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Safety and efficacy assessment of a GLP-1 mimetic: insulin glargine combination for treatment of feline diabetes mellitus

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ABSTRACT

A commonly used therapeutic strategy for type 2 diabetes mellitus (DM) in humans involves the use of synthetic incretin hormone-based therapies including exenatide, a glucagon-like pepetide-1 hormone agonist. Glucagon-like pepetide-1 agonists can be used alone or as an ancillary therapy with other agents, including insulin and oral antihyperglycemics. Little is known about the role of these therapies for DM in cats. Therefore, the primary objective of this study was to evaluate the safety and efficacy of short-acting exenatide combined with insulin, as compared to placebo and insulin for the treatment of DM in cats. Treatment with exenatide was well tolerated; only 2 cats developed side effects requiring dose reduction. Two cats (25%) went into diabetic remission while receiving exenatide and insulin, whereas remission was not reported during placebo treatment. The average change in the daily exogenous insulin dose was significant ($\beta = -0.56$ U/kg, 95% confidence interval, −0.96 to −0.15, $P = 0.007$), and the dose of insulin administered was lower during exenatide treatment. The average weight loss experienced on exenatide was significantly higher than on placebo ($\beta = 0.65$ kg, 95% confidence interval, 0.09–1.21, $P = 0.02$). There was no significant difference in any of the hormone concentrations evaluated for cats on exenatide vs placebo treatments. Overall, the treatment of diabetic cats with insulin and a fixed dose of exenatide was found to be safe. The weight loss and decreased exogenous insulin requirement experienced with exenatide treatment could be a significant benefit for overweight diabetic cats and warrants further evaluation.

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1. Introduction

Diabetes mellitus (DM) is a common endocrinopathy in cats, the incidence of which is increasing globally [1,2]. The pathophysiology of feline DM is not completely known; however, it closely resembles type 2 DM (T2DM) in humans. With T2DM in both species, obesity-induced insulin resistance, β-cell toxicity, and a decrease in the number of β-cells has been documented [3–6]. In both species, the ultimate goal of medical therapy is to achieve diabetic remission. The cornerstones of current treatment in cats include administration of exogenous insulin [7,8] as well as feeding a specialized low-carbohydrate, high-protein diet [9]. Unfortunately, many diabetic cats receiving this treatment combination do not achieve optimal glycemic control. In overweight cats, this may be due in part to weight gain secondary to the anabolic effects of insulin therapy [10]. Such weight gain may become problematic, as increasing body weight (BW) has clearly been documented to decrease insulin sensitivity and could decrease the chance of obtaining diabetic remission [11–13].

Recently, diminished secretion of incretin hormones including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) have been identified to play a role in the development of T2DM in people [14–16]. These gastrointestinal hormones are secreted in both humans and cats in response to ingestion of nutrients [15–19]. They are known as incretin hormones as both enhance glucose-stimulated insulin secretion [15–17]. This augmented insulin secretion is known as the "incretin effect", which refers to greater insulin secretion following isoglycemic administration of an oral vs intravenous glucose load [15,17]. It has been well documented that humans with T2DM still respond to exogenous GLP-1 with increased insulin secretion but that the effect of GIP is lost [20,21].

These discoveries have led to the development of GLP-based therapies for use in people with T2DM that offer benefits over other therapies, including the promotion of weight loss, decreased risk of hypoglycemia, and potential for β-cell mass expansion [14,22,23]. Incretin-based therapies for human T2DM include short- and long-acting GLP-1 mimetics that are given either as sole or adjunct therapy [14,22,23]. The most studied short-acting synthetic GLP-1 mimetics include liraglutide and exenatide. Exenatide has been shown to improve glycemic control by reducing fasting and postprandial glucose though promotion of glucose- or meal-dependent insulin secretion, restoration of the first-phase insulin response, suppression of glucagon secretion, delayed gastric emptying, and improved satiety [22–24]. In humans, the most common side effects of short-acting exenatide are mild nausea and vomiting, which generally resolve within weeks of starting therapy [23]. Other rare adverse effects include diarrhea, hypoglycemia, and possibly pancreatitis [23].

Preliminary work with various GLP-based therapies in cats suggests that administration of both short-acting and extended-release exenatide are safe in healthy lean [18,19,25–28] and obese [29] cats. Substantial weight loss has been reported with the short-acting formulation when administered to healthy lean and obese cats [19,29]. It has recently been reported that the use of extended-release exenatide in newly diagnosed diabetic cats is safe and does not result in weight gain when compared to placebo [30].

The primary objective of this study was to assess the safety of exenatide and the differences in glycemic control between cats with DM treated with either exenatide or placebo in combination with insulin glargine. This study also aimed to evaluate the effect of a GLP-1 mimetic on BW and serum concentrations of hormones involved in glucose homeostasis: insulin, glucagon, leptin, and adiponectin. We hypothesized that overweight diabetic cats treated with a combination of short-acting exenatide and insulin glargine would have improved glycemic control and experience greater weight loss compared with those treated with placebo and insulin glargine. In addition, treatment with exenatide was hypothesized to decrease serum leptin and glucagon concentrations and increase postprandial endogenous insulin and adiponectin concentrations compared to placebo.

2. Materials and methods

2.1. Animals

The University of Saskatchewan Animal Care and Use Committee approved all animal use, and clients signed a consent form agreeing to enroll their cat in this study. Eight client-owned diabetic cats were recruited from the patient population at the Veterinary Medical Center (VMC), including 4 spayed females and 4 neutered males with a median age of 12 yr (range, 6–15 yr). Six were domestic short hair cats, one was a domestic medium hair, and one was a Siamese cross. Body condition score (BCS) was determined based on a validated body condition scoring system [31], and only cats with a BCS $\geq$ 5/9 were eligible for enrollment. The median BCS at presentation was 6/9 (range, 5–7/9). The median BW of the cats on presentation was 6.35 kg (range, 5.7–7.5 kg).

The only medication that was being administered to any of the cats at the time of enrollment was insulin glargine. Cats were also only eligible to be included if they had been treated with insulin glargine twice daily (BID) for a minimum of 1 mo before enrollment and had a stable BW. Three cats were diagnosed with DM less than 6 mo before enrollment; 3 had been diagnosed between 7 and 9 mo before enrollment; 2 had been diabetic for several years before enrollment. Diabetic cats with stage 3 or higher chronic kidney disease based on the International Renal Interest Society staging system, neoplasia, uncontrolled hyperthyroidism, evidence of significant concurrent infection (ie, hepatitis, pyelonephritis, etc), or treatment with any medications known to influence glycemic regulation (ie, glucocorticoids) were excluded.

Throughout the study, owners administered the medications and closely monitored appetite, food and water intake and reported any clinical side effects at each visit. Owners were provided with both dry and wet formulations of the same commercial prescription high-protein, low-carbohydrate diabetic diet (Purina DM Diabetic Maintenance; Nestle Purina Pet Care Company, St Louis, MO, USA) and were instructed to transition their cat onto this new

diet over a 3 d period. Before enrollment, only one cat was being fed a diet specifically formulated for treatment of DM; the remainders were being fed a variety of over-the-counter diets. The amount fed per day for each cat was calculated using the formula $(70 \times \text{BW kg})^{0.75}$, divided as 2 equal meals, given 12 h apart. Cats were fed most of their daily caloric requirement as canned food with the remaining allotted calories provided by 1/4 of cup of dry food per day.

2.2. Study design

A double-blinded, randomized, placebo-controlled, crossover study design was utilized. The primary investigators (M.A.S. and E.C.S.) were blinded to the identity of treatment for all cats until the study was complete. Using a random number generator, each cat was assigned to initially receive either exenatide and insulin glargine or placebo and insulin glargine. Exenatide (0.25 mg/mL, Byetta, Eli Lilly) was removed from the prefilled pen and transferred to a sterile generic glass vial using aseptic technique. Each vile containing exenatide was labeled treatment A with instructions on the volume and frequency of administration (1 μg/kg subcutaneously [SQ] BID for 6 weeks). The placebo (0.9% sterile saline) was provided in an identical glass vial labeled treatment B with the same instructions related to volume and frequency. Both exenatide and placebo vials sent home with owners were prepared in a sterile fume hood by a licensed pharmacist. The daily dose of short-acting exenatide used was identical to that given to healthy cats by Seyfert et al [25]. To best ensure we were able to document a weight loss effect, if present, a treatment period of 42 d was chosen based on the 6% weight loss that occurred in healthy cats given exenatide SQ BID for a 28 d period [25]. Each owner was provided with a 12 weeks supply of 100 unit insulin syringes. The washout period between the 2 arms of the study was 6 weeks. In a previous study of healthy cats, the peak concentration of short-acting exenatide occurred 45 min after SQ injection, and serum concentrations were detectable in 3 of 5 cats 8 h after injection [19]. Based on these findings, a washout period of 42 d was considered more than ample for complete elimination of exenatide, and also for cats to regain any weight lost during the first arm of the study. After the washout period, cats received the alternate treatment. At the end of the study, owners were asked to return any remaining exenatide and placebo so compliance could be objectively assessed.

At the time of enrollment and again after the 6 weeks washout period between the two arms of the study, all cats were assessed using routine laboratory tests (complete blood count, serum biochemistry, serum total thyroxine, serum fructosamine, and urinalysis) along with peripheral blood pressure measurements and fundoscopic examinations. A urine culture was performed at the time of enrollment to screen for any possible urinary tract infections. Samples for routine laboratory testing were analyzed at a commercial laboratory (Idexx Laboratories, Markham, ON, Canada). Enzyme-Linked Immunosorbent Assay (ELISA) testing for feline leukemia virus and feline immunodeficiency virus (SNAP FIV/FeLV Combo Test, Idexx Laboratories, Markham, ON, Canada) confirmed negative retrovirus status in all enrolled cats. Thoracic radiographs and abdominal ultrasound were preformed on all cats before enrollment to exclude those with significant concurrent disease.

During the study, cats were presented to the VMC for evaluation 8 times (Fig. 1); during these visits, they were housed in a quiet room on their own. Packed cell volume, plasma total solids, blood urea nitrogen (Azostix Reagent Strips; Bayer Healthcare, USA), and fasting blood glucose (BG) concentration were obtained and recorded at the beginning of each visit along with the findings of a complete physical examination and assessment of BW. Owners were asked about their cat's clinical signs, including those that could indicate changes in glycemic regulation (ie, subjective degree of polyuria, polydipsia, and polyphagia) or that could relate to an adverse reaction to the exenatide (ie, vomiting, diarrhea, and anorexia) at each visit. Blood samples were collected for hormone analysis preprandially and postprandially, and a 12-h BG curve during which samples were obtained every hour was performed at each visit. One hand-held, point-of-care glucometer (AlphaTRAK2 Blood Glucose Monitoring System; Abbott Laboratories, Abbott Park, IL, USA) was used for all glucose measurements.

Owners were instructed to report signs consistent with hypoglycemia (ie, lethargy, weakness, decreased mentation or seizures), and an additional BG curve was performed at the VMC if such clinical signs were reported. Blood glucose

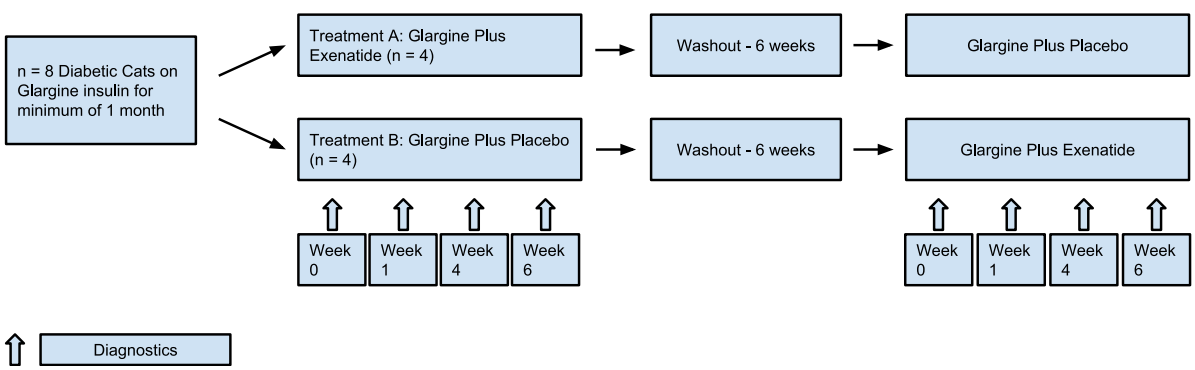

Fig. 1. Flow diagram outlining the study design.

curves, along with the owner's perception of glycemic control (improvement in polyuria, polydipsia, polyphagia, and weight loss), and serum fructosamine concentration were used to determine if changes of the insulin dose were required. If hypoglycemia was detected, and depending on the severity, the dose of glargine insulin was reduced by 25% to 50%. The insulin dose was decreased to 0.5 U per cat once daily for any cats that achieved remission, described as a normal fasting BG concentration and sustained euglycemia and/or hypoglycemia (3.5–6.3 mmol/L) during a 12-h BG curve and subsequent curves thereafter. These cats remained on the prescribed exenatide or placebo dose (1 μg/kg) and the diabetic diet unless adverse effects suspected to be related to the treatment protocol were reported.

2.3. Hormone concentrations

Blood samples for baseline hormone concentrations were collected at each sampling point in a fasted state (glucagon, leptin, adiponectin, and insulin) and 1 h postprandial (glucagon and insulin) following feeding a fixed amount (1/4 can) of wet food (Purina DM Diabetic Maintenance) and administration of insulin and exenatide or placebo. The size of this meal was representative of the cat's normal morning meal and was a standardized volume that could easily be consumed in 10 min permitting postprandial resampling 1 h later. Immediately following collection, blood samples were added to chilled tubes containing a protease inhibitor cocktail (10 μL/mL blood; P2714, Protease inhibitor cocktail, Sigma, MO, USA), Dipeptidyl peptidase-IV inhibitor (10 μL/mL blood; DPP-IV inhibitor, EMD Millipore, CA, USA), and EDTA (1 mg/mL blood) and kept on ice. Samples were centrifuged within 10 to 15 min of collection at 3,600 rpm for 9 min at −3°C and the resulting plasma stored at −80°C until analysis.

The adiponectin, leptin, glucagon, and insulin assays used in this study had all been previously validated for use in cats [11,27,32–35]. Briefly, assays were conducted following manufacturer's guidelines, and all samples were measured in duplicate and randomized so that there was equal representation of both treatment groups on each ELISA plate. To estimate interassay CV, pooled plasma was included in each ELISA assay plate. Leptin serum concentrations were measured in a single RIA with standards ranging from 0 to 50 ng/mL human equivalent (HE) (Multispecies Leptin RIA Kit, Millipore, MO, USA). The sensitivity of the assay was 0.8 ng/mL HE, and intraassay CVs were 13.5% and 5.0% for control sera of 3.3 and 12.6 ng/mL HE, respectively. Serum adiponectin was measured using a human high sensitivity ELISA (BioVender LLC, NC, USA) with standards ranging from 1 to 150 ng/mL. The feline samples were diluted 60× with the dilution buffer provided, while the kit control samples were diluted as per the manufacturer's instructions to a final dilution of 300×. The interassay CV for a control serum of 6.50 μg/mL was 1.0%, and the assay sensitivity is given as 0.47 ng/mL. Preprandial and postprandial glucagon concentrations were measured using a glucagon RIA (MP Biomedicals LLC, OH, USA). The intraassay and interassay CVs were found to be 8.9% and 12.0% and 2.9% and 3.4% for control sera with concentrations of 63.1 and 310.8 pg/mL, respectively. Feline insulin concentrations were measured using a feline insulin-specific ELISA (Feline Insulin ELISA, Mercodia AB Uppsala, Sweden) with standards ranging from 35.7 to 702 pg/mL. The intraassay and interassay CVs were 15.0% and 3.3% for control sera of 420.6 and 1,249.1 pg/mL, respectively. The assay sensitivity was 9.2 pg/mL. Positive controls (predetermined quantity of peptide) provided with the kits were tested with each assay, and the values obtained were within the quality control ranges provided by vendors. This assay has not been reported to have any cross-reactivity with exenatide in previous studies where both were measured [19,25,28,29].

2.4. Evaluation of glycemic control

Parameters of glycemic control evaluated during the study included serum fructosamine, fasting and 1 h postprandial BG concentrations, mean BG concentration for a 12-h BG curve, and the daily dose of exogenous insulin per kilogram of BW. Serum fructosamine (Prairie Diagnostic Services Ltd, Saskatoon, SK) and triglyceride concentration (Catalyst Dx Chemistry Analyzer, Idexx Laboratories, Markham, ON, Canada) were measured at the beginning of weeks 0, 4, and 6 of each treatment protocol. The other glycemic parameters mentioned were measured, and an owner's perception of diabetic control was recorded at each visit.

2.5. BW and adverse events

BW (kg) changes for exenatide vs placebo treatments were monitored throughout the study. Adverse events associated with either insulin therapy (hypoglycemia, weight gain) and/or exenatide/placebo (hypoglycemia, nausea, vomiting, anorexia, injection site reactions) were monitored and recorded by both the researcher and owner.

2.6. Statistical analysis

Statistical analysis was performed using a commercially available statistics software program (SAS ver 9.3, SAS Institute Inc Cary, NC, USA). The patient population, clinical, and laboratory test results were summarized using descriptive statistics with values presented as means and SDs or medians and range (minimum and maximum), depending on whether the data were normally or non-normally distributed, respectively. A Wilcoxon signed-rank test was used to examine the differences in baseline blood chemistry values of the cats after receiving exenatide vs placebo.

Generalized estimating equations (Proc Genmod, SAS ver 9.3, Cary, NC, USA) assuming a normal distribution were used to compare outcomes of interest between the 2 treatment protocols (exenatide vs placebo). Generalized estimating equations were needed to account for repeated measures on individual cats due to the crossover design and, in some models, for repeated measurements on individual cats within each treatment. A first-order autoregressive correlation structure was chosen as the best fit to the study design and data based on optimization of Quasilikelihood under the Independence model Criterion.

A fixed effect for measurement time was included in all models when there were multiple measurements within a single protocol. Time was evaluated as an ordered categorical fixed effect to avoid the assumption of linearity. Differences between specific time points were reported only if they were significant as changes between individual measurements within treatment were not part of the primary hypotheses for the study. Where protocol differences and time were both significant, first-order interactions were examined and reported if $P < 0.05$. For all parameters evaluated a $P \leq 0.05$ was accepted as significantly significant.

3. Results

3.1. Body parameters and adverse effects

Treatment with exenatide was well tolerated. Only 2 cats displayed adverse signs that required a decrease in dose of exenatide. The reported signs included anorexia (n = 2) and weakness associated with hypoglycemia (n = 1). After a temporary decrease in the dose of exenatide for 48 h, one-time administration of subcutaneous fluids (100 mL lactated ringers solution), and a single dose of the antiemetic Maropitant citrate (1 mg/kg SQ, Cerenia, Pfizer Animal Health, New York, NY, USA), the 2 affected cats resumed eating normally and showed no further gastrointestinal signs. Neither owner believed that the clinical signs were severe enough to warrant removal of their cat from the study.

Six cats lost weight while receiving exenatide (median: −0.72 kg, range, −1.4 to −0.3 kg), whereas 2 cats gained a minimal amount of weight (median 0.1 kg, range, 0.1–0.12 kg). Five cats gained weight while receiving the placebo (median: 0.48 kg, range, 0.14–1.14 kg), whereas 3 cats lost weight (median: −0.26 kg, range, −0.57 to −0.03 kg) (Fig. 2). The average weight loss experienced for cats receiving exenatide was significantly greater than that for placebo (β = 0.65 kg, 95% confidence interval (CI), 0.09–1.21, $P = 0.02$).

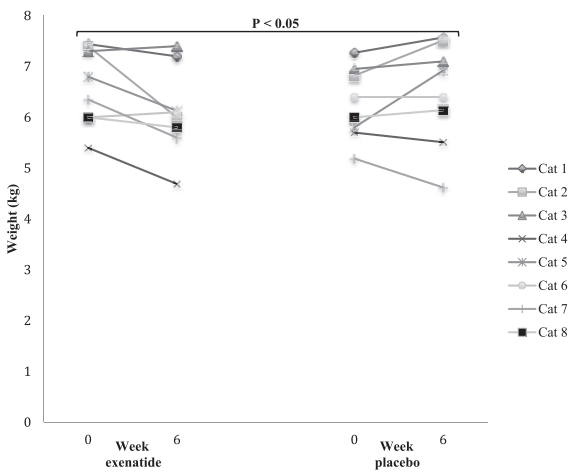

Fig. 2. Body weights of cats at the start and end of each treatment. The average weight loss was greater with exenatide as compared to placebo treatment (β = 0.65 kg, 95% CI 0.09 to 1.21, $P = 0.02$).

3.2. Hematological, biochemical, and glycemic parameters

Laboratory test results (complete blood count, serum biochemistry, and urinalysis) collected to screen for concurrent disorders before treatment with exenatide and placebo are summarized in Table 1. There was a statistically significant difference in the median creatinine ($P = 0.028$) and chloride ($P = 0.028$) concentrations between the 2 baseline time points for the 8 cats, but this was not considered to be biologically important.

Two of the 8 diabetic cats were diagnosed with International Renal Interest Society stage 2 (nonproteinuric, nonhypertensive) chronic kidney disease at the time of enrollment. The median urine-specific gravity for all 8 cats before administration of exenatide and placebo was 1.040 (range, 1.015–1.058) and 1.033 (range, 1.019–1.060), respectively. The median total T4 before administration of exenatide and placebo was 19.5 mmol/L (range, 15.5–25.3 mmol/L) and 20.1 mmol/L (range, 13.5–25.0 mmol/L), respectively.

Asymptomatic hypoglycemia (BG < 3.5 mmol/L) was documented in 5 of the 8 cats during the study; one while receiving exenatide, 3 while receiving placebo, and one during both treatment arms of the study. Two of the 8 cats (25%) went into diabetic remission while receiving exenatide compared to none while receiving placebo.

The median fasted BG concentration while cats were receiving exenatide or placebo was 15.8 mmol/L (range, 3.2–41.7 mmol/L) and 19.3 mmol/L (range, 4.1–40.0 mmol/L), respectively. The median 1 h postprandial BG concentration while cats were receiving exenatide or placebo was 13.6 mmol/L (range, 3.2–29.9 mmol/L) and 16.7 mmol/L (range, 3.6–28.5 mmol/L), respectively. Neither the average fasting (β = −0.51 pg/mL, 95% CI: −4.67 to 3.64, $P = 0.80$) nor 1 h postprandial BG concentrations (β = −0.91 pg/mL, 95% CI: −3.32 to 1.50, $P = 0.46$) were significantly lower across time when cats received exenatide compared to when they received placebo. The mean BG for cats during each scheduled 12-h BG curve while receiving exenatide and placebo was 12.3 mmol/L (SD = 7.0) and 13.9 mmol/L (SD = 7.0), respectively. Over the course of treatment, cats receiving exenatide did not have a significantly lower mean BG compared to when receiving placebo (β = −0.51 pg/mL, 95% CI: −5.11 to 4.08, $P = 0.83$).

The median dose of exogenous insulin glargine administered was 1.5 U/kg/d (range, 0.54–3.8 U/kg/d) on admission and 1.24 U/kg/d (0–2.2 U/kg/d) at the end of the study. The median change in the total daily dose (TDD) of insulin glargine given during the study was −0.3 U/kg/d (range, −1.0 to 1.1 U/kg/d). Four cats had a decrease in TDD of insulin while on exenatide, as compared to only one cat while on placebo (Fig. 3). The median change in TDD of insulin was −0.1 U/kg/d (range, −0.8 to 0.3 U/kg/d) for cats receiving exenatide and 0.1 U/kg/d (range, −0.3 to 1.4 U/kg/d) for placebo, respectively. The average change in the daily exogenous insulin dose was significantly lower during exenatide treatment compared to placebo (β = −0.56 U/kg, 95% CI: −0.96 to −0.15, $P = 0.007$).

Table 1
The laboratory parameters for the cats at the beginning of both treatment arms.

Parameter	Exenatide				Placebo				Reference interval	*P*-value
	Median	Range	Elevated (n)	Low (n)	Median	Range	Elevated (n)	Low (n)		
WBC ($\times 10^9$/L)	7.1	4.7–12.3	0	1	7	4.1–19.1	1	1	4.8–15.6	0.917
Neutrophils ($\times 10^9$/L)	5.9	2.5–7.7	0	0	4	2.3–7.8	0	1	2.5–12.5	0.345
Lymphocytes ($\times 10^9$/L)	2	0.9–6.3	0	1	1.6	0.7–2.0	0	1	1.5–7.0	0.5
Monocytes ($\times 10^9$/L)	0.3	0.0–0.9	1	0	0.2	0.0–0.4	0	0	0.0–0.85	0.498
Eosinophils ($\times 10^9$/L)	0.5	0.2–0.9	0	0	0.7	0.1–0.9	0	0	0.0–1.5	0.336
Basophils ($\times 10^9$/L)	0	0.0–0.2	1	0	0	0.0–0.0	0	0	0.0–0.1	0.109
RBC ($\times 10^{12}$/L)	10	7.7–11.9	3	0	9.75	9.1–11.9	1	0	6.0–10.0	0.345
Hemoglobin (g/L)	142	108–168	2	0	134	122–157	2	0	95.0–150	0.917
Hematocrit (L/L)	0.4	0.34–0.49	2	0	0.42	0.37–0.46	2	0	0.29–0.45	0.916
Total Protein (g/L)	75.5	72.0–82.0	0	0	80	71.0–91.0	1	0	60.0–85.0	0.115
Albumin (g/L)	36	32.0–41.0	1	0	36	32.0–41.1	1	0	26.0–40.0	0.68
Globulin (g/L)	40	29.0–46.0	0	0	44	36.0–50.0	0	0	25.0–51.0	0.116
Total bilirubin (μmol/L)	1.8	0.9–2.6	0	0	1.6	0.9–3.6	0	0	0.0–4.5	1
ALP (IU/L)	36.5	22.0–61.0	0	0	31	22.0–45.0	0	0	10.0–85.0	1
ALT (IU/L)	45	35.0–69.0	0	0	46	29.0–169	1	0	5.0–110	0.499
AST (IU/L)	25.8	12.0–50.0	0	0	20	10.0–47.0	0	0	5.0–71.0	0.865
Amylase (IU/L)	1,102	676–2,431	1	0	932	723–1,695	0	0	300–1,700	0.398
Lipase (IU/L)	99.5	21.0–150	0	0	142	72.0–203	1	0	0.0–200	0.176
GGT (IU/L)	0	0.0–1.0	0	0	0	0.0–2.0	0	0	0.0–6.0	1
Cholesterol (mmol/L)	6.1	3.2–7.7	2	0	5.8	4.0–10.4	2	0	2.0–7.0	0.237
Triglycerides (mmol/L)	0.7	0.4–12.2	3	0	1.4	0.7–5.0	4	0	0.1–1.2	0.735
Glucose (mmol/L)	15.9	9.1–27.4	8	0	20.9	8.0–33.9	7	0	3.9–8.0	0.31
Urea (mmol/L)	9	7.7–14.4	1	0	12.2	9.6–19.1	2	0	5.0–13.0	0.063
Creatinine (mmol/L)	129	98.0–202	2	0	109	80.0–127	0	0	49.0–173	0.028
Sodium (mmol/L)	152	147–153	0	0	148	147–152	0	0	145–158	0.058
Potassium (mmol/L)	4.4	3.9–5.0	0	0	4.5	4.0–5.0	0	0	3.7–5.8	0.799
Chloride (mmol/L)	115	111–119	0	0	110	109–118	0	0	109–130	0.028
Calcium (mmol/L)	2.6	2.3–2.7	0	0	2.4	2.4–3.0	1	0	2.0–2.9	0.595
Phosphorus (mmol/L)	1.4	1.2–1.6	0	0	1.4	1.0–1.6	0	0	0.9–2.8	0.581
Bicarbonate (mmol/L)	18	16.0–22.0	0	0	15	15.0–19.0	0	0	15.0–23.0	0.248
Magnesium mmol/L	0.9	0.7–1.0	0	1	0.9	0.8–1.0	0	0	0.8–1.0	0.498

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; RBC, red blood cells; WBC, white blood cell.

The median fructosamine for the 8 enrolled cats before treatment with exenatide and placebo was 581 μmol/L (range, 366–690 μmol/L) and 531 μmol/L (range, 283–778 μmol/L), respectively. The median change in fructosamine during treatment with exenatide was 24.5 μmol/L (range, −107 to 227 μmol/L) and −11 μmol/L (range, −316 to 58 μmol/L) during treatment with placebo. The change in serum fructosamine from the beginning to the end of each treatment arm was not significantly different between exenatide and placebo treatments (β = −23 μmol/L, 95% CI, −52 to 5.3, $P = 0.11$).

3.3. Hormones

There was no significant difference across time in the median fasting endogenous insulin concentration for exenatide vs placebo (β = −8.4 pg/mL, 95% CI, −57.2 to 40.4, $P = 0.73$) treatments. The median 1 h postprandial endogenous insulin concentration for diabetic cats while receiving exenatide was 94.4 pg/mL (range, 15.5–741.1 pg/mL) compared to 86.7 pg/mL (range, 25.2–628.3 pg/mL) for placebo. There was no significant difference across time in the 1 h postprandial endogenous insulin concentration for exenatide vs placebo (β = 32.3 pg/mL, 95% CI, −27.2 to 91.8, $P = 0.29$) treatments.

The median-fasted serum glucagon concentration for diabetic cats receiving exenatide or placebo was 182.3 pg/mL (range, 92.5–678.1 pg/mL) and 202.0 pg/mL (range, 112.6–711.4 pg/mL), respectively. The median 1 h postprandial serum glucagon concentration for diabetic cats receiving exenatide and placebo was 211.4 pg/mL (range, 113.3–739.5 pg/mL) and 201.1 pg/mL (range, 136.7–880.1 pg/mL), respectively. There was no significant difference between the mean fasting or 1 h postprandial

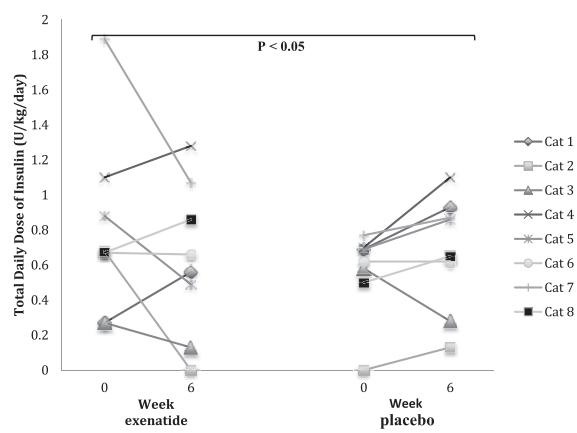

Fig. 3. Total daily doses (TDD) of exogenous insulin glargine administered to cats at the start and end of each treatment. The average change in the TDD was lower with exenatide compared to placebo treatment (β = −0.56 U/kg, 95% CI: −0.96 to −0.15, $P = 0.007$).

glucagon concentrations (β = −92.7 pg/mL, 95% CI, −195.2 to 9.80, $P = 0.08$) or any difference in the change from fasting to 1 h postprandial glucagon levels (β = 116 pg/mL, 95% CI, −41.7 to 275, $P = 0.15$) with exenatide compared to placebo treatments.

The median adiponectin concentration for diabetic cats receiving exenatide and placebo was 0.83 ng/mL (range, 0.4–1.2 ng/mL) vs 0.84 ng/mL (range, 0.5–1.2 ng/mL), respectively. Over time, the average adiponectin concentration did not significantly differ between cats treated with exenatide vs placebo (β = −0.007 ng/mL, 95% CI: −0.035 to 0.021, $P = 0.62$) (Fig. 4).

The median leptin concentration for diabetic cats receiving exenatide and placebo was 4.5 ng/mL human equivalent (HE) (range, 2.0–13.8 ng/mL HE) and 4.0 ng/mL HE (range, 2.1–10.5 ng/mL HE), respectively. Throughout the study, the average concentration of leptin measured in cats while receiving exenatide was not significantly different than when receiving a placebo (β = 0.29 ng/mL, 95% CI: −0.71 to 1.30, $P = 0.57$) (Fig. 5).

4. Discussion

This is the first report examining the safety and efficacy of a short-acting GLP-1 mimetic (exenatide) and insulin glargine combination for treatment of diabetic cats. Both short-acting and extended-release GLP-1 mimetics have previously been evaluated for safety, glycemic, and insulinotropic effects in healthy lean cats [18,19,25–28]. The insulinotropic activity of short-acting exenatide has also been evaluated in obese cats [29]. More recently the effects on glycemic regulation and safety of extended-release exenatide in combination with insulin glargine have been evaluated in newly diagnosed diabetic cats [30].

In this study in diabetic cats, as in other studies using healthy lean and obese cats, we found the use of exenatide did not result in a high frequency of side effects at a dose of 1 μg/kg SQ BID [19,25,28,29]. No injection site reactions were observed in our study. Adverse clinical signs were not reported while cats were treated with placebo but 2 cats experienced anorexia while receiving exenatide, which prompted a temporary dose reduction of 25% and 50%, respectively. In both cats, clinical signs were short-lived,

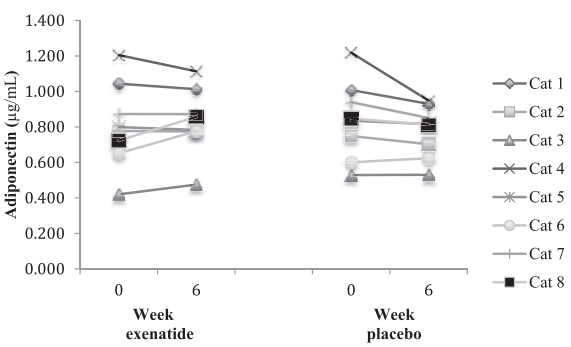

Fig. 4. Adiponectin concentrations at the start and end of each treatment. The average adiponectin concentration did not differ significantly between cats treated with exenatide compared to placebo (β = −0.007 ng/mL, 95% CI: −0.035 to 0.021, $P = 0.62$).

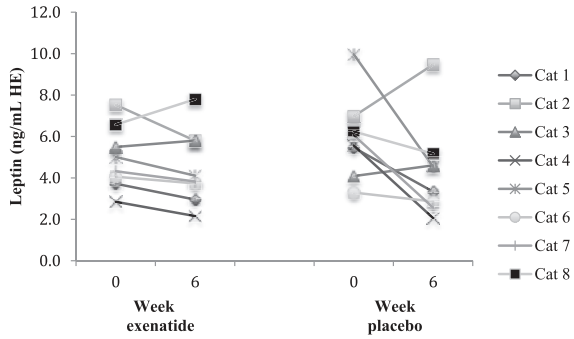

Fig. 5. Leptin concentrations of cats at the start and end of each treatment. The average concentration of leptin did not differ significantly between cats treated with exenatide compared to placebo (β = 0.29 ng/mL, 95% CI: −0.71 to 1.30, $P = 0.57$). HE, human equivalent.

and both returned to their original exenatide dose within 2 d with no further episodes reported by clients. The etiology of the anorexia was unknown in these 2 cases; however, in a placebo-controlled randomized study evaluating newly diagnosed diabetic cats (n = 30) treated with extended-release exenatide, anorexia was reported in 60% of cats [30]. The adverse effects seen in that study were self-limiting, and the incidence was not significantly different from cats treated with insulin glargine and placebo [30]. Pancreatitis has been reported in human patients with T2DM treated with both short-acting and extended-release exenatide [22–24]. It was not been reported in 30 diabetic cats treated with extended-release exenatide for 16 weeks [30] or in 12 obese cats treated for 12 weeks [29]. Should anorexia have persisted in our patients, measuring feline pancreatic lipase immunoreactivity concentrations and performing an abdominal ultrasound would have been considered to look for evidence of pancreatitis. Based on our study and work by others, we recommend that diabetic cats receiving exenatide in combination with insulin be monitored for anorexia.

We hypothesized, based on previous studies demonstrating weight loss with GLP-1 analogues in both humans and cats, that cotreatment with exenatide would promote weight loss in diabetic cats [14,19,29,36–39]. Weight loss in humans treated with GLP-1 analogues is secondary to promotion of satiety and delayed gastric emptying [39]. Six of 8 cats enrolled in this study lost weight while on the exenatide and insulin combination. Three of 8 cats also lost weight while receiving placebo and insulin; however, the average weight loss was significantly greater while receiving exenatide. Despite the low study power, weight loss appears to be associated with exenatide therapy through promotion of insulin glargine's efficacy. Additional support that pharmacologic doses of short-acting exenatide results in weight loss have recently been documented in a study where obese cats had decreased appetite while on therapy, which resulted in weight loss. [29]. The definitive reason for weight loss in our patients is unclear, but it is possible that this effect is secondary to decreased caloric intake. In our study, cats were managed at home, and therefore, the actual amount of food offered and consumed was not closely tracked. Cats in our study may also have lost

weight for other reasons including unappreciated chronic illnesses missed by preenrollment screening.

Hypoglycemia is a rare but serious side effect of treatment with GLP-1 analogues in combination with either exogenous insulin or oral antidiabetic medications such as sulfonylureas [14,15,24]. Asymptomatic hypoglycemia has been reported in both healthy lean and obese cats treated with short-acting exenatide, and in newly diagnosed diabetic cats treated with a combination of extended-release exenatide and insulin glargine [19,29,30]. In our study during 12-h BG curves, 5 of 8 cats had asymptomatic hypoglycemia documented (BG $<$ 3.5 mmol/L), one while receiving exenatide, 3 while receiving placebo, and in one cat during both treatment arms. Asymptomatic hypoglycemia is a known and common complication of insulin therapy, especially with insulin glargine in diabetic cats [13]. It is suspected that hypoglycemia was more likely induced by insulin therapy and not exenatide. Generalized weakness was reported in one cat that developed anorexia while on exenatide therapy; weakness was most likely attributed to hypoglycemia, based on a BG $<$ 3.5 mmol/L. While it cannot be ruled out that hypoglycemia was not caused in part by the exenatide, it is more likely attributable to insulin glargine since despite specific instructions to decrease the dose of insulin if anorexia was encountered, the owner had administered the regular prescribed dose that day. An initial period of BG monitoring to determine the effect of combined exenatide and insulin glargine therapy may allow for adjustments in the insulin dose to decrease the risk of hypoglycemia. If clinical hypoglycemia is detected with combination therapy, a reduction of the dosage of insulin and exenatide is recommended as there are concerns that further insulin administration will predispose to hypoglycemia.

The TDD of insulin (U/kg) administered was significantly lower during treatment with exenatide vs placebo. Improved glycemic control with adjunct exenatide therapy given with insulin in people with T2DM is hypothesized to be secondary to the glucose-lowering effects of exenatide. This, however, was not supported in our study, as we did not detect a significant reduction in the mean BG during a 12-h BG curve or serum fructosamine during treatment with exenatide compared to placebo. The lack of significant improvement in serum fructosamine and the mean 12-h BG curve over the 6 weeks of treatment with exenatide did not correlate in all cases with the cat owner's perceptions of glycemic control. Four of 8 noted subjective improvements that can be used to indicate glycemic regulation included decreases in polyuria and polydipsia despite a lack of significant changes in 12-h BG curves and serum fructosamine concentrations. In another study evaluating the effects of extended-release exenatide and insulin combination therapy as compared to a placebo and insulin combination in diabetic cats over 16 weeks, serum fructosamine concentrations also were not significantly different between groups; however, in both groups, the serum fructosamine dropped substantially [30]. Given these findings, serum fructosamine should not be evaluated as an independent measure of metabolic control as it is known to have significant variability between individual animals [40], but instead considered in combination with other assessments including BG and resolution of clinical signs.

Historically cats are less likely to go into diabetic remission if they have been treated for diabetes for longer than 6 mo [13]. In our study, 2 cats treated with exenatide achieved remission compared to none on placebo. Both cats had been diagnosed within 6 mo of enrollment. One cat, diagnosed and started on treatment approximately 2 mo before enrollment, was treated with exenatide and insulin during the first arm when he went into remission on weeks 4 of the study. The other cat that was diagnosed and started on treatment 1 mo before enrollment did not go into remission until entering into the second arm of the study and starting treatment with exenatide and insulin. Limitations of the study to evaluate remission and glycemic control were low power and the variability in time in which cats were diagnosed with DM and treated with insulin glargine before enrollment. It is possible and likely that the severity of T2DM and subsequent beta cell toxicity and endogenous insulin secretion differed in our population and may have played a factor in remission rates and glycemic control in our study. While it appears that treatment with exenatide was correlated with remission, a larger prospective study would be necessary to determine if exenatide in combination with insulin results in an increased likelihood of remission in cats vs insulin therapy alone. As suspected, our study suggests that diabetic cats treated with short-acting exenatide may require a lower TDD of insulin; hence, combination treatment could potentially reduce the insulin dose needed and make hypoglycemia less likely.

Interestingly, we found that the *P*-values for glucagon approached significance between treatment groups, and the information within the 95% CI suggests the potential for an increase in secretion of endogenous insulin and decrease in glucagon concentrations with exenatide treatment. This may imply that exogenous administration of GLP-1 had the expected insulinotropic effect and that the GLP-1 analogue inhibited glucagon secretion, playing a role in perceived glycemic regulation. Substantial heterogeneity in endogenous insulin secretion has been described in healthy, diabetic, and obese cats [27–29] and in healthy cats receiving exenatide [26]. This variability, along with only measuring the endogenous insulin concentration at one time point postprandially, could explain our lack of significant findings. Also while the dose of short-acting exenatide used in this study was 15 times greater than the dose approved for use in people and was sufficient to increase insulin secretion in previous studies involving healthy cats [18,25], it did not produce a significant change in insulin secretion in obese cats [29]. This suggests that overweight cats may be less responsive to GLP-1 mimetics. It is also possible that changes in the feline diabetic pancreatic beta cells require higher doses to induce increased insulin secretion or to overcome GLP-1 resistance [26,28,30]. The dose of liraglutide, an exogenous GLP-agonist, in adult obese patients with or without T2DM is 1.6 times greater than the max dose approved for treatment of T2DM [41]. Therefore, our exenatide dose may not have been adequate to produce the expected increase in endogenous insulin release in the overweight, diabetic cats used in this study.

Obesity-related peripheral insulin resistance may arise secondarily to the dysregulation of adipokines, which are

known to play a role in energy metabolism and glucose homeostasis [37,42]. Previous studies have found elevated leptin levels in both obese and insulin-resistant humans and cats, consistent with leptin resistance [37,42–44]. High leptin concentrations reduce the ability to regulate energy homeostasis, leading to further weight gain and insulin resistance [32]. Weight loss normalizes leptin levels, and therefore, serum leptin concentration is an indicator of fat mass [44]. By contrast, obese humans and cats, as well as diabetic cats have decreased adiponectin levels, and recent human studies indicate a potential link between low adiponectin levels, insulin resistance, and T2DM [45,46]. It was suspected that adiponectin would increase, and leptin decrease in diabetic cats treated with exenatide that lost weight compared to those treated with placebo. However, there was no significant difference in the adiponectin or leptin concentrations despite significant weight loss during treatment with exenatide. This could be due to the lack of a substantial enough weight loss to normalize leptin concentration, possibly from an insufficient dose of exenatide, or due to the low sample size. Until recently, adipokines had not been measured in diabetic cats during treatment when weight loss was intended, and therefore, the amount of weight loss needed to significantly change adipokine concentrations was not known. Adipokine concentrations, especially leptin, can also vary significantly between individuals and with sex, which could account for the lack of detectable difference between exenatide and placebo in this study. Although not directly comparable, the cats treated with exenatide in this study had leptin levels similar to what has been reported for clinically healthy lean cats (4.6 ng/mL HE) [35] and lower than what has been previously reported in obese cats (24.5 ng/mL HE) [32]. Adiponectin levels, however, were lower than previously reported in lean cats (3.0 ng/mL) [11] but similar to obese cats (1.0 ng/mL) [11] and obese client-owned cats treated with exenatide [29]. Our findings could be due to the enrollment of normal and overweight, rather than truly obese cats in this study, and not a lack of a treatment effect. This is possible because leptin is known to be a good indicator of fat mass. Low study power, variable weight loss, and inappropriate GLP-1 dose for diabetic patients could have also contributed to the lack of significant changes in adipokines seen in this study.

There are several limitations to this study that could influence our findings. Financial constraints limited our sample size and the resulting power to detect differences between the treatment and placebo protocols. In addition, there was variability in the patient population with regard to chronicity of diabetes and the intensity of its management before enrollment. Studies investigating the effects of incretin hormone analogues in humans with T2DM are generally longer (ie, many months), and the pharmacological effect of GLP-1 mimetics may be different in cats with beta cell glucose toxicity due to colocalization of GLP-R in the pancreatic beta cells.

5. Conclusions

In conclusion, this study reveals that a treatment protocol for diabetic cats including insulin glargine and a fixed dose of a GLP-1 mimetic did not have a high frequency of AE. Overweight diabetic cats receiving combination therapy experienced significant weight loss and a decrease in the TDD of insulin administered. However, in this group of 8 diabetic cats, there were no observed significant benefits on glycemic control when evaluating fructosamine and BG concentrations with the use of short-acting exenatide and insulin glargine at the dose or duration of therapy used in this study when compared to placebo and glargine.

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References

[1] Baral R, Rand J, Catt M, Farrow H. Prevalence of feline diabetes mellitus in a feline private practice [abstract] J Vet Intern Med 2003; 17:433–4.
[2] Prahl A, Guptill L, Glickman NW, Tetrick M, Glickman LT. Time trends and risk factors for diabetes mellitus in cats presented to veterinary teaching hospitals. J Feline Med Surg 2007;9:351–8.
[3] Nelson RW, Reusch CE. Animal models of disease: classification and etiology of diabetes in dogs and cats. J Endocrinol 2014;222:T1–9.
[4] Osto M, Zini E, Reusch CE, Lutz TA. Diabetes from humans to cats. Gen Comp Endocrinol 2013;182:48–53.
[5] Hoenig M. The cat as a model for human obesity and diabetes. J Diabetes Sci Technol 2012;6:525–33.
[6] Yano BL, Hayden DW, Johnson KH. Feline insular amyloid: association with diabetes mellitus. Vet Pathol 1981;18:621–7.
[7] Sparkes AH, Cannon M, Church D, Fleeman L, Harvey A, Hoenig M, Peterson ME, Reusch CE, Taylor S, Rosenberg D. ISFM consensus guidelines on the practical management of diabetes mellitus in cats. J Feline Med Surg 2015;17:235–50.
[8] Martin G, Rand J. Current understanding of feline diabetes: part 2, treatment. J Feline Med Surg 2000;2:3–17.
[9] Zoran DL, Rand JS. The role of diet in the prevention and management of feline diabetes. Vet Clin North Am Small Anim Pract 2013; 43:233–43.
[10] Reusch CE. Feline diabetes mellitus. In: Feldmean EC, Nelson RW, Reusch CE, Scott-Moncrieff JCR, Behend EN, editors. Canine & feline Endocrinology. 4 ed. St. Louis, MO: Elsevier Saunders; 2015. p. 289.
[11] Hoenig M, Thomaseth K, Waldron M, Ferguson DC. Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss. Am J Physiol Regul Integr Comp Physiol 2007;292:R227–34.
[12] Nelson EW. Diabetes mellitus. In: Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine-disease of the dog and cat. 7 ed. St. Louis, MO: WB Saunders Co.; 2010. p. 1796–816.
[13] Roomp PK, Rand JS. Evaluation of detemir in diabetic cats managed with a protocol for intensive blood glucose control. J Feline Med Surg 2012;14:566–72.
[14] João AL, Reis F, Fernandes R. The incretin system ABCs in obesity and diabetes - novel therapeutic strategies for weight loss and beyond. Obes Rev 2016;17:553–72.
[15] Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 2013;17:819–37.
[16] Irwin DM, Prentice KJ. Incretin hormones and the expanding families of glucagon-like sequences and their receptors. Diabetes Obes Metab 2011;13:69–81.

[17] Gilor C, Graves TK, Gilor S, Ridge TK, Weng HY, Dossin O. The incretin effect in cats: comparison between oral glucose, lipids and amino acids. Domest Anim Endocrinol 2011;40:205–12.
[18] Nishii N, Takashima S, Iguchi A, Murahata Y, Matsuu A, Hikasa Y, Kitagawa H. Effects of sitagliptin on plasma incretin concentrations after glucose administration though an esophagostomy tube or feeding in healthy cats. Domest Anim Endocrinol 2014;49:14–9.
[19] Gilor C, Graves TK, Gilor S, Ridge TK, Tge RM. GLP-1 mimetic exenatide potentiates insulin secretion in healthy cats. Domest Anim Endocrinol 2011;41:42–9.
[20] Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. Diabetologia 2002;45:1111–9.
[21] Højberg PV, Vilsbøll T, Rabøl R, Knop FK, Bache M, Krarup T, Holst JJ, Madsbad S. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia 2009;52:199–207.
[22] Frias JP, Edelman SV. Incretins and their role in the management of diabetes. Curr Opin Endocrinol Diabetes Obes 2007;14:269–76.
[23] Garg K. The role of basal insulin and glucagon-like peptide-1 agonists in the therapeutic management of type 2 diabetes – a comprehensive review. Diabetes Technol Ther 2010;12:11–24.
[24] Iltz JL, Baker DE, Setter SM, Keith Campbell R. Exenatide: an incretin mimetic for the treatment of type 2 diabetes mellitus. Clin Ther 2006;28:652–65.
[25] Seyfert TM, Brunker JD, Maxwell LK, Payton ME, McFarlane D. Effects of a glucagon-like Peptide-1 mimetic (exenatide) in healthy cats. Intern J Appl Res Vet Med 2012;10:147–56.
[26] Rudinsky AJ, Adin CA, Borin-Crivellenti S, Rajala-Schultz P, Hall MJ, Gilor C. Pharmacology of the glucagon-like pepetide-1 analog Exenatide extended-release in healthy cats. Domest Anim Endocrinol 2015;51:78–85.
[27] Hall MJ, Adin CA, Borin-Crivellenti S, Rudlinsky AJ, Rajala-Schultz P, Lakritz J, Gilor C. Pharmacokinetics and pharmacodynamics of the glucagon-like pepetide-1 analog liraglutide in healthy cats. Domest Anim Endocrinol 2015;51:114–21.
[28] Padrutt I, Lutz TA, Reusch CE, Zini E. Effects of the glucagon-like peptide analogues exenatide, exenatide extended-release, and of the dipeptidylpeptidase-4 (DPP-4) inhibitor sitagliptin on glucose metabolism in healthy cats. Res Vet Sci 2015;99:23–9.
[29] Hoelmkjaer KM, Wewer Albrechtsen NJ, Holst JJ, Cronin AM, Nielsen DH, Mandrup-Poulsen T, Bjornvad CR. A placebo-controlled study on the effects of the glucagon-like peptide-1 mimetic, exenatide, on insulin secretion, body Composition and adipokines in obese, client-owned cats. Gonzalez-Bulnes A PLoS One 2016;11: e0154727.
[30] Reiderer A, Zini E, Salesov E, Fracassi F, Padrutt I, Macha K, Stöcjle TM, Lutz TA, Reusche CE. Effect of the glucagon-like peptide-1 analogue exenatide extended release in cats with newly diagnosed diabetes mellitus. J Vet Intern Med 2016;30:92–100.
[31] Laflamme D. Development and validation of a body condition score system for cats: a clinical tool. Feline Pract 1997;25:13–8.
[32] Appleton DJ, Rand JS, Sunvold GD. Plasma leptin concentrations in cats: reference range, effect of weight gain and relationship with adiposity as measured by dual energy X-ray absorptiometry. J Feline Med Surg 2000;2:191–9.
[33] Strage EM, Holst BS, Nilsson G, Jones B, Lilliehook I. Validation of an enzymes-linked immunosorbent assay for measurement of feline serum insulin. Vet Clin Pathol 2012;41:518–28.
[34] Wewer Albrechtsen NJ, Hartmann B, Veedfald S, Windelov JA, Plamboeck A, Bojsen-Moller KN, Idorn T, Feldt-Rasmussen B, Knop FK, Madbad S, Deacon CF, Holst JJ. Hyperglucagonaemia analyzed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? Diabetologia 2014;57:1919–26.
[35] Shibata H, Sasaki N, Honjoh T, Ohishi I, Takiguchi M, Ishioka K, Ahmed M, Soliman M, Kimura K, Saito M. Feline leptin: immunogenic and biological activities of the recombinant protein, and its measurement by ELISA. J Vet Med Sci 2003;65:1207–11.
[36] German AJ, Ryan VH, German AC, Wood IS, Trayhurn P. Obesity, its associated disorders and the role of inflammatory adipokines in companion animals. Vet J 2010;185:4–9.
[37] Vilsbøll T, Chistensen M, Junker AE, Knop FK, Gluud LL. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ 2012;334:d7771.
[38] Dushay J, Gao C, Gopalakrishnan GS, Crawley M, Mitten EK, Wilker E, Mullington J, Maratos-Flier E. Short-term exenatide treatment leads to significant weight loss in a subset of obese women without diabetes. Diabetes Care 2012;35:4–11.
[39] Taylor K, Gurney K, Han J, Pencek R, Walsh B, Trautmann M. Exenatide once wkly treatment maintained improvements in glycemic control and weight loss over 2 years. BMC Endocr Disord 2011;11:9.
[40] Gilor C, Graves TK, Lascelles BD, Thomson AE, Simpson W, Halpern DS. The effects of body weight, body condition score, sex, and age on serum fructosamine concentrations in clinically healthy cats. Vet Clin Pathol 2010;39:322–8.
[41] Astrup A, Rössner S, Van Gaal L, Rissanen A, Niskanen L, Al Hakim M, Madsen J, Rasmussen MF, Lean MEJ. Effects of liraglutide in the treatment of obesity: a randomized, double-blinded, placebo-controlled study. Lancet 2009;374:1606–16.
[42] Radin MJ, Sharkey LC, Holycross BJ. Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. Vet Clin Pathol 2009;38:136–56.
[43] McMillan CJ, Zapata RC, Chelikani PK, Snead EC, Cosford K. Circulating concentrations of glucagon-like peptide 1, glucose-dependent insulinotropic peptide, peptide YY, and insulin in client-owned lean, overweight, and diabetic cats. Domest Anim Endocrinol 2016;54:85–94.
[44] Martin L, Siliart B, Dumon H, Backus R, Biourge V, Nguyen P. Leptin, body fat content and energy expenditure in intact and gonadectomized adult cats: a preliminary study. J Anim Physiol Anim Nutr 2001;85:195–9.
[45] Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930–5.
[46] Zapata RC, Meachem MD, Cardoso NC, Mehain SO, McMillan CJ, Snead ER, Chelikani PK. Differential circulating concentrations of adipokines, glucagon and adropin in a clinical population of lean, overweight and diabetic cats. BMC Vet Res 2017;13:85.