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

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Running title: Metabolic response in lean and obese cats

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Abstract

Obesity is a major health problem in cats and a risk factor for diabetes. It has been postulated 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 diet (HP) on glucose and fat metabolism during euglycemic hyperinsulinemic clamps, adipocytokines, and fat distribution in 12 lean cats (LEAN) and 16 obese cats (OBESE) before and after weight loss. Feeding HP led to greater heat production in LEAN but not in OBESE. Regardless of diet, OBESE had markedly decreased glucose effectiveness and insulin resistance, yet, greater suppression of non-esterified fatty acid during the euglycemic hyperinsulinemic clamp was seen in OBESE on HC compared to LEAN on either diet or OBESE on HP. In contrast to humans, OBESE had abdominal fat equally distributed subcutaneously and intra-abdominally. Weight loss normalized insulin sensitivity; however, increased non-esterified fatty acid suppression was maintained and fat loss was less in cats on HC. Adiponectin was negatively and leptin positively correlated with fat mass. LEAN, and cats during weight loss, but not OBESE, adapted to the varying dietary carbohydrate/protein content with changes in substrate oxidation. We conclude that HP is beneficial through maintenance of normal insulin sensitivity of fat metabolism in OBESE, facilitating the loss of fat during weight loss, and increasing heat production in LEAN. These data also show that insulin sensitivity of glucose and fat metabolism can be differentially regulated in cats.

Introduction

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. In order to be able to develop treatment regimens for obesity, it is important to understand the pathophysiological mechanisms that lead to its manifestation. Using the euglycemic hyperinsulinemic clamp, 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 have recently shown that obese cats preferentially partition fatty acids to muscle tissue resulting in increased muscular lipid content (49). 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 co-morbidities of obesity (29,34,38,42,46). In people, 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 gluteo-femoral fat accumulation pattern called 'gynoid' which is not associated with similar co-morbidities.

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). Contrary 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 if 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 efficaceous 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 if 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

12 lean (6 females and 6 males) and 16 obese (8 females and 8 males) adult neutered purpose-bred cats (Harlan Sprague Dawley, Madison, WI) were used. The age of the lean cats was 4.3 ± 0.4 years, and that of the obese cats was 5.1 ± 1.2 years. The obese cats had been obese for >1 year. 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, using 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 one of 2 diets (Table 1, diet HC and HP) 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 2 diets. Each diet was fed for a period of 4 months 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 months, the cats were fed the amount necessary to maintain their weight within a narrow range (Table 2). After a total of 8 months, the obese cats were maintained on the same diet that had been fed during the preceding 4 months, however food intake was decreased to obtain approximately a 1.5% decrease in body

weight per week. As is shown in Table 2, the food intake during that time was similar for both groups. Insulin sensitivity was tested with the euglycemic hyperinsulinemic clamp (EHC) after 4 months (T1), after 8 months (T2), and 6 months later when the obese cats returned to their original lean weight (T4). Magnetic resonance imaging of abdominal and subcutaneous fat was performed after 4 months (T1), after 8 months (T2), and after the obese cats had lost approximately 50% of their excess weight (T3).

Blood sampling

To allow blood sampling, catheters were placed in the jugular vein of cats 36-40 hours before each of the EHC and in the cephalic vein prior to the clamp. Catheter patency was maintained by injection of 0.5 ml of 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate, Sigma Co., MO) every 8 hours. 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 benzamidine (1M) and the samples were centrifuged immediately. Serum and plasma samples were stored at -20°C until assayed. 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 Corporation, 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 (19).

Testing of insulin sensitivity and modeling of data

Insulin sensitivity was examined with the EHC, using the minimal model of glucose disappearance (New Reference Bergman) modified to account for exogenous glucose administration, as described in detail (19). Tracer glucose was omitted. Briefly, saline was infused for 120 min. At time 120 min, the clamp was started and lasted for 120 minutes using 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 Lily, Indianapolis, IN) was administered IV and a solution of saline with added glucose (20% w/v) was infused for 120 min at a rate necessary to maintain the cats' fasting blood glucose concentrations.

The analysis was carried out using 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

$$dG(t)/dt = -(S_G + X(t))\, G(t) + S_G\, G_b + u_G(t)/V_G$$

$$dX(t)/dt = -P_2\,(X(t) - S_I\,(I(t) - I_b))$$

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 of from basal according to the rate constant P_2; G_b, and I_b are the basal concentrations of glucose and insulin, respectively; S_G and S_I are the main parameters called *glucose effectiveness* and *insulin sensitivity*, respectively, that 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 administered 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 to values estimated from a previous study with FSIGT (1); the basal concentrations G_b, and I_b, were assigned to the measurements taken at t=0.

Parameter estimates (logtransformed values) of each individual experiment (viewed as independent from the others) were obtained by fitting a non-linear mixed effects (NLME) population model simultaneously to all available data. The fixed effects (population averages) were found significantly dependent upon 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 (BLUP). The evaluation of possible dependencies of individual glucose kinetics parameters from the diet (intraindividual variability) was subsequently assessed by linear mixed effects (LME) modeling. Nonlinear and linear mixed effects analysis was 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 ± SD recovery of 77.4 ± 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 using a colorimetric glucose oxidase method (glucose trinder kit; Diagnostic Chemicals limited. Charlottetown, PE, Canada).

Serum concentrations of non-esterified fatty acids (NEFA) were measured by use of an enzymatic test kit (NEFA C; Wako Diagnostic, Richmond, PA). The percentage of the suppression of NEFA concentrations was calculated as follows:

Percentage suppression = 100-NEFA concentration (t)/NEFA concentration (t0) x 100 where t0 represents the data collection point prior to 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 have been described previously (20).

All cats had been accustomed to the calorimetry chamber over a 5 day period. Baseline measurements were taken for 2 hours. The cats were then fed and measurements were continued for another 22 hours. Lean cats ate their food within a 15 min period. Obese cats did not eat their food within a certain time period. The 24 hour period consisted of a 12 hour light and 12 hour dark cycle. The data were extrapolated to 24 hours to allow a comparison between different time points, among groups, and 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 intramuscularly; Fort Dodge Animal Health, Fort Dodge, IO). All cats were investigated on a Varian/Inova 4.7T horizontal spectroscopy and imaging system as described previously (49) with a 16 X16 cm field of view and a standard spin-echo sequence. The slice thickness was 3mm with 256x256 points. We obtained 7 slices centered on the abdomen with the number of averages equal to 4. Repetition time (Tr) was 0.4s and echo time (TE) was 20ms. 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 $\pm$ SEM 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 body weight was found between lean and obese cats at T1, T2, and T4 (Table 2). There was no difference between the weight of lean cats (T1 and T2) and the weight of the cats at T4 compared to lean cats in T1 and T2. At T3, the weight of the cats on diet HP and HC was 4.6 $\pm$ 0.2 kg 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 to lean cats, i.e. S_G(lean) = 0.0494(28%) min^{-1} (mean (CV%)), S_G(obese) = 0.0181 (37%)min^{-1} , i.e. only 36.6% that of lean ($p = 0.03$). Similarly, S_I(lean) = 10.71 10^{-5} (23%)min^{-1} $pmol^{-1}$ L and S_I(obese)=3.73 10^{-5} (25%)min^{-1} $pmol^{-1}$ L, i.e. 34.8% that of lean ($p = 0.002$).

There was no diet effect in lean or obese cats on either S_G or S_I (data not shown)**.**

The estimated effect of body weight (BW) on log S_I and log S_G is shown in table 3. The intercept represents the reference value of log S_I at BW=0 kg. Since BW is a continuous variable the above table predicts that log $S_I = -7.8567 - 0.3694$ BW, i.e. that for each kg increase S_I decreases to 69% (= $e^{-0.369458}$) or by 31%. Similarly, for each kg increase S_G 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 hour RER was significantly higher in lean cats on diet HC than HP, and lean and obese cats on diet HC. There was no difference in 24 hour RER values of obese cats. Dark cycle values were not different than 24 hour values (not shown).

The baseline heat production per MBS ($kg^{0.75}$) per 24 hours was not significantly different among the groups and ranged from 49.1 $\pm$ 1.7 in obese cats on HP at T2 to 54.8 $\pm$ 3.7 in lean cats on HC at T2. Heat production increased significantly during the first hour after food was offered but returned to baseline levels in lean cats fed HC diet, whereas it remained higher than baseline in lean cats fed diet HP at T2 (Fig. 1). The difference between diet HC and HP was significant during the 22 hour evaluation period ($p < 0.05$). Cats that had lost weight (T4) also increased heat production during food intake; this was, however, not significant. Obese cats did not increase their heat production per MBS after food intake (49.1 $\pm$ 1.7 and 50.2 + 1.2 during baseline and 50.9 $\pm$ 2.2 and 50.3 + 1.7 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 1 shows the fat distribution of an obese cat. There was also no effect of diet on the distribution of fat between those 2 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 fat (0.004) 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 (Figure 2). 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 than the concentrations of lean cats (T1 and T2).

Contrary 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.3). There was no effect of diet (data not shown) and the diet groups were therefore combined.

Non-esterified fatty acids

The suppression of non-esterified fatty acid (NEFA) concentrations during the EHC is shown in table 6. There was no diet effect in lean cats in 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 the dietary protein or carbohydrate content, led to severe insulin resistance in cats and a marked decrease of glucose effectiveness. The results obtained in this analysis confirm the findings of previous studies that in cats, body weight appears to be the major determinant of variations in insulin sensitivity and glucose effectiveness (19,20). Based on our calculations, each kg increase in weight led to approximately 30% loss in insulin sensitivity and glucose effectiveness. Insulin resistance is an important risk factor in the development of diabetes (36) and has been associated with the distribution of fat. In people, individuals with increased central adiposity develop more co-morbidities than individuals with a peripheral body fat distribution (24). There is some controversy if abdominal subcutaneous fat is more critical for changes in insulin sensitivity (1,14,25) or if the intra-abdominal fat is more important as has been recently suggested (7) based on the findings that intra-abdominal fat leads to increased flux of adipocytokines and hormones and has increased lipolytic activity which increases free fatty acids. Fatty acids not only impair first-passage absorption of insulin in the liver (48) but also alter insulin signaling (23). However, in a recent study in old rodents, intra-abdominal obesity could only account for a portion of insulin-resistance suggesting that other factors might play a role (6). This is the first study, to our knowledge, that investigated abdominal fat distribution in cats. We had an indication that abdominal fat was the primary site of fat accumulation in cats because we have previously shown that girth correlates well with dual emission x-ray absorptiometry (DEXA) scans (18). However, DEXA scans are not sensitive enough to distinguish intra-abdominal fat from abdominal subcutaneous fat. Using 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 gender 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. While 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 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, it may 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 are 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 previously stated 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 using 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 to 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 to 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 have previously shown that NEFA concentrations decrease during the EHC (20). Contrary to findings in people, 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 we showed that obese cats have increased fat deposits in muscle tissue (49), 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 people, where obesity diminishes the effect of insulin on both glucose and fatty acid clearance, in obese cats, glucose metabolism is also affected negatively by obesity, however, fatty acid clearance is increased indicating an enhanced effect of insulin. One can conclude form 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 to cats fed a high protein diet.

There was no dietary effect on leptin levels; however, leptin levels were several-fold increased in obese cats compared to lean cats and decreased to lean levels when the fat mass was reduced. This suggests that leptin, like in man 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 a high body mass index, and a positive correlation with glucose clearance and high-density lipoproteins. Weight loss in people leads to an increase in adiponectin levels (27). Our results indicate that the regulation of adiponectin is similar in cats and man. 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 than 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, cats had abdominal fat deposits which 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 people, 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 months 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 to 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 to a high carbohydrate/low protein diet but did not influence heat production in obese cats.

One can conclude that, contrary to popular believe, 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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Figure legends

Figure 1. Average heat production (Kcal/hour) in lean cats fed either HC (n=5; closed triangles) or HP (n=5; open circles). The value at time 0 is the average heat production during the 2 hour baseline period. The cats were fed at time 0 and monitored for an additional 22 hours.

Figure 2. Nuclear magnetic image of abdominal fat distribution in an obese cat. The image was taken caudal to the last rib.

Figure 3. Adiponectin concentrations (mean $\pm$ SEM) in 12 lean and 16 obese cats before (T1,T2) and after weight loss (T4) (Values with the same superscript letter differ significantly, ^{a} $p < 0.016$; ^{b} $p < 0.001$; ^{c} $p < 0.02$)

Figure 4. Leptin concentrations (mean $\pm$ SEM) in 12 lean and 16 obese cats before (T1,T2) and after weight loss (T4) (Values with the same superscript letter differ significantly, $^{a\text{-}c}$ $p < 0.001$)

Tables

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

Nutrient composition, g/100 g	Diet HC	Diet HP
Protein	27.5	45.2
Fat	15.7	15.8
Carbohydrate	38.1	24.7
Crude fiber	1.1	1.3
Ash	5.7	7.6
Metabolizable energy (Kcal/g)*	4.01	4.04

* Determined by bomb calorimetry

	Diet HC (%)	Diet HP (%)
Rice, brewers	33	16
Chicken, whole	20.5	26
Corn gluten meal	6	25
Poultry byproduct 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
Soduim chloride	0.25	0.25
Phosphoric acid	0.75	0.75
Mineral PMX	0.25	0.25
Choline Chloride	0.15	0.15
Vitamine E	0.2	0.2
Vitamin PMX	0.08	0.08

Table 2. Body weight (kg) and energy intake (Kcal/kg body weight) in 12 lean (L) and 16 obese (O) cats on 2 different diets (HC and HP) measured at T1, T2, and T4 (see Materials and Methods).

cats	Body Weight	Energy Intake
HCLT1	3.4 ± 0.3^{a}	51.6 ± 4.2^{g}
HCLT2	3.3 ± 0.2^{c}	50.6 ± 4.5^{i}
HPLT1	3.3 ± 0.2^{b}	55.4 ± 6.0^{h}
HPLT2	3.6 ± 0.2^{d}	49.3 ± 3.8^{j}
HCOT1	6.6 ± 0.3^{a}	42.1 ± 1.4^{g}
HCOT2	$6.2 \pm 0.4^{c, e}$	$35.8 \pm 2.0^{l,k}$
HPOT1	6.3 ± 0.4^{b}	41.7 ± 1.6^{h}
HPOT2	$6.3 \pm 0.4^{d,f}$	$38.8 \pm 2.1^{j,l}$
HCT4	3.7 ± 0.3^{e}	24.3 ± 1.5^{k}
HPT4	3.5 ± 0.1^{f}	23.4 ± 0.7^{l}

Values with the same superscript letter differ significantly ($^{a-f,l}$ $p < 0.001$; ^{g,h} $p < 0.04$; ^{i} $p < 0.009$; ^{j} $p < 0.03$; ^{k} $p < 0.002$ ^{j} $p < 0.03$)

Table 3. Effects of body weight of 24 cats on individual estimates of log S_G (glucose effectiveness) and log S_I (insulin sensitivity) during the euglycemic hyperinsulinemic clamp

	value	Std.Error	p-value
log S_G (Intercept)	-1.7807	0.4956	9.00e-04
Δ log S_G (BW)	-0.3528	0.0990	1.00e-03
log S_I (Intercept)	-7.8567	0.4586	1.00e-04
Δ log S_I (BW)	-0.3694	0.0916	3.00e-04

Table 4: Evaluation of respiratory exchange ratio using indirect calorimetry in 12 lean cats at T2 and 16 obese cats at T2, and T4 (see Matarials and Methods) fed either diet X or diet Z (mean $\pm$ SEM).

Time	Lean X T2	Lean Z T2	Obese X T2	Obese Z T2	X T4	Z T4
baseline	$0.88 \pm 0.02^{a,b}$	0.83 ± 0.02^{d}	$0.81 \pm 0.01^{a,c}$	0.82 ± 0.02^{e}	$0.76 \pm 0.01^{b,c}$	$0.75 \pm 0.01^{d,e,j}$
24 hours	$0.89 \pm 0.02^{f,g,h}$	$0.84 \pm 0.01^{f,i}$	0.83 ± 0.01^{g}	0.82 ± 0.02	$0.81 \pm 0.01^{n,h}$	$0.79 \pm 0.01^{i,j}$

Values with the same superscript letter differ significantly (^{a,} $p < 0.012$; $^{b-d}$ $p < 0.029$; ^{e} $p < 0.004$; $^{f}p < 0.033$; $^{g}p < 0.03$; $^{h}p < 0.002$; $^{i,j}p < 0.001$)

Table 5: Magnetic resonance imaging of abdominal (subcutaneous and intra-abdominal) fat (%; mean ± SEM) in 12 lean (L) cats at T2, and 16 obese (O) cats before (T2) and after approximately 50% weight loss (T3; see Materials and Methods; mean ± SEM). The cats were either fed a diet high in carbohydrate (X) or high in protein (Z).

cats	Total fat	Abdominal fat	Subcutaneous fat	Body Weight, kg
XLT2	26.6 ± 1.9*	13.8 ± 2.1*	12.8 ± 1.6*	3.3 ± 0.2*
XOT2	58.7 ± 2.3 *,[d]	29.0 ± 1.8*	29.6 ± 2.0*	6.2 ± 0.4 *,[e]
ZLT2	31.3 ± 3.2*	15.5 ± 2.9*	15.8 ± 1.1*	3.6 ± 0.2*
ZOT2	57.3 ± 1.8 *,[f]	29.1 ± 1.0 *,[g]	28.2 ± 1.1 *,[h]	6.3 ± 0.4*
XT3	51.7 ± 2.7[a,d]	26.8 ± 1.4 [b,]	24.9 ± 1.7 [c,]	4.7 ± 0.4 [e]
ZT3	41.2 ± 2.2 [a,f]	21.3 ± 1.7 [b,g]	19.9 ± 1.2 [c,h]	4.5 ± 0.2

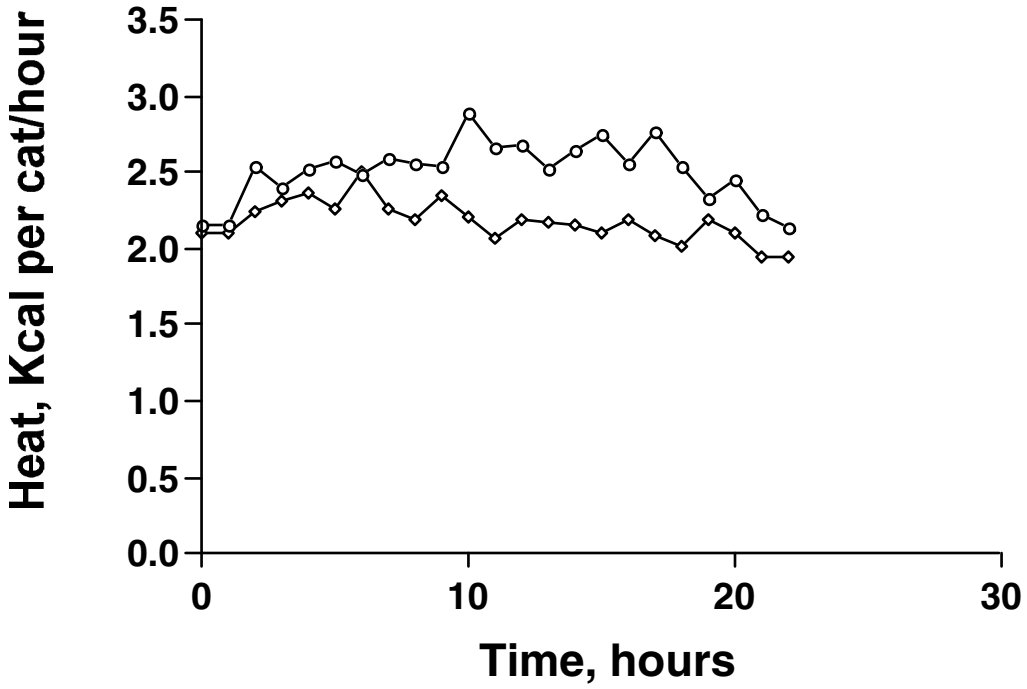
* Comparison between lean and obese cats at T2 of total, abdominal, subcutaneous fat, and weight was $p < 0.001$ for all parameters. The comparison was made between cats fed the same diet.

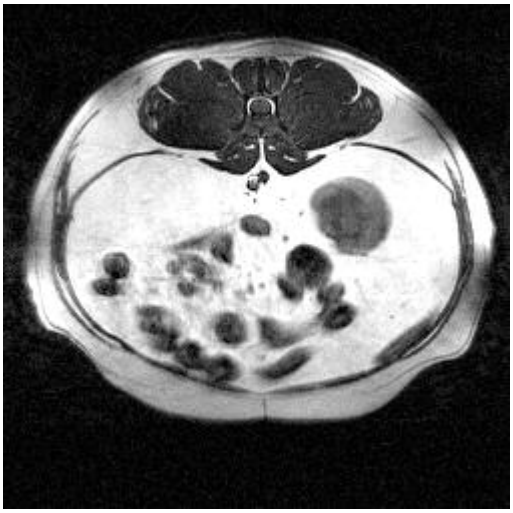
Other significant differences between obese cats on the same diet and between obese cats on different diets at T2 and T3 are indicated by the same superscript letters ([a] $p < 0.023$; [b] $p < 0.034$; [c] $p < 0.05$; [d] $p = 0.07$; [e] $p < 0.034$; [f] $p < 0.004$; [g] $p < 0.002$; [h] $p < 0.011$)

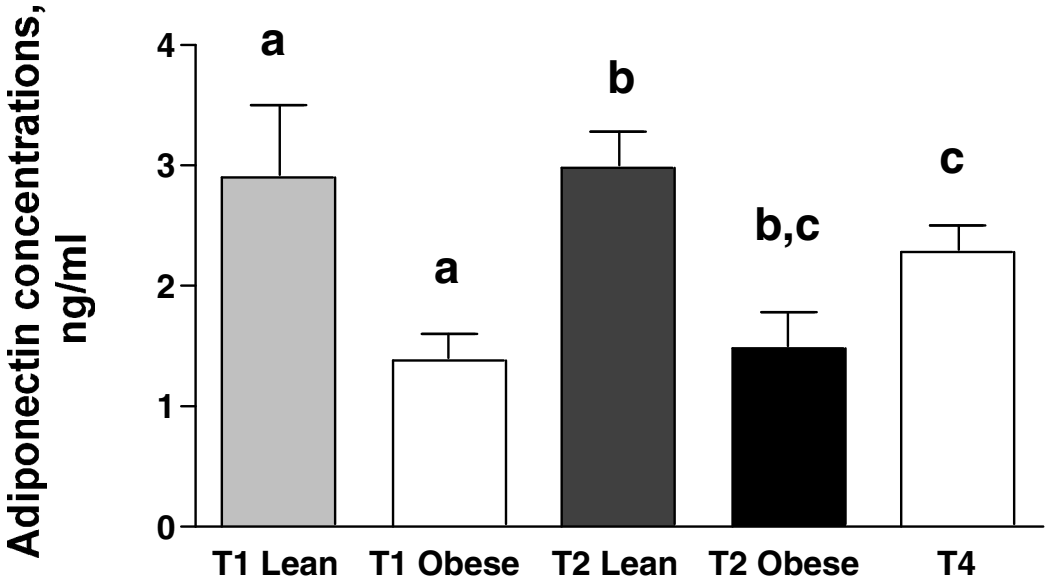
Table 6. Area under the curve of %NEFA suppression (mean ± SEM) in 12 lean and 16 obese neutered cats on a diet high in carbohydrates (X) and high in protein (Z).

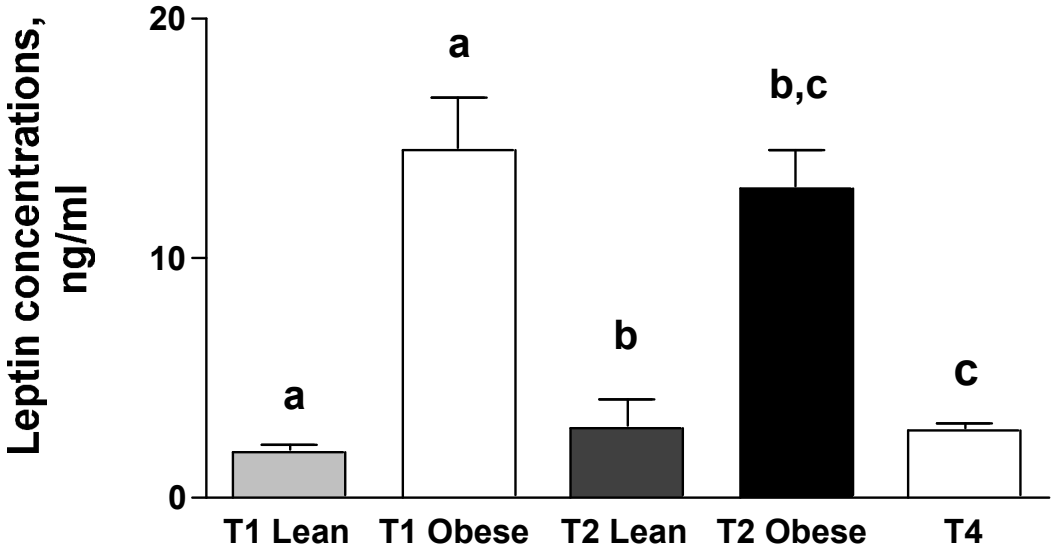
cats	AUC % suppression X	AUC % suppression Z
T1 Lean	43.23 ± 4.7 [a]	48.5 ± 5.1
T1 Obese	30.25 ± 3.3 [a,b]	44.6 ± 3.9[b]
T2 Lean	46.3 ± 8.9[e]	54.9 ± 3.2
T2 Obese	28.7 ± 2.8 [c,e]	40.3 ± 4.8 [c]
T4 Lean	31.9 ± 3.5 [d]	44.0 ± 2.2 [d]

Values with the same superscript letter differ significantly ([a] $p < 0.05$; [b] $p < 0.021$; [c] $p < 0.055$; [d] $p < 0.022$; [e] $p < 0.05$)


Heat, Kcal per cat/hour
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
Time, hours




Adiponectin concentrations, ng/ml
4
3
2
1
0
a
a
b
b,c
c
T1 Lean
T1 Obese
T2 Lean
T2 Obese
T4


Leptin concentrations, ng/ml
20
10
0
a
a
b
b,c
c
T1 Lean
T1 Obese
T2 Lean
T2 Obese
T4