

# Effects of Pioglitazone on Insulin Sensitivity and Serum Lipids in Obese Cats

M. Clark, K. Thomaseth, L. Dirikolu, D. C. Ferguson, and M. Hoenig

**Background:** Pioglitazone is a thiazolidinedione (TZD) insulin sensitizer approved for use in human type 2 diabetes mellitus. Therapeutic options for diabetes in cats are limited.

**Objective:** To evaluate the effects of pioglitazone in obese cats, which are predisposed to insulin resistance, to assess its potential for future use in feline diabetes mellitus.

**Animals:** A total of 12 obese purpose-bred research cats (6 neutered males and 6 spayed females, 5–7 years of age, weighing 5.4–9.8 kg).

**Methods:** Randomized, placebo-controlled 3-way crossover study. Oral placebo or pioglitazone (Actos™; 1 or 3 mg/kg) was administered daily for 7-week periods, with IV glucose tolerance testing before and after each period.

**Results:** Three mg/kg pioglitazone significantly improved insulin sensitivity (geometric mean [95% CI] 0.90 [0.64–1.28] to 2.03 [1.49–2.78] min<sup>-1</sup>pmol<sup>-1</sup>L; *P* = .0014 versus change with placebo), reduced insulin area under the curve during IVGTT (geometric mean [range] 27 [9–64] to 18 [6–54] min-nmol/L; *P* = .0031 versus change with placebo), and lowered serum triglyceride (geometric mean [range] 71 [29–271] to 48 [27–75] mg/dL; *P* = .047 versus change with placebo) and cholesterol (geometric mean [range] 187 [133–294] to 162 [107–249] mg/dL; *P* = .0042 versus change with placebo) concentrations in the obese cats. No adverse effects attributable to pioglitazone were evident in the otherwise healthy obese cats at this dosage and duration.

**Conclusions and Clinical Importance:** Results of this study support a positive effect of pioglitazone on insulin sensitivity and lipid metabolism in obese cats, and suggest that further evaluation of the drug in cats with diabetes mellitus or other metabolic disorders might be warranted.

**Key words:** Diabetes; Feline; IVGTT; Thiazolidinedione.

Diabetes mellitus is a disorder of carbohydrate, lipid, and protein metabolism arising from absolute or relative insulin deficiency. It occurs in 0.4–1% of domestic cats, and prevalence appears to be increasing.<sup>1</sup> The most common form of diabetes in cats is similar to type 2 diabetes in humans; first, diabetes in cats often involves a combination of insulin resistance and  $\beta$ -cell dysfunction, and second, obesity, which leads to insulin resistance,<sup>2</sup> is a predisposing factor.<sup>1</sup>

Current treatment options for diabetes in cats are limited to insulin and the sulfonylureas. Although insulin is effective in many cases, it must be given by injection and carries the risk of hypoglycemia, both of which can be significant concerns for owners.<sup>3</sup> Glipizide, a sulfonylurea insulin secretagogue, has been used in diabetic cats, but long-term treatment with glipizide promotes amyloid deposition in pancreatic islets, and does not enhance potential for  $\beta$ -cell recovery.<sup>4</sup> More

## Abbreviations:

AUC <sub>0-inf</sub>	area under the curve from time 0 to infinity
AUC	area under the curve
C <sub>max</sub>	maximum plasma concentration
HDL	high-density lipoprotein
IVGTT	intravenous glucose tolerance test
K-value	percent glucose disappearance per minute
MM	minimal model of glucose disappearance
NEFA	nonesterified fatty acid
NLME	nonlinear mixed-effects modeling
PPAR $\gamma$	peroxisome proliferator-activated receptor gamma
RER	respiratory exchange ratio
RIA	radioimmunoassay
S <sub>I</sub>	insulin sensitivity of glucose disappearance
S <sub>IN</sub>	insulin sensitivity of NEFA suppression
TZD	thiazolidinedione
VLDL	very low-density lipoprotein

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treatment choices for diabetes in cats, particularly for owners unable or unwilling to attempt insulin therapy, would be beneficial.

The thiazolidinedione (TZDs) are oral insulin sensitizers marketed for use in human type 2 diabetes. They are agonists of the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), a nuclear transcription factor that is a key regulator of glucose metabolism, lipid metabolism, and adipogenesis.<sup>5</sup> Treatment of humans and rodents with TZDs increases whole-body insulin sensitivity and promotes uptake and storage of circulating lipids by adipocytes; in type 2 diabetics, the result is improved glycemic control, reduced plasma lipid concentrations, and redistribution of lipids from sites of ectopic accumulation (muscle and liver) to adipose tissue.<sup>5,6</sup> TZDs also lower hepatic lipid in human

nonalcoholic fatty liver disease, a chronic steatotic hepatopathy associated with insulin resistance.<sup>5</sup> Unlike sulfonylureas, TZDs have beneficial long-term effects on pancreatic  $\beta$ -cells, and as insulin sensitizers, they are associated with a low risk of hypoglycemia.<sup>7</sup>

Because the TZDs are effective for type 2 diabetes in humans, they may prove useful for treatment of diabetes in cats. An experimental TZD, darglitazone, increased glucose and nonesterified fatty acid (NEFA) clearance during glucose challenge in obese, insulin-resistant cats.<sup>8</sup> Although darglitazone did not complete clinical development in either humans or animals, the TZD pioglitazone was approved for human use in 1999, and has remained commercially available.<sup>9</sup> The purpose of this study was to evaluate the effects of pioglitazone in obese cats, to assess its potential for future use in feline diabetes or hepatic lipidosis. We hypothesized that pioglitazone would increase insulin sensitivity and lead to enhanced glucose and lipid disposal.

## Materials and Methods

### Animals

Twelve neutered Domestic Shorthair cats (equal sex distribution; 5–7 years of age), were used for this study. Cats weighed 5.4–9.8 kg (median 6.2 kg), and were classified as obese based on gain of  $\geq 50\%$  of their adult lean body weight. Obesity was originally induced by ad libitum feeding. Cats were individually housed at the University of Illinois veterinary medical animal care facility, in a 70–72°F room with a 12-hour light/dark cycle, and were fed a commercial dry maintenance diet<sup>a</sup> once a day. Diet composition was 40% protein (min.), 16% fat (min.), 2% fiber (max.), and 12% moisture (max.). Food intake was recorded daily and adjusted to maintain body weight, which was recorded weekly. Study procedures were approved by the university's Institutional Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Treatments

Cats were stratified by sex and percent gain above their adult lean body weight, and randomly allocated to 3 groups. A treatment sequence, consisting of 7 weeks of oral placebo, 1 mg/kg pioglitazone, and 3 mg/kg pioglitazone (each followed by a 7-week washout period), was randomly assigned to each group. All cats received all treatments, in a 3-way crossover design uniform within period and sequence.

Pioglitazone<sup>b</sup> or placebo was administered once daily with a small amount of canned food.<sup>c</sup> Pioglitazone tablets were divided using a pill cutter, weighed, and placed in gelatin capsules before administration. Placebo capsules contained approximately 186 mg powdered lactose,<sup>d</sup> a quantity equivalent to the approximate amount in the greatest number of pioglitazone tablets to be received by any cat during any period of the study. Feeding of the daily ration was conducted without regard to the time of dosing.

### Physical Examination, Laboratory Testing, and Echocardiography

Before each dosing period and during weeks 6–7 of dosing, a physical examination, complete blood count, biochemistry panel,

and echocardiogram were performed on each cat. Serum total thyroxine concentration was also measured before the first dosing period. Laboratory testing was performed through the Clinical Pathology Laboratory, and echocardiograms were performed by a board-certified veterinary radiologist, at the University of Illinois College of Veterinary Medicine.

### Indirect Calorimetry

For detection of any species-specific effects of pioglitazone on substrate metabolism or energy expenditure, indirect calorimetry was performed (as previously described<sup>10</sup>) before each dosing period and during the 6th week of dosing. Cats remained in the calorimetry chambers for ~8 h, after an overnight fast, and were fed on removal from the chamber.

### IV Glucose Tolerance Testing

IV glucose tolerance tests (IVGTTs) were performed before each dosing period, and during the 7th week of dosing. Details are available as supporting information. Briefly, blood samples were collected via jugular catheter from 0 to 180 min after IV administration of 0.8 g/kg dextrose, and plasma was aliquoted for measurement of glucose, insulin, NEFAs, and baseline concentrations of adiponectin and leptin. During each posttreatment IVGTT, blood samples were collected for pioglitazone measurement at 0 (predose), 2, 3, 4, 5, 8, and 11 h after drug administration. Samples were stored at  $-20^{\circ}\text{C}$ .

### Assays

Glucose was measured by a colorimetric glucose oxidase method,<sup>e</sup> insulin by a porcine insulin radioimmunoassay with human insulin standards,<sup>f</sup> and NEFAs with an enzymatic colorimetric kit.<sup>g</sup> Adiponectin was measured by ELISA,<sup>h</sup> and leptin by a commercial radioimmunoassay,<sup>i</sup> both of which were validated in our laboratory for use in cats.<sup>2,11</sup> All samples were assayed in duplicate. For adiponectin and leptin, all samples were processed in the same assay. For insulin, the standard curve for diluted feline samples was parallel to the curve for human insulin standards. Recovery of insulin from spiked samples in blank feline plasma was 103% at low and 94% at high concentrations. Interassay and intra-assay coefficients of variation were 6.3% and 11.9%, respectively. Pioglitazone concentrations were determined by high-performance liquid chromatography using a previously validated procedure.<sup>12</sup>

### Data Analysis

Area under the curve (AUC) for glucose, insulin and NEFAs, AUC for percent NEFA suppression, and percent glucose disappearance per minute ( $K$ -value) were calculated for each IVGTT using statistical software.<sup>j,k</sup> Formulas for  $K$ -value and percent NEFA suppression have been described previously.<sup>2,13</sup> Respiratory exchange ratio (RER) and heat production were calculated for each cat as the average of measurements obtained during hours 4–6 of calorimetry.  $C_{\text{max}}$ ,  $\text{AUC}_{0-\text{inf}}$ , and terminal elimination half-life of pioglitazone were estimated for each cat using commercial software.<sup>l</sup>

Insulin sensitivity, defined as the response of both glucose and NEFAs to endogenous insulin secreted during IVGTT, was quantified using the minimal model (MM) of glucose disappearance<sup>14</sup> and a modified version of a previous model of NEFA kinetics.<sup>15</sup> MM and NEFA kinetic model parameters were estimated, and changes in  $S_I$  (insulin sensitivity of glucose disappearance) and  $S_F$

$N$  (insulin sensitivity of NEFA suppression) were compared among treatments, using nonlinear mixed-effects modeling (NLME). Changes in other outcome measures were compared among treatments using linear mixed-effects modeling with logarithmic data transformation. Both models incorporated fixed effects of sequence, period, and treatment; details are available as supporting information.

All model identifications, parameter estimations, and significance tests were carried out using a statistical software package.<sup>k</sup> Mathematical model equations and simulation code were generated using the modeling software tool PANSYM.<sup>16</sup> Significance was set at 0.05. Placebo was used as a reference for contrasts; therefore, all reported changes with pioglitazone take into account the corresponding change with placebo.

## Results

### Clinical Observations, Clinicopathologic Data, and Echocardiography

Throughout the study, no important abnormalities were detected on physical examinations. No differences were found among treatments with regard to changes in clinicopathologic or echocardiographic measurements, with the exception of decreases in triglycerides ( $P = .047$ ), cholesterol ( $P = .0042$ ), eosinophils ( $P = .026$ ), and phosphorus ( $P = .018$ ) with 3 mg/kg pioglitazone, and a decrease in cholesterol ( $P = .034$ ) with 1 mg/kg pioglitazone (Table 1). Values for these variables remained above the lower limit of the reference interval in all cats.

One or more episodes of vomiting occurred in 10/12 cats during the 8 months of the study, but frequency was not different for either dosage of drug versus placebo (all  $P \geq .36$ ). One cat died during sedation for catheter placement at the end of the last study period; necropsy and histopathology revealed myocardial fiber disarray characteristic of hypertrophic cardiomyopathy.

**Table 1.** Serum triglyceride, cholesterol, and phosphorus concentrations, and blood eosinophil concentrations (geometric mean [range]), in obese cats ( $n = 11$ – $12$ ) before and after 6 weeks of oral placebo, 1 mg/kg pioglitazone, or 3 mg/kg pioglitazone.

	Placebo	1 mg/kg Pioglitazone	3 mg/kg Pioglitazone
Triglycerides (mg/dL)			
Before	81 (37–1666 <sup>b</sup> )	72 (36–454)	71 (29–271)
After	87 (39–614)	61 (36–112)	48 (27–75) <sup>a</sup>
Cholesterol (mg/dL)			
Before	192 (112–273)	186 (120–295)	187 (133–294)
After	191 (124–286)	171 (109–250) <sup>a</sup>	162 (107–249) <sup>a</sup>
Phosphorus (mg/dL)			
Before	4.3 (3.7–4.9)	4.5 (4.0–4.8)	4.5 (4.0–5.3)
After	4.3 (3.5–4.9)	4.3 (3.9–4.7)	4.2 (3.6–4.6) <sup>a</sup>
Eosinophils (K/ $\mu$ L)			
Before	0.39 (0.08–0.97)	0.44 (0.26–1.01)	0.54 (0.16–1.63)
After	0.51 (0.24–1.06)	0.46 (0.19–1.55)	0.31 (0.07–0.74) <sup>a</sup>

<sup>a</sup>Significant change from pretreatment value versus change with placebo ( $P < .05$ ).

<sup>b</sup>Outlier.

### Glucose and NEFA Disposal

Glucose and insulin concentrations during IVGTT are shown in Figures 1 and 2, respectively. AUC for glucose, insulin, NEFAs, and percent NEFA suppression are shown in Table 2. There were no differences among treatments with respect to the change in fasting glucose or NEFA concentrations or  $K$ -value (data not shown), or glucose AUC<sub>0-180</sub>. However, there was a significant decrease in insulin AUC<sub>0-180</sub> with 3 mg/kg pioglitazone ( $P = .0031$ ). Fasting insulin was also lower after 3 mg/kg pioglitazone, but the  $P$ -value for this change was borderline ( $P = .052$ ).

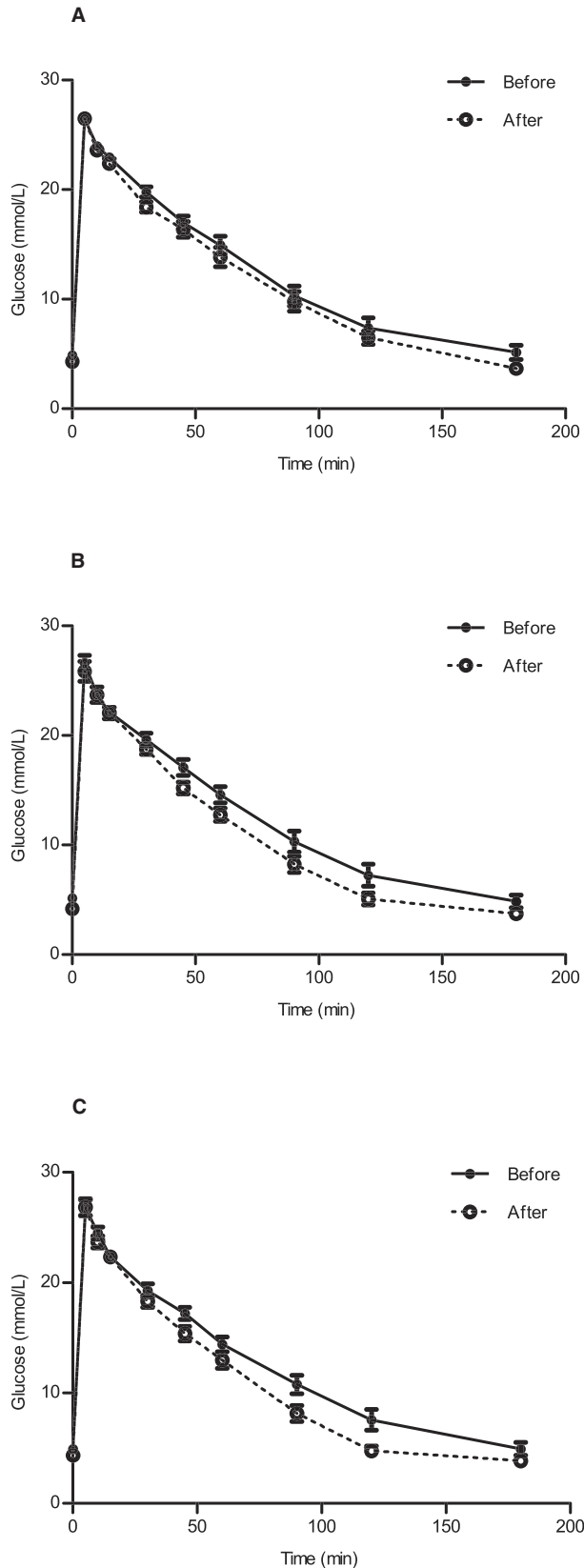
In the plots of percent NEFA suppression, a subjectively greater initial suppression and earlier, more precipitous rebound from suppression were evident as drug dosage increased (Fig 3). The early rebound was also apparent in the NEFA concentration-time profiles (data not shown). Because of the possible interference of the rebound with the analysis of AUC, AUC for percent NEFA suppression was calculated both as AUC<sub>0-180</sub> (total NEFA suppression) and as AUC<sub>0-90</sub> (initial NEFA suppression; Table 2). AUC<sub>0-180</sub> increased with 1 mg/kg pioglitazone ( $P = .036$ ), but the change with the 3 mg/kg dosage was not significant ( $P = .26$ ). AUC<sub>0-90</sub> increased with both 1 and 3 mg/kg pioglitazone ( $P = .025$  and  $P = .048$ , respectively). For all calculations involving AUC for percent NEFA suppression, it was necessary to eliminate 2 profiles with an average suprabasal NEFA increment from analysis, as they yielded negative AUCs that were not amenable to log transformation. Because one of these profiles occurred after placebo, and one after 1 mg/kg pioglitazone, their exclusion had the potential to artificially improve the measures of percent NEFA suppression for both placebo and the 1 mg/kg dosage.

### Insulin Sensitivity

$S_I$  increased with 3 mg/kg, but not 1 mg/kg, pioglitazone (geometric mean [95% CI] fold increase 2.4 [1.4–4.1],  $P = .0014$ , and 1.5 [0.9–2.4],  $P = .15$ , respectively). Likewise,  $S_{IN}$  increased with 3 mg/kg pioglitazone (2.1 [1.2–3.7] fold increase,  $P = .014$ ), but the change with 1 mg/kg was not significant (1.3 [0.7–2.6] fold increase,  $P = .37$ ; Table 2).

### Adipocytokines, Energy Expenditure, Body Weight, Food Intake, and Substrate Metabolism

Adiponectin concentration increased with both 1 and 3 mg/kg pioglitazone ( $P = .0024$  and  $P < .0001$ , respectively; Table 2). Pioglitazone did not affect leptin concentration (Table 2), body weight, average daily food intake, heat production (kcal/h), or heat production per metabolic body size (kcal/h/kg; data not shown). There was a statistically significant but very small decrease in RER with both 1 and 3 mg/kg pioglitazone ( $P = .014$  and  $P = .036$ , respectively; Table 2).



**Fig 1.** Plasma glucose concentrations (mean  $\pm$  SEM) versus time during intravenous glucose tolerance test in obese cats ( $n = 11$ – $12$ ) before and after 6 weeks of oral placebo (A), 1 mg/kg pioglitazone (B), or 3 mg/kg pioglitazone (C).

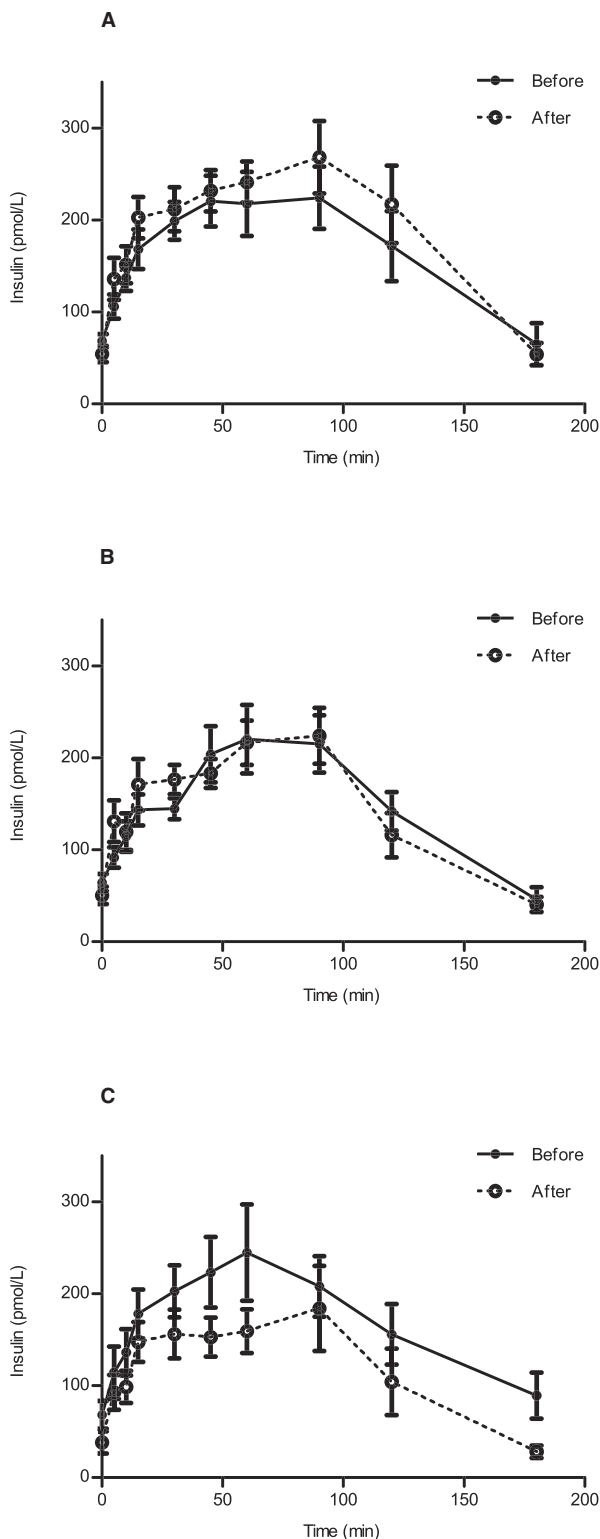
### Pioglitazone Concentrations

Plasma concentration-time profiles for pioglitazone are shown in Figure S1.  $C_{max}$  and  $AUC_{0-inf}$  were greater after 3 mg/kg than after 1 mg/kg administration (mean  $\pm$  SD,  $1528 \pm 527$  versus  $997 \pm 208$  ng/mL, and  $11.9 \pm 5.4$  versus  $7.44 \pm 2.25$  h  $\mu$ g/mL, respectively, both  $P < .001$ ), although the increases were less than proportional. Half-life did not differ between dosages (mean  $\pm$  SD,  $3.65 \pm 0.65$  versus  $3.67 \pm 0.56$  h for 1 and 3 mg/kg, respectively;  $P = .997$ ).

### Discussion

The insulin-sensitizing properties of pioglitazone have been well described in obese and type 2 diabetic humans, and results of this placebo-controlled study demonstrate that pioglitazone, at 3 mg/kg, increases insulin sensitivity in obese cats. Increased insulin sensitivity was evident in terms of both the insulin sensitivity index ( $S_I$ ), which reflects the actions of insulin to stimulate peripheral glucose uptake and suppress hepatic glucose production, and its counterpart ( $S_{IN}$ ) in the model of NEFA kinetics, which describes the potency of insulin to promote fatty acid uptake and inhibit peripheral lipolysis. The dual improvement in these indices in the obese cats is consistent with the fact that pioglitazone amplifies insulin signaling in multiple organs, including the muscle, adipose tissue, and liver, in other species.<sup>5,7,17,18</sup> In addition, it is encouraging with respect to the therapeutic potential of pioglitazone in diabetic cats, and perhaps in cats with hepatic lipodosis. In humans and rodents, the glycemic effects of the TZDs are more pronounced in diabetic than in obese individuals,<sup>18,19</sup> and the same doses of pioglitazone that increase insulin sensitivity in human obesity lower glucose and NEFA concentrations in type 2 diabetics.<sup>9,20</sup> Thus, it is possible that the insulin sensitization produced by pioglitazone in the obese cats would be manifest as a favorable impact on glucose and NEFA concentrations in diabetic cats. One caveat, however, is that the TZDs are clinically ineffective in the absence of insulin,<sup>17,18</sup> and diabetic cats may have low or undetectable insulin concentrations.<sup>21</sup> Consequently, effects of pioglitazone in diabetic cats remain speculative without further investigation. Additionally, while pioglitazone might improve insulin sensitivity in obese diabetic cats, weight loss would still be warranted to minimize other obesity-related disorders.

The decrease in insulin AUC with 3 mg/kg pioglitazone represents alleviation of compensatory hyperinsulinemia, an allostatic response to insulin resistance that is present in obese cats both during IVGTT and in the basal state.<sup>22,23</sup> Relief of compensatory hyperinsulinemia also occurred with the more potent experimental TZD darglitazone in obese cats,<sup>8</sup> and is a predictable outcome of TZD administration in obese humans and rodents.<sup>18,20</sup> In addition, insulin sensitization with pioglitazone enhanced NEFA suppression during the first 90 min of IVGTT; potential mechanisms for this change include greater inhibition of lipolysis, increased



**Fig 2.** Plasma insulin concentrations (mean  $\pm$  SEM) versus time during intravenous glucose tolerance test in obese cats ( $n = 11-12$ ) before and after 6 weeks of oral placebo (A), 1 mg/kg pioglitazone (B), or 3 mg/kg pioglitazone (C).

NEFA uptake, or some combination of the two. In Zucker fatty rats, TZDs enhance both lipolytic inhibition and NEFA uptake (primarily into adipocytes)

under insulin-stimulated conditions,<sup>24</sup> leading to a net flux of NEFAs into peripheral adipose depots and a corresponding depletion of ectopic lipid from other tissues.<sup>24,25</sup> Thus, it might be postulated that preferential adipose tissue NEFA uptake was a component of NEFA suppression in the obese cats, with the possible sequel of reduced ectopic lipid deposition, although further study would be required to confirm this.

An interesting feature of the postpioglitazone NEFA profiles in the obese cats was the brisk suprabasal rebound that followed initial suppression. In humans, this has been attributed to lipolytic hormone release secondary to rapid glucose clearance,<sup>26</sup> and is faster in lean than in obese individuals.<sup>27</sup> A similar phenomenon has been described after pharmacological doses of glucose administered PO, and the magnitude of the NEFA rebound in one study was positively correlated with insulin sensitivity.<sup>28</sup> The subjectively steeper rebound with pioglitazone in the cats, therefore, appears compatible with other evidence of increased insulin sensitivity, and may actually reflect a biologically significant increase in glucose clearance.

The changes in serum lipids that occurred with pioglitazone are comparable in some respects to those that occur in pioglitazone-treated humans. In diabetic humans, pioglitazone lowers triglycerides by increasing clearance of very low-density lipoproteins (VLDL), ostensibly through enhanced activity of lipoprotein lipase, an insulin-regulated enzyme that mediates VLDL-triglyceride hydrolysis.<sup>29</sup> A similar mechanism may have been responsible for the decrease in triglycerides in the obese cats. With regard to cholesterol, the effects of pioglitazone differ between humans and cats: pioglitazone increases high-density lipoprotein (HDL) cholesterol in humans, but does not affect total cholesterol.<sup>20,29</sup> This difference may relate to the fact that (1) cats carry most of their cholesterol in HDL, and (2) activity of cholesteryl ester transfer protein, which catalyzes the exchange of cholesterol between HDL and other lipoproteins, appears to be very low in cats.<sup>30</sup> As hypercholesterolemia is a negative predictor of diabetic remission in cats,<sup>31</sup> it is possible that the cholesterol-lowering effect of pioglitazone, as observed in the obese cats, would ultimately impart clinical benefit in diabetic cats. Furthermore, the effects of pioglitazone on serum lipids and NEFAs may eventually prove useful in cats with other dyslipidemias. Cats with idiopathic hepatic lipidosis have increased triglyceride content in all lipoproteins, and elevated NEFA concentrations because of excessive peripheral lipolysis.<sup>32</sup> Depending on the role of insulin resistance in feline hepatic lipidosis, pioglitazone could theoretically lead to improved lipid metabolism and mobilization of hepatic triglyceride in this disorder.

Although most other outcomes of pioglitazone administration were significant only at the 3 mg/kg dosage, even 1 mg/kg pioglitazone caused a robust increase in plasma concentrations of adiponectin. Adiponectin is an adipose-derived cytokine with insulin-sensitizing, anti-apoptotic, and anti-inflammatory effects,<sup>33</sup> and concentrations are abnormally low in

**Table 2.** Geometric mean (range) AUC for glucose, insulin, NEFAs, and % NEFA suppression, adipocytokine concentrations, and NLME-derived estimates of insulin sensitivity (geometric mean [95% CI]) during IVGTT in obese cats (n = 11–12) before and after 6 weeks of oral placebo, 1 mg/kg pioglitazone, or 3 mg/kg pioglitazone. Body weight and food intake, and heat production and RER during indirect calorimetry (geometric mean [range]) are also shown.

	Placebo	1 mg/kg Pioglitazone	3 mg/kg Pioglitazone
Glucose AUC <sub>0–180</sub> (min mol/L)			
Before	2.1 (1.5–2.7)	2.1 (1.5–3.2)	2.1 (1.6–3.0)
After	2.0 (1.5–2.4)	1.8 (1.5–2.4)	1.8 (1.6–2.2)
Insulin AUC <sub>0–180</sub> (min nmol/L)			
Before	28 (18–52)	25 (16–43)	27 (9–64)
After	33 (19–53)	25 (14–39)	18 (6–54)*
NEFA AUC <sub>0–180</sub> (min mEq/L)			
Before	49.7 (35.8–81.5)	54.4 (33.0–98.8)	54.2 (38.6–83.7)
After	57.7 (40.1–100.9)	54.0 (40.3–74.1)*	62.1 (32.2–95.2)
% NEFA suppression AUC <sub>0–180</sub> ([min-%]/1000)			
Before	10.0 (7.8–11.7)	9.0 (5.1–13.5)	8.7 (6.7–10.4)
After	7.8 (5.3–9.5)	9.4 (4.5–11.1)*	7.8 (3.8–11.1)
% NEFA suppression AUC <sub>0–90</sub> ([min %]/1000)			
Before	4.8 (2.9–6.2)	4.3 (1.7–6.5)	4.1 (2.4–5.5)
After	3.9 (1.9–6.1)	5.1 (3.2–6.3)*	4.7 (2.3–6.3)*
Adiponectin (µg/mL)			
Before	1.3 (0.5–4.9)	1.4 (0.3–4.7)	1.2 (0.4–4.5)
After	1.6 (0.3–6.5)	3.6 (0.8–11.5)*	6.1 (2.7–14.2)*
Leptin (ng/mL)			
Before	16 (7–41)	14 (4–31)	14 (7–27)
After	15 (6–29)	13 (5–26)	15 (7–27)
Body weight (kg)			
Before	6.3 (5.0–9.7)	6.3 (5.2–9.7)	6.3 (5.4–9.9)
After	6.4 (5.0–9.9)	6.3 (5.0–9.7)	6.4 (5.3–9.9)
Daily food consumption (g)	61 (47–82)	61 (43–81)	61 (41–81)
Heat production (kcal/h/cat)			
Before	11.0 (8.3–12.7)	11.2 (8.0–14.0)	11.0 (8.9–13.4)
After	10.8 (8.2–13.8)	10.9 (8.1–14.0)	10.8 (7.9–13.2)
RER			
Before	0.76 (0.73–0.81)	0.76 (0.74–0.80)	0.76 (0.73–0.79)
After	0.76 (0.74–0.80)	0.75 (0.73–0.79)*	0.75 (0.73–0.78)*
S <sub>I</sub> (min <sup>-1</sup> pmol <sup>-1</sup> L) 10 <sup>4</sup>			
Before	1.03 (0.77–1.36)	1.07 (0.79–1.44)	0.90 (0.64–1.28)
After	0.86 (0.64–1.16)	1.39 (1.07–1.79)	2.03 (1.49–2.78)*
S <sub>IV</sub> (min <sup>-1</sup> mEq <sup>-1</sup> L) 100			
Before	3.8 (2.9–5.1)	3.5 (2.8–4.4)	1.9 (1.3–2.7)
After	1.9 (1.4–2.5)	2.3 (1.4–3.5)	1.9 (1.5–2.4)*

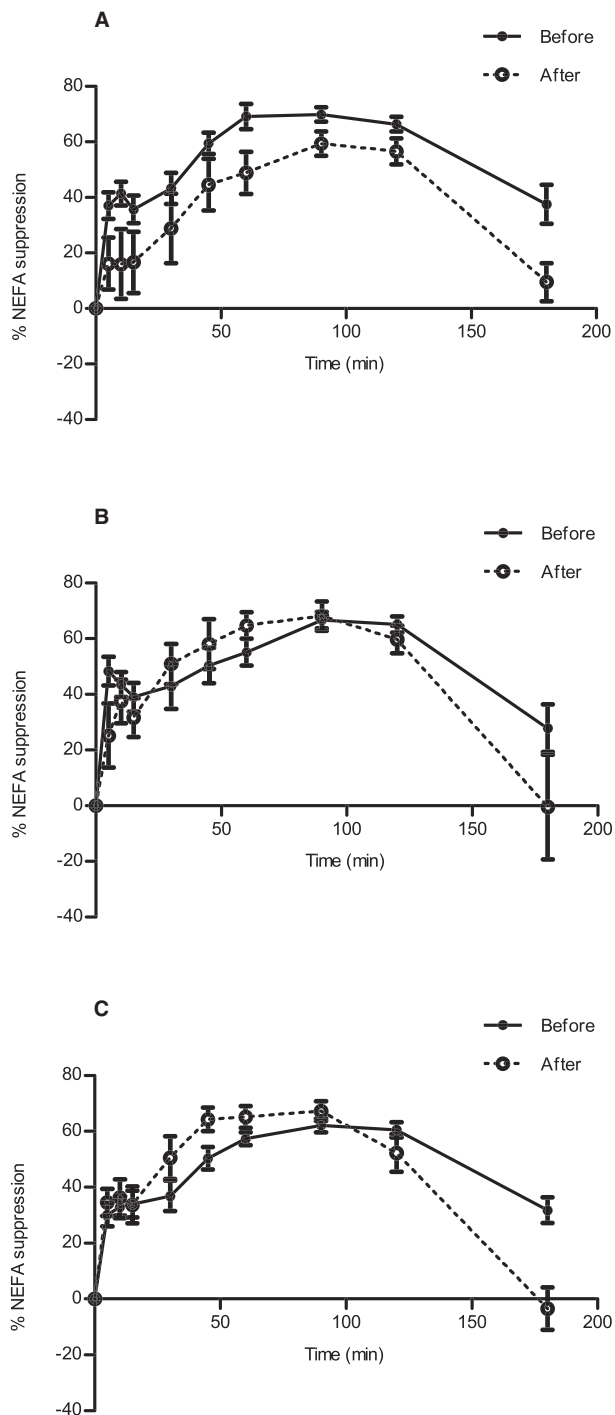
\*Significant change from pretreatment value versus change with placebo ( $P < .05$ ).

obese cats.<sup>2</sup> The increase in adiponectin with pioglitazone signifies activation of PPAR $\gamma$ , the molecular target of the TZDs; TZDs directly increase adiponectin expression through a PPAR $\gamma$  response element in the adiponectin promoter, and a dose-dependent increase in this cytokine is a reliable sequel of PPAR $\gamma$  agonist administration in humans and rodents.<sup>34</sup> Additionally, adiponectin is thought to mediate some of the effects of pioglitazone on insulin sensitivity.<sup>35</sup>

In the cats of this study, pioglitazone produced no change in energy expenditure or mean body weight, and the small decreases in RER are unlikely to be biologically significant. The calorimetry results are consistent with findings in obese, diabetic humans.<sup>36</sup> The lack of a change in body weight in the cats, which were fed a fixed ration, is also similar to reports in humans that weight gain (considered a dose-dependent

side effect of the TZDs) did not occur when pioglitazone was administered with a portion-controlled diet<sup>37</sup>

In addition to weight gain, the adverse effects profile of pioglitazone in humans includes fluid accumulation (manifest as peripheral edema or, more rarely, congestive heart failure), mild, reversible decreases in PCV, and increased risk of distal limb fractures in women.<sup>9</sup> PCV decrements were also observed in dogs and rodents during preclinical testing, and cardiac hypertrophy and thoracic effusion (considered secondary to volume overload rather than direct cardiac toxicity) occurred in these species at  $\geq 5$  times the human exposure or after durations of  $\geq 1$  year.<sup>38,39</sup> In view of these potential adverse effects, cats in this study were monitored by physical examination, laboratory evaluation, and echocardiography. All cats appeared healthy before study entry based on these criteria; however,



**Fig 3.** Percent non esterified fatty acid suppression (mean  $\pm$  SEM) versus time during intravenous glucose tolerance test in obese cats ( $n = 11-12$ ) before and after 6 weeks of oral placebo (A), 1 mg/kg pioglitazone (B), or 3 mg/kg pioglitazone (C).

myofiber disarray was identified in 1 cat that experienced cardiac arrest under sedation. This histologic abnormality is unlikely to have been caused by short-term pioglitazone administration, although exacerbation of pre-existing disease cannot be ruled out. In the

other obese cats, pioglitazone did not cause echocardiographic changes or clinical signs of toxicity at the dosages and duration used here. The clinical significance of the decreases in eosinophils and phosphorus is unclear, as phosphorus did not decrease below the reference range in any cat, and such changes do not appear to occur with pioglitazone in other species. Mild decreases in leukocyte count were observed in 1 study of pioglitazone in obese humans, in conjunction with decreases in other markers of systemic inflammation, but specific leukocyte fractions were not reported.<sup>40</sup> It must be noted that in the present study, no correction for multiple comparisons was applied during analysis of clinicopathologic or echocardiographic data, as minimizing type II error was considered a priority.

The dosages of pioglitazone used in this study were selected based on a previous pharmacokinetic evaluation, and were designed to bracket the range of human therapeutic concentrations.<sup>12</sup>  $C_{max}$  and  $AUC_{0-inf}$ , as assessed by limited sampling, were similar to concentrations achieved previously in cats and to concentrations considered therapeutic in humans,<sup>12,41</sup> but increases in these parameters between dosages were less than proportional. Subproportional increases in  $C_{max}$  and  $AUC_{0-inf}$  have also been reported for pioglitazone in dogs and rats, at dosages ranging from 0.1 to 30 mg/kg.<sup>41</sup> Either changes in bioavailability or changes in elimination with increasing dose may account for lack of dose proportionality; in the cats of this study, given the lack of a difference in half-life between dosages, a likely explanation for this phenomenon is a relative decrease in oral bioavailability, perhaps because of saturable absorption, at the higher dosage.

In summary, oral pioglitazone significantly improved insulin sensitivity and lowered plasma cholesterol and triglyceride concentrations in obese, insulin-resistant cats, after 6 weeks of daily dosing at 3 mg/kg. No changes in energy expenditure were noted, and no overt clinical toxicity attributable to pioglitazone was evident in otherwise healthy obese cats at this dosage and duration. Based on these results, further investigation of pioglitazone in diabetic cats, or in cats with other lipid metabolic disorders, might be warranted.

## Footnotes

<sup>a</sup> Purina ProPlan Chicken and Rice™, Nestlé Purina, St. Louis, MO

<sup>b</sup> Actos™, Takeda Pharmaceuticals North America Inc, Deerfield, IL

<sup>c</sup> Hill's a/d™, Hill's Pet Nutrition, Topeka, KS, and Purina DM™, Nestlé Purina

<sup>d</sup> D-lactose monohydrate, USP-NF, Fisher Scientific, Pittsburgh, PA

<sup>e</sup> Glucose (Trinder) Assay, Genzyme Diagnostics, Charlottetown, PEI

<sup>f</sup> Porcine Insulin RIA, Millipore, Billerica, MA

<sup>g</sup> NEFA-HR2, Wako Diagnostics, Richmond, PA

- <sup>h</sup> Human Adiponectin ELISA Kit, B-Bridge International Inc, Sunnyvale, CA
- <sup>i</sup> Multispecies Leptin RIA kit, Linco, St. Charles, MO
- <sup>j</sup> GraphPad Prism Version 5.00 for Windows, Graph Pad Software, San Diego, CA
- <sup>k</sup> R version 2.15.1; R Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org>
- <sup>l</sup> Phoenix WinNonlin version 6.1, Pharsight Corporation, Cary, NC

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Pioglitazone concentrations (mean  $\pm$  SEM) versus time in obese cats ( $n = 11$ – $12$ ) after 6 weeks of 1 or 3 mg/kg oral pioglitazone (Actos<sup>TM</sup>). Note logarithmic  $y$ -axis.

**Data S1.** Description of intravenous glucose tolerance testing and mathematical modeling of outcome variables in obese cats administered oral placebo, 1 mg/kg pioglitazone, and 3 mg/kg pioglitazone for 7-week periods in a crossover design.