Effect of macronutrients, age, and obesity on 6- and 24-h postprandial glucose metabolism in cats

Margarethe Hoenig,1,2 Erin T. Jordan,1 John Glushka,3 Saskia Kley,1 Avinash Patil,4 Mark Waldron,4,5 James H. Prestegard,3 Duncan C. Ferguson,6 Shaoxiong Wu,7 and Darin E. Olson8

1Department of Physiology and Pharmacology, University of Georgia College of Veterinary Medicine, Athens, Georgia; 2Department of Veterinary Clinical Medicine, University of Illinois College of Veterinary Medicine, Urbana-Champaign, Illinois; 3Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia; 4Nestlé Purina Petcare, St. Louis, Missouri; 5Nestlé Research Center, Lausanne, Switzerland; 6Department of Veterinary Biosciences, University of Illinois College of Veterinary Medicine, Urbana-Champaign, Illinois; 7Emory NMR Research Center, Emory University, Atlanta, Georgia; 8Division of Endocrinology, Metabolism, and Lipids, Department of Medicine, Emory University School of Medicine and the Atlanta Veterans Affairs Medical Center, Atlanta, Georgia

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Hoenig M, Jordan ET, Glushka J, Kley S, Patil A, Waldron M, Prestegard JH, Ferguson DC, Wu S, Olson DE. Effect of macronutrients, age, and obesity on 6- and 24-h postprandial glucose metabolism in cats. Am J Physiol Regul Integr Comp Physiol 301: R1798–R1807, 2011. First published September 21, 2011; doi:10.1152/ajpregu.00342.2011.—Obesity and age are risk factors for feline diabetes. This study aimed to test the hypothesis that age, long-term obesity, and dietary composition would lead to peripheral and hepatic insulin resistance, indicated by higher endogenous glucose production (EGP) in the fasted and postprandial state, higher blood glucose and insulin, and higher leptin, free thyroxine, and lower adiponectin concentrations. Using triple tracer—2H2O, [U-13C3]-propionate, and [3,4-13C2]-glucose infusion, and indirect calorimetry—we investigated carbohydrate and fat metabolic pathways in overnight-fasted neutered cats (13 young lean, 12 old lean, and 12 old obese), each fed three different diets (high protein with and without polyunsaturated fatty acids, and high carbohydrate) in a crossover design. EGP was lowest in fasted and postprandial obese cats despite peripheral insulin resistance, indicated by hyperinsulinemia. Gluconeogenesis was the most important pathway for EGP in all groups, but glycogen contributed significantly. Insulin and leptin concentrations were higher in old than in young lean cats; adiponectin was lowest in obese cats but surprisingly highest in lean old cats. Diet had little effect on metabolic parameters. We conclude that hepatoportal insulin resistance does not develop in the fasted or postprandial state, even in long-term obese cats, allowing the maintenance of euglycemia through lowering EGP. Glycogen plays a major role in EGP, especially in lean fasted cats, and in the postprandial state. Aging may predispose to insulin resistance, which is a risk factor for diabetes in cats. Mechanisms underlying the high adiponectin of healthy old lean cats need to be further explored.

nuclear magnetic resonance spectroscopy; adipocytokines; indirect calorimetry; glycogenolysis; insulin resistance; endogenous glucose production

In man and in cats, obesity and age are risk factors in the development of diabetes (8, 69). As in people, the incidence of feline obesity and diabetes has increased dramatically in the past 3 decades (2, 8). Obesity is now the most common nutritional disorder in cats, and diabetes is one of the most common endocrinopathies in aging cats. Little is known about the metabolic changes in obese cats and the influence of age and food intake on metabolic parameters that contribute to increasing obesity and diabetes.

Cats are obligatory carnivores that naturally subsist on a high-protein and high-fat diet. It has been stated anecdotally that glycogen contributes less to glucose production in cats than other species. Rogers et al. (61) reported that cats cannot readily adapt their sources of glucose production, a notion that has been refuted by others (25, 26, 65, 66). We have previously shown that, in fasted cats, fractional gluconeogenesis (GNG) and glycogenolysis is similar to humans (41). However, specific feline carbohydrate metabolism responses to a meal or to different diets has, to date, been unexplored.

Using nuclear magnetic resonance technology and an infusion method of three stable isotopes, we investigated key steps in glucose metabolism with a single blood sample. [3,4-13C2]-glucose was used to measure glucose turnover by conventional indicator dilution; 2H2O was used to measure the fractional contribution of glycogen, glycerol, and the TCA cycle to endogenous glucose production (EGP), and [U-13C3]-propionate was used as a anaplerotic tracer to measure fluxes through pathways associated with the TCA cycle.

We tested the hypothesis that age, long-term obesity, and dietary composition would lead to peripheral and hepatic insulin resistance, indicated by higher EGP in the fasted and postprandial state, higher glucose and insulin, leptin, and free thyroxine, and lower adiponectin concentrations.

MATERIALS AND METHODS

Animals and Diets

In these studies, we included 25 lean (L; 12 female, 13 male) and 12 obese (obese; 6 female, 6 male) neutered adult cats. Thirteen of the lean cats were younger at ∼1 yr old (YL; 7 male and 6 female). Twelve of the lean cats were middle-aged to old (OL; equal gender distribution). The age for the OL females was 9.5 ± 0.2 yr, the age for the OL males was 8.3 ± 0.4 yr. All obese cats were middle-aged to old. The age for the obese females was 9.4 ± 0.2 yr, and for the obese males, the age was 8.2 ± 0.6 yr. The obese animals had been obese for over 5 yr prior to the beginning of the study. Obesity was originally induced by allowing ad libitum food intake, whereas lean animals were fed only the amount needed to maintain their body weight. The weight of the obese cats was ∼100% higher than their original lean adult weight. Cats were maintained at the University of Georgia, College of Veterinary Medicine Animal Care Facility under...
approved colony conditions. They were housed individually and were given free access to water. The University of Georgia Animal Care and Use Committee approved all animal studies and they were 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. We determined that animals were healthy on the basis of results of a physical examination and clinical laboratory data. All cats were socialized with daily interactions. All cats ate the same diet before the beginning of the study (Nestlé Purina ProPlan, St. Louis, MO). They were then randomly allocated to start on one of three diets. Each cat received each of the three diets in a serial rotation for 5 mo at a time in a crossover design. The three diets included a high-protein regimen (HP), a high-carbohydrate regimen (HC), and a HP regimen with added polyunsaturated fatty acids (HPPUFA). Only two of the investigators (M. Waldron and A. Patil) knew the diet composition (Table 1), and they were not involved in the execution of the experiments and data analyses. Food intake was recorded at each feeding. Caloric content of the ingested food was calculated using standard Atwater factors applied to the diet contents from Table 1 (49). We monitored the weight of the cats weekly and adjusted food intake to maintain body weight within a narrow range (<5%).

We measured girth circumference (cm) and body mass index (BMI; expressed in kg/m²), as previously described (37) before each infusion experiment by the same person to minimize variability (E. Jordan). Dual emission X-ray absorptiometry was performed as previously described (75) once before the beginning of the infusion experiments during the second diet rotation.

Stable Isotope Method

Twice during each dietary rotation, the cats received infusions of stable nonradioactive isotopes, which were applied to study carbohydrate metabolism. After 4 mo on each of the three diets, we performed infusion experiments 5 h after feeding (postprandial groups) and, after an additional month, repeated the infusion procedures after fasting the animals for 24 h (fasting groups) using the methodology described in detail by Kley et al. (41) with minor modifications. We collected blood samples from the jugular vein, via a catheter, which was placed 48 h earlier, and maintained catheter patency by injection of 0.5 ml of 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate; Sigma, St. Louis, MO) every 8–12 h. For the postprandial experiments, cats were initially fasted for 24 h, then fed their daily ration and mildly sedated [0.3–0.5 mg/kg body wt tiletamine/zolazepam (Telazol; Fort Dodge Animal Health, Fort Dodge, IA) intravenously]. We have previously shown that Telazol has no effect on glucose tolerance and insulin sensitivity (25). We collected a baseline blood sample (2.0 ml) and administered 6.0 ml/kg of a deuterium oxide ([2H₂O, 99.9%]/ sodium [U-13C₃] propionate, 99%; Cambridge Isotopes, Andover, MA) solution (200:1) via gastric tube. The cats then received a bolus intravenous infusion of [3,4-13C₂] glucose (3.69 mg/kg as a 2 mg/ml in normal saline, 99%; Omicron Biochemicals, South Bend, IN) immediately followed by a continuous infusion (0.083 mg/kg·min⁻¹·10⁻³) via the jugular vein catheter for 60 min. We collected another blood sample (12.0 ml) 60 min after the infusion to prepare for spectroscopy studies. For the experiments in the fasted state, the infusion of [3,4-13C₂] glucose and oral administration of deuterium oxide and sodium propionate was performed after a 24-h fast.

Sample Processing

For the collection of plasma, we placed whole blood into chilled tubes containing EDTA and centrifuged the samples immediately at 4°C, and 890 g. We stored plasma samples at −80°C until further processing. For analysis by NMR, we isolated plasma glucose from the 60-min postinfusion blood sample and converted it to monooctone glucose (MAG) as described in detail (41).

NMR Spectroscopy

We collected all NMR spectra with a Varian INOVA 14.1 T spectrometer (Varian Instruments, Palo Alto, CA) equipped with a 3-mm broadband probe (3 mm PFG Dual Broadband Probe, Varian, Palo Alto, CA), as previously described (32, 41). Spectral analysis was processed using the curve-fitting program MestRe-C (Mestrelab Research SL, Santiago de Compostela, Spain) a PC-based NMR spectral analysis program.

Metabolic Analysis

We estimated glucose turnover from the dilution of infused [3,4-13C₂] glucose using 13C NMR of the MAG samples, as previously described (30). The fluxes from glycogen, glyceral, and phosphoenol pyruvate (PEP) into plasma glucose were estimated from the deuterium enrichment at position 2, 5, and 6 s (H₂, H₅, and H₆, respectively) based on the 2H NMR spectra (36, 46). 13C NMR analysis of the multiplets of carbon 2 of MAG yields the relative fluxes in the TCA cycle, as described previously (32, 35).

Other Assays

Insulin and glucose measurements were performed in samples taken before [3,4-13C₂] glucose infusion in fasted and postprandial cats. Glucose was also measured on samples from the end of the 1-h infusion experiment. We measured glucose using a colorimetric glucose oxidase method (Genzyme Diagnostics, Cambridge, MA, USA), and insulin by radioimmunoassay, as described previously (23). We have previously shown that [3,4-13C₂] glucose does not change plasma glucose concentrations (41). We measured adiponectin, leptin, and free thyroxine by equilibrium dialysis (FT4) concentrations in fasted cats with assays previously validated in our laboratory for use in cats (Refs. 17, 22, and 26, respectively). Plasma nonesterified fatty acids (NEFAs) were measured in fasted cats by use of an enzymatic test kit (NEFA-C; Wako Chemicals, Richmond, VA).

<table>
<thead>
<tr>
<th>Table 1. Diet composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet composition HP HPPUFA HC</td>
</tr>
<tr>
<td>Protein, % 48.10 46.60 32.60</td>
</tr>
<tr>
<td>Fat, % 11.40 12.90 10.50</td>
</tr>
<tr>
<td>CHO (by subtraction), % 24.16 23.37 37.74</td>
</tr>
<tr>
<td>Fiber (crude), % 0.94 1.19 1.32</td>
</tr>
<tr>
<td>Ash, % 6.68 6.25 6.94</td>
</tr>
<tr>
<td>Moisture, % 7.82 8.99 10.30</td>
</tr>
<tr>
<td>ME, kcal/g (by calculation) 3.57 3.61 3.41</td>
</tr>
<tr>
<td>Fatty acid analysis, % of fat</td>
</tr>
<tr>
<td>14:0 1.32 2.86 2.02</td>
</tr>
<tr>
<td>14:1 0.20 0.14 0.29</td>
</tr>
<tr>
<td>16:0 22.20 19.60 21.30</td>
</tr>
<tr>
<td>16:1n-7 3.15 4.40 2.47</td>
</tr>
<tr>
<td>18:0 9.61 7.88 13.20</td>
</tr>
<tr>
<td>18:1n-9 1.90 1.29 3.32</td>
</tr>
<tr>
<td>18:2n-6 22.70 17.90 18.80</td>
</tr>
<tr>
<td>18:3n-3 1.32 1.50 1.04</td>
</tr>
<tr>
<td>20:4n-6 0.10 0.19 0.23</td>
</tr>
<tr>
<td>20:5n-3 &lt;0.10 0.17 0.10</td>
</tr>
<tr>
<td>20:6n-3 0.45 0.67 0.28</td>
</tr>
<tr>
<td>Total n-6 23.36 19.09 19.08</td>
</tr>
<tr>
<td>Total n-3 1.95 9.05 1.64</td>
</tr>
</tbody>
</table>

HP, high-protein diet; HPPUFA, high-protein enriched in n-3 polyunsaturated fatty acids diet; HC, high-carbohydrate diet; CHO, carbohydrates; ME, malic enzyme.
Old lean 3.6 different diets after fasting for 24 h

The different diets. In YL, the weight was 3.2

eters (Table 2). There was no difference in cat weight among significantly larger than age-matched OL controls in all four param-

eries were carried out using Systat 13 (Systat, Chicago, IL).

6. h postfeeding for calculation of postprandial heat production.

Statistical Analysis

Preliminary analyses, including summary statistics, tests of nor-
mality (Anderson-Darling and Shapiro-Wilks tests), multinormality (Mardia’s skewness and kurtosis coefficients, and the Henze-Zirkler test statistic), probability and quartile plots, and evaluation of transformations were carried out using Systat 13 (Systat, Chicago, IL).

Analysis of repeated measures was carried out using PROC GLIM-

MIX (SAS 9.1.3; SAS Institute, Cary, NC). If sex, age, diet, or weight

formations were carried out using Systat 13 (Systat, Chicago, IL).

0.1 during each

Indirect Calorimetry

We performed indirect calorimetry in each cat 1 wk before the postprandial infusion procedures (in the laboratory of Dr. William P.

Flatt, Department 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 (25). The ambient temperature was 23°C. Cats were

acclimated to the calorimetry chamber, baseline measurements were taken for 2 h after a 24-h fast, then the cats were fed their daily ration, and measurements continued for another 6 h. We used the measurements during the 6th h postfeeding for calculation of postprandial heat production.

Glucose and NEFA Concentrations

There was no significant difference in baseline glucose concentrations between any of the experimental groups, nor was there any difference due to diet or between fasting and postprandial experiments (data not shown). There was also no change in glucose concentrations after the 1-h infusion (data not shown). Fasted NEFA concentrations were highest in obese cats (Table 4).

Hormonal Concentrations

Insulin and leptin concentrations were higher, and adiponectin concentrations were lower in obese cats compared with OL, as expected (Table 4). Insulin and leptin were also higher in OL cats compared with YL controls, but the adiponectin levels were also significantly increased in OL. The postprandial insulin levels were not significantly higher compared with fasting in all groups. Free thyroxine concentrations were highest in obese; however, this difference did not achieve significance (Table 4; P = 0.068).

Indirect Calorimetry

RER was not different among diets (data not shown). RER was not different between lean cats (0.77 ± 0.01 for both) but

body weight, girth, % fat, and body mass index in 13 young lean, 12 old lean, and 12 old obese cats fed three different diets after fasting for 24 h

Table 2.

<table>
<thead>
<tr>
<th>Body Weight, kg</th>
<th>Girth, cm</th>
<th>% Fat</th>
<th>Body Mass Index, kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young lean</td>
<td>3.2 ± 0.1*</td>
<td>30.8 ± 0.4</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Old lean</td>
<td>3.6 ± 0.1*#</td>
<td>31.6 ± 0.5#</td>
<td>20 ± 2#</td>
</tr>
<tr>
<td>Obese</td>
<td>7.0 ± 0.3#</td>
<td>51.5 ± 1.2#</td>
<td>61 ± 1#</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. The results were combined because there was no difference among the diets. The % fat was measured in all cats only once at the end of the second diet rotation. * and #Significant differences between lean young and old cats and lean old and obese cats, respectively (P < 0.01).

RESULTS

Body Weight, BMI, Girth, % Fat, and Food Intake

Weight and BMI were higher in OL than YL groups, but % fat mass and girth were not different. Obese cats were significantly larger than age-matched OL controls in all four parameters (Table 2). There was no difference in cat weight among the different diets. In YL, the weight was 3.2 ± 0.1 during each of the three diet periods; in OL, it was 3.6 ± 0.1 kg when feeding HP and HC, and 3.5 ± 0.1 when feeding HPPUFA. In obese, it was 7.2 ± 0.3 in HPPUFA and 7.0 ± 0.3 in HP and HC. Total food consumed was greater in the obese cats compared with both YL and OL for all of the diets, and there was no significant difference between any of the food intake parameters for each diet preceding either postprandial or fasting experiments (data not shown), so the results presented combine both postprandial and fasting measurements. However, the food intake normalized to total body weight was consistently less in the obese cats. The dietary composition had only a modest effect on caloric intake. There was slightly greater intake of the HC diet (significant only for OL and obese) and slightly lower intake of the HPPUFA diet (significant only for OL) (Table 3).

Table 3. Caloric intake in 13 young lean, 12 old lean, and 12 old obese cats fed three different diets

<table>
<thead>
<tr>
<th>HP, kcal/day</th>
<th>HPPUFA, kcal/day</th>
<th>HC, kcal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young lean</td>
<td>159 ± 7</td>
<td>163 ± 10</td>
</tr>
<tr>
<td>Old lean</td>
<td>167 ± 7#abc</td>
<td>162 ± 7#abc</td>
</tr>
<tr>
<td>Obese</td>
<td>237 ± 8#</td>
<td>223 ± 9#δ</td>
</tr>
<tr>
<td>Young lean</td>
<td>50 ± 2</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Old lean</td>
<td>47 ± 1#abcδ</td>
<td>45 ± 1#abcδ</td>
</tr>
<tr>
<td>Obese</td>
<td>35 ± 1#</td>
<td>33 ± 1#abc</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. #Significant differences between lean old and obese cats. *Significant differences between young and old cats

was significantly lower in obese cats (0.74 ± 0.01). There was an expected significant increase in RER measured 6 h postfeeding (P < 0.01) compared with baseline in all groups with a shift toward carbohydrate metabolism in the fed state. The total heat production per hour is shown in Fig. 1. The heat production per metabolic body weight [kcal/hr/body wt]0.75 in the fasted state was not different between YL and OL (2.5 ± 0.1 in YL, 2.5 ± 0.1 in OL) but was lower in obese cats (2.3 ± 0.1; P < 0.01). Postprandially, there was a significant increase in YL (2.9 ± 0.1) and OL (2.8 ± 0.1; P < 0.01 for both). In obese cats, postprandial heat production was not different compared with fasting; however, it was lower (2.2 ± 0.1) compared with OL (P < 0.01).

**Glucose Turnover and Metabolic Fluxes**

Postprandial EGP was significantly higher than fasting EGP in all cat groups. Comparing obese with OL, it was found that total EGP (mg/min) during fasting and postprandial experiments was higher, but EGP was significantly lower in obese cats, when it was expressed on a body weight basis (milligrams per kilograms per minute) (Fig. 2).

**Origin of blood glucose by 2H NMR analysis.** Glucose produced by GNG was the major contributor to EGP in all groups, but there was also a considerable contribution from glycogenolysis in all groups (Fig. 3). There were no diet effects in any of the fluxes that were measured, and the data were combined.

In fasting, the percentage of GNG and the % flux from PEP were significantly higher in obese cats compared with OL, whereas the percent glycogenolysis contributing to EGP was lower in obese cats than OL (Fig. 3). There was no difference in the % flux between OL and YL, and there was no difference in the % flux from glycerol to glucose among the three groups. In contrast to fasting, postprandial values for the percentage of GNG, glycogenolysis, and % flux from glycerol and PEP to glucose were not different among any of the groups. This was due to the fact that GNG was lower in obese cats than it had been in fasting, and % glycogenolysis was higher.

Absolute fluxes were obtained by combining the relative fluxes with the measured EGP (36). In fasting, the absolute flux from glycogen was significantly lower in obese than lean but increased postprandially and was similar to the postprandial values seen in the lean cats. The fasting fluxes from glycerol or from PEP to glucose were not different among the groups (Table 5). Postprandially, the absolute flux from glycerol to glucose increased in lean cats, but this was not the case in obese cats. The postprandial absolute flux from PEP to glucose was higher in all cats compared with fasting. (Table 5).

**TCA cycle and related fluxes measured by 13C NMR analysis.** The fluxes through the TCA cycle and related fluxes measured by 13C NMR are shown in Table 5. Analyzing the C2 multiplets of the 13C NMR spectrum of MAG provides an estimate of fluxes through pyruvate carboxylase/phosphoenolpyruvate carboxykinase (PC/PEPCK) and pyruvate kinase/aldolase enzyme (PK/ME)-mediated pyruvate exchange with TCA cycle intermediates (pyruvate cycling), and GNG from the TCA cycle, all relative to citrate synthase flux. There was no effect of diet on these anaplerotic/cataplerotic fluxes through PC/PEPCK, pyruvate cycling, or GNG from the TCA cycle relative to the flux through citrate synthase, so the data were combined. Obese cats had higher fluxes through PEPCK/CS in the postprandial state compared with fasting, whereas there was no difference in the lean cats. There were no differences among fasting or postprandial groups in the flux through pyruvate kinase/CS (pyruvate cycling). GNG from the TCA cycle was higher in the postprandial state in all cats, but this was only significant for YL and obese cats.

Combining the results of fractional sources of glucose production by 2H NMR, TCA cycle fluxes obtained by 13C NMR and of the measured EGP yields the absolute fluxes through multiple pathways of the TCA cycle (Table 5). The results of the absolute fluxes were similar among all three groups in fasting, and there were no diet differences within any of the groups. Postprandial flux through PEPCK was higher in OL and obese cats, and pyruvate cycling tended to be higher in obese cats (P = 0.0650). There was a strong relationship between the absolute flux through PEPCK and the TCA cycle flux (citrate synthase) in fasting (r^2 = 0.4303; P < 0.0001) and in the postprandial state (r^2 = 0.3150; P < 0.0001).
DISCUSSION

This is the first report to our knowledge comparing the metabolic status of cats of different ages and body condition, and the metabolic consequences of food intake. We found that obese cats maintained euglycemia in the fasted and postprandial condition by adjusting EGP despite peripheral insulin resistance. While we did not measure insulin resistance directly in this study with the gold standard method—the euglycemic hyperinsulinemic clamp—we have previously shown that each increase in body weight by 1 kg leads to a decrease in insulin sensitivity by $\sim 30\%$ (26). We also documented in the current study that glycogen plays a major role in glucose metabolism in cats in the fasted and postprandial state.

The long-term obese cats in the current study demonstrate insulin resistance characterized by fasting hyperinsulinemia, but maintain normal fasting and postprandial glucose concentrations, perhaps because they were able to lower EGP in response to the elevated insulin levels. We previously examined cats in the fasted state that had been obese for 1 yr, and also demonstrated lower fasting EGP in those obese cats compared with age-matched lean controls (41). We suggested that this might explain, in part, why obese cats maintain normal

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**Fig. 2.** Endogenous glucose production (mg/min and mg·kg$^{-1}$·min$^{-1}$) in 13 young lean, 12 old lean, and 12 old obese cats fed three different diets. The results were combined because there was no difference among the diets. *#*Significant differences between lean old and obese cats. $^a$,$^b$,$^c$Values with the same superscript letters indicate differences between fasting and postprandial values within a cat group ($P < 0.05$).

**Fig. 3.** Relative fluxes through pathways in glucose production measured by 2H NMR analysis. Values are expressed as means ± SE for 13 young lean, 12 old lean, and 12 old obese cats fed three different diets. The results from each diet were combined because there was no difference among the diets. PEP, phosphoenolpyruvate; LY, young lean cats; LO, old lean cats; obese, old obese cats. *#*Significant differences between lean old and obese cats. $^a$Values with the same superscript letter indicate differences between fasting and postprandial values within a cat group ($P < 0.05$).
Baseline glucose concentrations and glycosylated hemoglobin concentrations, and our current study confirms that obese cats compensate well for the lower peripheral insulin sensitivity. In that study, the low EGP was due to lower GNG and glycogenolysis; in the current study, only lower glycogenolysis was observed in obese cats. Other researchers have found that increased glycogenolysis and increased GNG in obese people was also associated with an increase in hepatic glycogen content (50). Our data suggest that obese cats reduce glycogenolysis but do not indicate the stoichiometric effects of intracellular glucose substrates. The relevance of glycogen to EGP in cats is also supported by early studies showing the presence of glycogen in liver of fasted cats (10, 59). We have recently measured glycogen in OL and old obese fasted cats and found that glycogen comprises -5% of the liver weight (unpublished data), which is similar to what is found in dogs (12) and humans (52). It is also noteworthy that, contrary to data from rats (33), glycogen is not depleted in cats after a 24-h fast and, even in fasted obese cats, glycogenolysis still contributes about 30% to glucose production. In a study of 22-h fasted people, glycogenolysis accounted for 36% to total glucose production (63), a value similar to cats in this study fasted for 24 h. It was not until a fast of 42 h that GNG accounted for essentially all of the glucose production in people (63). The lower glycogenolysis in fasted obese cats could be due to reduced hepatic glycogen content. We found that glycogen content in old obese fasted cats was ~10% lower than that seen in lean age-matched cats, but this was not significant (unpublished data). Decreased glycogenolysis and increased GNG in obese people was actually associated with an increase in hepatic glycogen content (50). When net glycogenolysis was followed 5–12 h after a mixed meal, liver glycogen increased over 30% at 5 h. It was calculated that ~19% of the carbohydrate content in the meal was deposited as glycogen in one study (71). Others found that

### Table 5. Relative and absolute fluxes through pathways of glucose production

<table>
<thead>
<tr>
<th>Flux through PEPCK/CS</th>
<th>Young Lean</th>
<th>Old Lean</th>
<th>Obese</th>
<th>13C NMR: Fluxes Relative to Citrate Synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>4.45 ± 0.36</td>
<td>5.25 ± 0.53</td>
<td>4.37 ± 0.27*</td>
<td></td>
</tr>
<tr>
<td>Postprandial</td>
<td>4.25 ± 0.25</td>
<td>4.86 ± 0.51</td>
<td>5.41 ± 0.37*</td>
<td></td>
</tr>
<tr>
<td>Pyruvate cycling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>3.25 ± 0.34</td>
<td>4.03 ± 0.47</td>
<td>3.47 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Postprandial</td>
<td>2.62 ± 0.24</td>
<td>3.42 ± 0.41</td>
<td>4.09 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Flux from PEP to glucose/CS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>1.20 ± 0.08b</td>
<td>1.24 ± 0.12#</td>
<td>0.90 ± 0.06b#</td>
<td></td>
</tr>
<tr>
<td>Postprandial</td>
<td>1.63 ± 0.09b</td>
<td>1.44 ± 0.13</td>
<td>1.31 ± 0.11c#</td>
<td></td>
</tr>
</tbody>
</table>

**Derived Fluxes**

| Flux from glycogen to glucose | Fasting | 0.84 ± 0.07a | 1.00 ± 0.09#| 0.39 ± 0.06b#|
|                              | Postprandial | 1.40 ± 0.13a | 1.04 ± 0.11| 0.80 ± 0.06c#|
| Flux from glycogen to glucose | Fasting | 0.67 ± 0.14a | 0.36 ± 0.04a#| 0.38 ± 0.05|
|                              | Postprandial | 1.04 ± 0.18a | 0.91 ± 0.13a#| 0.38 ± 0.06b#|
| Flux from PEP to glucose      | Fasting | 1.52 ± 0.11a | 1.32 ± 0.10b| 1.16 ± 0.06b|
|                              | Postprandial | 2.62 ± 0.19a | 2.66 ± 0.25b#| 1.79 ± 0.13b#|
| Flux through PEPCK            | Fasting | 5.75 ± 0.51 | 6.46 ± 0.64b| 6.15 ± 0.45b|
|                              | Postprandial | 6.97 ± 0.61 | 8.31 ± 0.61b| 7.99 ± 0.67b|
| Pyruvate cycling              | Fasting | 4.25 ± 0.48 | 5.11 ± 0.60| 4.99 ± 0.42b|
|                              | Postprandial | 4.38 ± 0.51 | 5.63 ± 0.50| 6.23 ± 0.60b|
| TCA cycle turnover            | Fasting | 1.41 ± 0.12 | 1.42 ± 0.17b| 1.52 ± 0.13|
|                              | Postprandial | 1.71 ± 0.13 | 2.03 ± 0.20b| 1.61 ± 0.15|

Values are means ± SE for 13 lean young, 12 lean old, and 12 old obese cats fed three different diets. Relative and absolute fluxes are given in mg·kg⁻¹·min⁻¹. The results from each diet were combined because there was no difference among the diets. PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; CS, citrate synthase; OAA, oxaloacetate. * and #Significant differences between lean young and old cats and lean old and obese cats, respectively (P < 0.05). *#Values with the same superscript letter indicate differences within a group of cats (P < 0.05). $P = 0.0650.$
about 25% were deposited after an oral glucose load (58). Postprandial hepatic glycogen content has not been evaluated in cats. Because muscle is also involved in glycogen storage, it would be of interest to evaluate whether and how muscle glycogen stores change with fasting and feeding in cats, but this has not been reported. In people, it has been shown that muscle glycogen increases from 80 to 100 mmol/l by 5 h after a meal and then decreases (71), whereas in the liver, the postprandial increase is larger (from 265 mmol/l to 360 mmol/l) (45). Similarly, this study did not address gluconeogenesis by the kidney, and no previous data are available from cats. The contribution of the kidney in humans to EGP in the postprandial state has been small (~5%), and even after 60 h of fasting, only represents 20–25% of EGP (16). It has also been shown in dogs that an overnight fast does not lead to significant net output of glucose from the kidney (14). The liver, therefore, is the predominant site for glucose production in the fasted and even more so in the postprandial state. Although it may seem unexpected that EGP is elevated in postprandial cats, EGP within 10 h postfeeding in people is similarly about 50% higher than EGP after a 1-day fast (60).

Relative and absolute fluxes of PEP to glucose, oxaloacetate to glucose, and pyruvate cycling (which refers to the combined flux through two distinct futile cycles: oxaloacetate-PEP-pyruvate-oxaloacetate, and oxaloacetate-malate-pyruvate-oxaloacetate) (34) were similar to our previous measurements in fasted cats (41). In YL cats, no changes between fasting and postprandial absolute fluxes were seen. In obese cats, fluxes through PC/PEPCK and citrate synthase were significantly more active in the postprandial state, and this was accompanied by a significant increase (~30%) in pyruvate cycling, which was marginally significant ($P = 0.0650$). These data suggest that the hepatic TCA cycle is significantly more active in obese postprandial cats, but pyruvate cycling appears to modulate gluconeogenesis and EGP. A regulatory role for pyruvate cycling has also been described in Zucker ($fa/pha$) rats (34). We have previously shown that fasted female obese cats had similar changes, including higher pyruvate cycling (41). We hypothesized in that study that obese cats might compensate their overnutrition by enhancing these fluxes, creating a protective mechanism against oxidative stress (41). It is unclear why changes were not seen also in the fasted state in the current study. It is possible that prolonged obesity or the differences in the diets between the two studies led to this difference. Fluxes through PEPCK and TCA cycle fluxes were strongly correlated in all cats, an interdependence previously shown in several studies (7, 21) and consistent with the postulate that flux through PEPCK generates energy in the hepatic TCA cycle.

OL and obese cats had significantly higher insulin concentrations than the YL cats, whereas glucose concentrations were not different. This suggests that OL and obese cats have insulin resistance. We have previously shown in obese cats that the development of obesity leads to a change in insulin sensitivity (26). In a recent study, older cats (average age 5.8 yr) showed a higher area under the curve for insulin (AUCI) in the late phase of an intravenous glucose tolerance test than young cats (average age 1.1 yr). However, in that study, body fat mass was higher in the older cats and may have accounted for the higher AUCI. Fasting insulin and glucose concentrations were not different (3). An association between aging and peripheral insulin resistance has been known for many years in humans of both sexes (13, 18, 28, 64) and rodents (20, 51). Aging is also associated with changes in body composition, especially higher fat mass, making it difficult to isolate the role of aging on impaired insulin action. Several reports have shown that decreased insulin sensitivity occurs in nonobese elderly humans (13, 28, 64), others have shown that age-related changes in adiposity, and/or physical inactivity are the primary determinants of the declines in insulin sensitivity rather than chronological age (2, 5, 43). The higher insulin concentrations in the OL cats were not associated with a change in fat mass, nor did we document any specific changes in carbohydrate metabolism in the older cats with higher insulin levels. Studies using the gold standard method, the euglycemic hyperinsulinemic clamp, are necessary to confirm a decrease in insulin sensitivity in this age group. Insulin and glucose concentrations were not different in the postprandial state compared with the fasted state in all groups. We have recently shown that obese cats, unless prediabetic, do not have different glucose concentrations than lean cats and do not show great inter-day variability when monitored for a 7-day period during their daily routine with a continuous glucose monitoring system (27). Even when 40% of the diet consisted of glucose, only a small increase in blood glucose was seen at 1 h postfeeding but not at 3 or 6 h (39). Because only one blood sample was taken after food intake in the current study, it is possible that higher glucose and insulin concentrations might have been present at earlier time points.

It is interesting that leptin concentrations were also increased in OL cats. Our laboratory and others have shown that leptin increases with increasing fat mass (4, 26). However, fat mass was similar between the two lean groups, suggesting that not only body condition but also age, influence leptin concentrations in cats. In people, plasma leptin concentrations have been found to be low (29, 53), unchanged (1), or increased with age (20, 70). The OL cats in our study had higher fasting insulin concentrations compared with YL cats. Several studies have shown that insulin may modulate serum leptin levels. Serum leptin has been shown to correlate with fasting insulin (11, 19, 70) and indices of insulin resistance in humans (68) independent of obesity. Some investigators documented that insulin increases plasma leptin concentrations in rodents or humans (44, 47), whereas others were unable to show regulation of leptin expression or secretion by insulin (74). It is not possible to show a cause-effect relationship between the two hormones with our study design.

Adiponectin is a hormone that is primarily secreted from adipocytes but can also be secreted from skeletal muscle cells (55). It is very abundant in plasma, modulates glucose and lipid metabolism, and is thought to be anti-inflammatory, insulin-sensitizing, and anti-atherogenic. We have previously shown that adiponectin is negatively correlated with obesity in cats (26), and similar findings have been reported from other species, including humans (15). Weight reduction increases adiponectin in obese cats and humans (26, 67). Higher concentrations of adiponectin in lean cats compared with obese cats in this study were expected, but increased concentrations in OL compared with YL, is a novel finding. Discrepant results have been reported from studies in humans regarding the effect of age on adiponectin. Adiponectin levels have been reported to increase with age in normal-weight, middle-aged, and older women, and were independently related to age, leptin, and...
insulin resistance values (37, 42), while others have not seen an increase with age (67). A recent study (40) of adults surviving to advanced old age showed that plasma adiponectin concentration increased longitudinally with age and was also independently related to physical decline in this population. A similar negative association between plasma adiponectin concentration and total mortality, as well as cardiovascular mortality, has been described in a large cohort of older adults (57). Increased concentrations of circulating adiponectin have been observed repeatedly in patients with congestive heart failure (CHF) and are associated with disease severity and inverse outcome (73), although CHF is an insulin-resistant state in which decreased adiponectin levels might be expected. It has been speculated that increased adiponectin concentrations in CHF reflect a compensatory mechanism to overcome insulin resistance and inflammatory changes associated with the disease (48). In other studies, high concentrations of circulating adiponectin have been associated with reduced risks of cardiovascular disease (56, 62). Others have suggested that the increase in adiponectin seen with age is due to declining renal function, leading to decreased clearance of the hormone (30).

Because the biological significance of this elevation in plasma adiponectin in the elderly is not known, further investigation is necessary to clarify the role of increased adiponectin in older people and cats.

Diet only had a minor influence on any of the parameters measured in this study. This might be due to the fact that the differences among the diets were not very large. All diets had more than 30% protein, and the percentage of fat was approximately the same among the diets. In the cat, the minimum level to maintain nitrogen balance has been reported to be 10% (6). Neither age nor body condition led to changes in the RER, which is a measure of metabolic fuel source. Pure carbohydrate oxidation gives an RER of 1.00, whereas pure fat oxidation gives a ratio of ~0.71. In the fasted state, more fat was oxidized in all groups of cats, whereas feeding decreased its oxidation, regardless of diet. The significantly lower RER of obese fasted cats indicates a preference for lipids as fuel and might have contributed to the lower EGP. This supports our earlier findings during a euglycemic hyperinsulinemic clamp of a higher fat oxidation in younger obese females than in lean cats of either sex (25). We included a diet containing PUFAs because we were able to show previously a beneficial effect of PUFAs on insulin sensitivity, although at different concentrations (75).

The metabolic rate and caloric intake of obese cats based on metabolic body size and body weight, respectively, were less than that of lean cats, supporting earlier findings from our laboratory (26). One might argue that results from obese cats based on metabolic size or body weight lead to erroneous conclusions because fat mass is inert. This has been challenged recently, and it has been suggested that fat mass is important in whole body energy expenditure (38). In this study, total intake and heat production was higher in obese cats; however, while total body weight was twice as high in obese than lean cats, both total heat production and caloric intake were only ~50% higher, supporting the notion that fat contributes to energy production, but it is not a linear relationship.

The higher fasting NEFA concentrations in obese cats were likely caused by lower lipoprotein lipase activity (24) and may have led to the trend toward an increase in F4T concentration because we have previously shown that the in vitro addition of palmitate and oleate to cat plasma, within a physiological concentration range, leads to a significant increase of the free T4 fraction (17).

Perspectives and Significance

We have shown that long-term obese cats compensate well for peripheral insulin resistance by lowering endogenous glucose output in both the fasted and fed state. We have also shown that in lean and obese cats, the contribution of gluconeogenesis and glycogenolysis to endogenous glucose production was similar to that seen in humans. OL cats had higher insulin with normal glucose concentrations, suggesting that they were insulin resistant. Surprisingly, leptin and adiponectin levels were higher in OL cats than YL cats. The significance of these findings needs to be explored further. Diet had little influence on any of the parameters.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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