaneous fat. With MRI, it was seen that the abdominal fat mass of cats was equally distributed between subcutaneous tissue and the intra-abdominal area, suggesting that both might be involved in determining insulin sensitivity. There were no sex differences in fat distribution, which is in contrast with observations in humans. Although male neutered male cats are at increased risk of developing diabetes, from our study one can conclude that fat distribution is not a contributing factor.

Fig. 4. Leptin concentrations (mean ± SE) in 12 lean and 16 obese cats before (T1, T2) and after (T4) weight loss. Values with the same superscript letter differ significantly (P < 0.001).

accumulation in cats because we had previously shown (18) that girth correlates well with dual-emission X-ray absorptiometry (DEXA) scans. However, DEXA scans are not sensitive enough to distinguish intra-abdominal fat from abdominal subcutaneous fat. With MRI, it was seen that the abdominal fat mass of cats was equally distributed between subcutaneous tissue and the intra-abdominal area, suggesting that both might be involved in determining insulin sensitivity. There were no sex differences in fat distribution, which is in contrast with observations in humans. Although male neutered male cats are at increased risk of developing diabetes, from our study one can conclude that fat distribution is not a contributing factor.

It was surprising that diet had no effect on insulin sensitivity or glucose effectiveness. Although there are no scientific publications that have examined these parameters in cats on a high-protein diet until now, there is large anecdotal evidence that it leads to an improvement in glucose homeostasis and lowering of insulin requirements, implying that it affects insulin sensitivity. A high-protein diet is now the preferred dietary regimen for diabetic cats. Our finding that a high-protein diet leads to significantly higher heat production in cats supports the notion that this diet is beneficial in the long term. One might argue that a beneficial effect of the high-protein diet in this study should have been seen as a decrease in food intake because these cats were strictly weight controlled. However, even the above-mentioned anecdotal improvements do not occur quickly, and it is likely that the study was not long enough to see a significant decrease in food intake as a compensation for the increased heat production. A high-protein diet has also been shown to increase heat production in both lean and obese humans (8, 30). It is unclear why obese cats showed a lower response than lean cats; however, this may have been due to differences in the diet or the cats' response to the diet.

Table 5. Magnetic resonance imaging of abdominal (subcutaneous and intra-abdominal) fat

<table>
<thead>
<tr>
<th>Cats</th>
<th>Total Fat, %</th>
<th>Abdominal Fat, %</th>
<th>Subcutaneous Fat, %</th>
<th>BW, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLT2</td>
<td>36.6±1.9*</td>
<td>13.8±2.1*</td>
<td>12.8±1.6*</td>
<td>3.3±0.2*</td>
</tr>
<tr>
<td>XOT2</td>
<td>58.7±2.3**</td>
<td>29.0±1.8*</td>
<td>29.6±2.0*</td>
<td>6.2±0.4**</td>
</tr>
<tr>
<td>ZLT2</td>
<td>31.3±3.2*</td>
<td>15.5±2.9*</td>
<td>15.8±1.1*</td>
<td>3.6±0.2*</td>
</tr>
<tr>
<td>ZOT2</td>
<td>57.3±1.8*</td>
<td>29.1±1.0*</td>
<td>28.2±1.1*</td>
<td>6.3±0.4*</td>
</tr>
<tr>
<td>XT3</td>
<td>51.7±2.7*</td>
<td>26.8±1.4*</td>
<td>24.9±1.7*</td>
<td>4.7±0.4*</td>
</tr>
<tr>
<td>ZT3</td>
<td>41.2±2.2*</td>
<td>21.3±1.7*</td>
<td>19.9±1.2*</td>
<td>4.5±0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE for abdominal (subcutaneous and intra-abdominal) fat in 12 lean cats at T2 and 16 obese cats before (T2) and after (T3) ~50% weight loss (see MATERIALS AND METHODS). The cats were fed a diet either high in carbohydrate (X) or high in protein (Z). *Comparison between lean and obese cats at T2 of total, abdominal, subcutaneous fat, and weight was P < 0.001 for all parameters; the comparison was made between cats fed the same diet. Other significant differences between obese cats on the same diet and between obese cats on different diets at T2 and T3 are indicated by the same superscript letters (aP < 0.023, bP < 0.034, cP < 0.05, dP = 0.07, eP < 0.034, fP < 0.004, gP < 0.002, hP < 0.011).

Table 6. Area under the curve of % NEFA suppression

<table>
<thead>
<tr>
<th>Cats</th>
<th>AUC % Suppression X</th>
<th>AUC % Suppression Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 lean</td>
<td>43.2±4.7*</td>
<td>48.5±5.1</td>
</tr>
<tr>
<td>T1 obese</td>
<td>30.2±3.3*</td>
<td>44.6±3.9*</td>
</tr>
<tr>
<td>T2 lean</td>
<td>46.3±8.9*</td>
<td>54.9±3.2</td>
</tr>
<tr>
<td>T2 obese</td>
<td>28.7±2.8*</td>
<td>40.3±4.8*</td>
</tr>
<tr>
<td>T4 lean</td>
<td>31.9±3.5*</td>
<td>44.0±2.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 12 lean and 16 obese neutered cats on a diet high in carbohydrates (X) or high in protein (Z). NEFA, nonesterified fatty acids; AUC, area under the curve. Values with the same superscript letter differ significantly (aP < 0.05, bP < 0.021, cP < 0.055, dP < 0.022, eP < 0.05).
have been caused by the much slower food intake of obese cats, with the majority of intake occurring at night. In contrast, the lean cats of this study completed their food intake within 15 min. Therefore, the thermic effect in obese animals would not be seen unless they were studied for a longer time period.

In the present study we report that cats can adapt to different macronutrients. Cats on a high-carbohydrate diet had a significantly higher RER after food intake than cats on a high-protein diet, indicating increased glucose oxidation. It has been stated previously that cats do not adapt to dietary alterations and gluconeogenesis is always high (37). This has been questioned, however, by other investigators who found that cats respond to dietary protein by increasing net protein oxidation, as is seen in other species (38, 41). The capability of cats to change metabolism in response to diet was also clearly shown by our previous finding with tracer glucose (19) or indirect calorimetry (20) in combination with the EHC. In the first study, we documented increased glycolysis in the basal state but increased glycogen deposition when insulin concentrations were high. The second study showed that lean cats increased glycolysis, glycogen production, and lipogenesis under clamp conditions (20). In addition, cats have been shown to respond appropriately to increases in dietary fat content (28). The baseline RER of the obese cats after weight loss was significantly lower at all time points compared with lean weight-maintained cats, indicating that they were metabolizing more protein or fat. This would be expected in animals in a catabolic state and was expected because the measurements were taken directly at the end of the weight loss period but not after a time of weight maintenance.

In view of our finding of an increase in heat production in cats fed the high-protein diet, it could be suggested that, over time, cats with the same caloric intake develop less obesity and show less insulin resistance when fed a high-protein diet compared with cats fed a high-carbohydrate diet. A longer-term study is needed to address this issue. Our results that a high-protein diet leads to more fat loss during weight loss than a high-carbohydrate diet supports the findings of some investigators who have documented that a high-protein diet promotes fat loss and reduces loss of lean body mass in dogs and cats during weight loss (5, 26, 43), whereas others have not seen any effect (31).

We showed previously (20) that NEFA concentrations decrease during the EHC. Contrary to findings in humans, the suppression was greater in obese cats than in lean cats, and greater in obese males than obese females, despite similar insulin concentrations. In the present study, greater suppression in response to insulin during the EHC was only seen in obese cats on the high-carbohydrate diet, whereas NEFA suppression was similar among lean and obese cats on the high-protein diet and lean cats on a high-carbohydrate diet. This suggests that the high-carbohydrate diet leads to greater fatty acid disappearance, either fat oxidation or fat deposition. One might assume that there was greater lipid deposition, likely into muscle tissue and liver, in cats on the high-carbohydrate diet, although this was not examined in this study. However, in an earlier study (49) we showed that obese cats have increased fat deposits in muscle tissue, and it is common knowledge that obese cats develop fatty livers. In addition, when the EHC was combined with indirect calorimetry, we were able to document that the greater NEFA disappearance was associated with increased lipogenesis (20). These data illustrate that the sensitivity to insulin in cats is substrate specific. In contrast to humans, in whom obesity diminishes the effect of insulin on both glucose and fatty acid clearance, in obese cats glucose metabolism is also affected negatively by obesity but fatty acid clearance is increased, indicating an enhanced effect of insulin. One can conclude from these studies that increased sensitivity of fatty acids to the effect of insulin increases lipid deposition in organs. A high-protein diet, therefore, is beneficial because it maintains NEFA suppression at the same level as is seen in lean cats. The higher insulin sensitivity of fatty acid clearance in obese cats on the high-carbohydrate diet, even after weight loss, also provides an explanation for the decreased loss of fat mass compared with cats fed a high-protein diet.

There was no dietary effect on leptin levels; however, leptin levels were increased severalfold in obese cats compared with lean cats and decreased to lean levels when the fat mass was reduced. This suggests that leptin, as in humans and rodents, is an indicator of fat mass. It has been suggested that leptin is primarily an indicator of subcutaneous abdominal fat tissue because it was shown that intra-abdominal fat correlated with insulin resistance, whereas subcutaneous abdominal fat correlated with leptin concentrations (7). In our study lean and obese subcutaneous abdominal fat was increased proportionately with intra-abdominal fat, and it remains unclear which of the two fat depots is responsible for the increase in leptin. Weight loss led to a decrease in leptin to levels seen in lean cats. This is consistent with findings seen in humans (4, 27). Leptin has also been found to be a sensitive marker of body mass and weight reduction in children (35).

Diet did not affect adiponectin. This hormone has beneficial effects on glucose and lipid metabolism (22, 40). It stimulates fatty acid oxidation, suppresses hepatic gluconeogenesis, and inhibits inflammatory responses. In humans and animals, adiponectin levels are positively correlated with insulin sensitivity and inversely related to the degree of adiposity as manifested by a negative correlation of adiponectin with glucose, insulin, triglyceride concentrations, and high body mass index and a positive correlation with glucose clearance and high-density lipoproteins. Weight loss in humans leads to an increase in adiponectin levels (27). Our results indicate that the regulation of adiponectin is similar in cats and humans. Obese insulin-resistant cats showed markedly lower adiponectin concentrations associated with improvement of insulin sensitivity, whereas weight loss led to increased concentrations that were not different from the levels seen in lean cats.

In summary, we have shown that cats that have been obese long term were severely insulin resistant, and, in contrast to obese humans, the cats had abdominal fat deposits that were equally distributed subcutaneously and intra-abdominally. They consumed significantly less food than lean cats to maintain their body weight and produced less heat after eating than lean cats. Similar to what has been shown in humans, adiponectin was positively and leptin negatively associated with fat mass. Weight loss markedly improved the severe insulin resistance in obese cats and normalized the levels of adiponectin and leptin. Diet had no effect on insulin sensitivity in a 4-mo feeding trial; however, a high-protein diet led to more fat loss during calorie restriction due to lower insulin sensitivity of fatty acid clearance compared with cats fed a high-carbohydrate diet. Lean cats, but not obese cats, adapted to the varying
carbohydrate/protein content of the diet with changes in substrate oxidation. Weight loss caused greater fat oxidation irrespective of diet composition. Feeding a low-carbohydrate/high-protein diet led to greater heat production in lean cats compared with a high-carbohydrate/low-protein diet but did not influence heat production in obese cats. One can conclude that, contrary to popular belief, lean cats are capable to adapt to varying macronutrients; however, a high-protein diet is preferred for lean cats because it increases heat production. It has additional beneficial effects: it maintains normal insulin sensitivity of fat metabolism, which aids in increasing fat loss during calorie restriction, and it preserves lean body mass. As is seen in many species, weight loss completely normalized insulin sensitivity.

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GRANTS

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