Pharmacology of the GLP-1 Analog Liraglutide in Healthy Cats

Thesis

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Abstract

GLP-1 is an intestinal hormone that induces glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion and increasing beta cell mass, satiety and gastric-emptying time. Liraglutide is a fatty-acid derivative of GLP-1 with a protracted pharmacokinetic profile that is used in people for treatment of type II diabetes mellitus and obesity. The aim of this study was to determine the pharmacokinetics and pharmacodynamics of liraglutide in healthy cats.

Hyperglycemic clamps were performed on days 0 (HGC) and 14 (LgHGC) in eight healthy cats. Liraglutide was administered subcutaneously (0.6 mg/cat) once daily on days 8 through 14. Compared to the HGC (mean ± SD; 455.5 ± 115.8 ng/L), insulin concentrations during LgHGC were increased (760.8 ± 350.7 ng/L; P = 0.0022), glucagon concentrations decreased (0.66 ± 0.4 pmol/L during HGC vs. 0.5 ± 0.4 pmol/L during LgHGC; P = 0.0089) and there was a trend towards an increased total glucose infused [median (range) of 1.61 (1.11 – 2.54) g/kg during HGC vs. 2.25 (1.64 – 3.10) g/kg during LgHGC; P = 0.087]. Appetite reduction and decreased body weight (9% ± 3; P=0.006) were observed in all cats.
Liraglutide has similar effects and pharmacokinetics profile in cats to those reported in people. With a half-life of approximately 12 hours, once daily dosing might be feasible, however significant effects on appetite and weight loss may necessitate dosage or dosing frequency reductions. Further investigation of liraglutide in diabetic cats and overweight cats is warranted.
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List of Abbreviations

DM Diabetes mellitus
GLP-1 Glucagon-like peptide 1
T2DM Type 2 diabetes mellitus
SGLT-1 Sodium-glucose co-transporter protein
GLUT Glucose transporter
GIP Glucose-dependent insulinotropic peptide
DPP-4 dipeptidyl peptidase 4
GLP-1R Glucagon-like peptide 1 receptor
PYY Peptide YY
Epac Exchange proteins activated by cAMP
PKA Protein kinase A
PKB Protein kinase B
IP3 Inositol triphosphate
VGCC Voltage-gated calcium channels
RyR Ryanodine receptors
NFAT Nuclear factor of activated T-cells
ADA American Diabetes Association

HbA1c Hemoglobin A1c

LEAD trials Liraglutide Effect and Action in Diabetes trials

SMBG Self-monitoring of blood glucose

PBG Postprandial blood glucose

FBG Fasting blood glucose

AACE American Association of Clinical Endocrinologists

T1DM Type 1 diabetes mellitus

AUC Area under the curve

TGI Total glucose infused

GRPP Glicentin-related polypeptide
Diabetes mellitus (DM) is a common endocrine disease that is estimated to affect 0.25-1% of domestic cats[1]. Hospital prevalence of DM in the cat increased 15.5 fold from 1970 to 1999[2]. DM in the cat closely resembles and serves as a model for Type 2 diabetes mellitus (T2DM) in people. T2DM in people and DM in cats are characterized by insulin resistance, relative insulin deficiency, and islet amyloid depositions [3, 4]. Risk factors for DM in cats include obesity [5], male sex and increasing age [6]. A genetic influence has been purposed for Burmese cats because of the increased incidence of DM in this breed in the United Kingdom, Australia, and New Zealand, however the causative gene has not been identified [1, 7, 8]. Currently, treatment of DM in cats is focused on long acting insulin therapy in combination with dietary modifications. It is reported that 25% to 100% of cats diagnosed with T2DM will go into clinical remission. Practically, clinical remission likely occurs in approximately 50% of cats diagnosed with T2DM, and is dependent upon the duration of hyperglycemia prior to onset of treatment [4, 9-12].
Despite advances in monitoring, insulin therapy and oral antidiabetic drugs, many human and veterinary patients with DM fail to reach adequate glycemic control and often these treatments result in weight gain. In addition, most human patients will go on to develop cardiovascular and renal complications secondary to DM. A search for treatment aimed specifically at the underlying pathophysiology behind these deadly and costly diseases is warranted [13]. Incretin-based therapy for T2DM was first introduced in 2005 and has quickly become a widely used adjunctive therapy for T2DM. The American Diabetes Association recommends use of a glucagon-like peptide-1 receptor (GLP-1R) agonist as one of five additional therapeutic drugs to consider when lifestyle change and the drug metformin fail to achieve good glycemic control in patients with T2DM [14]. Benefits of incretin therapy for T2DM in people include: restoration of glucose sensitivity of pancreatic β-cells, induction of glucose-dependent stimulation of insulin secretion while simultaneously suppressing glucagon secretion (and thereby gluconeogenesis), inhibition of β-cell apoptosis and promotion of β-cell neogenesis, as well as inhibition of gastric secretion and motility, slowing of gastric emptying and enhancing satiety peripherally and centrally, with resultant weight loss. Recently, non-glucose-dependent benefits of incretin therapy in cardiovascular health in people have been recognized, and research continues into possible neuropathic effects of incretin hormones [15]. Here we review the pathophysiology of glycemic control with focus on incretin hormones, we review the available veterinary literature on incretin hormone
therapy, and we report, for the first time, the pharmacology of liraglutide, a GLP-1 (glucagon-like peptide-1) analog, in healthy cats.
Chapter 2: The Incretin Effect

Incretins are hormones released from the gastrointestinal tract during ingestion of a meal. The term “incretin effect” describes the greater release of insulin that occurs when glucose is administered orally compared to intravenously. The first described incretin, glucose-dependent insulinotropic peptide (GIP), was isolated by John C. Brown in 1971. GIP was later found to be synthesized and released from enteroendocrine cells (K cells) located in the duodenum and jejunum[16] in dogs, rats, pigs and humans in response to ingestion of glucose and fats[17], and to a lesser degree amino acids[18]. K cells in cats appear to be abundant throughout the entire intestinal tract, with the highest numbers in the ileum. GIP secretion is stimulated by amino acids and fat in cats but in contrast to other species, it is not stimulated by glucose [19]. GIP appears to be involved in many aspects of metabolism including stimulation of glucagon secretion, sensitization of insulin action in adipose tissue and a variety of effects in pancreatic beta cells. GIP effects on beta cells overlap with those of the other incretin, glucagon-like peptide-1 (GLP-1), and include: glucose-dependent stimulation of insulin secretion, promoting β-cell proliferation, and reducing β-cell apoptosis [20, 21]. The potential role of GIP receptor antagonists and agonists in obesity and diabetes mellitus (respectively)
has been extensively examined. In DM, β-cells become insensitive to the positive effects of GIP while its negative effects on metabolism (e.g. promoting glucagon secretion and weight gain) are unaffected by DM. Thus, GIP receptor agonists are not good candidates for treatment of DM [22].

Glucagon-like peptide-1 (GLP-1), on the other hand, has been extensively studied as a tool in the treatment of T2DM at supraphysiologic doses. In health, GLP-1 is produced in the enteroendocrine L cells of the distal small intestine and ascending colon of rats, pigs and humans and is secreted in response to a mixed meal [23]. In the dog, L cells are mainly found in the jejunum and ileum and are stimulated by GIP in a paracrine fashion, but are unaffected by glucose [24, 25]. A recent study demonstrated that L cells appear to be present most abundantly within the ileum of cats, but can also be found throughout the entire intestines in higher numbers than other species [19]. In addition to secreting GLP-1, L cells also secrete GLP-2 and peptide YY (PYY) both of which play multiple roles in intestinal health and satiety but are not incretins [19]. GLP-1 and GLP-2 are encoded by the proglucagon gene. This gene is expressed in a variety of tissues but its post-translational cleavage results in production of GLP-1 and GLP-2 largely in L cells [22]. GLP-1 is also produced in the central nervous system, namely the hypothalamus and brainstem [26]. Effects of GLP-1 found within the central nervous system include metabolic and neuroprotective actions which continue to be under investigation for future novel treatment modalities [27]. Multiple forms of GLP-1 are recognized in humans with the highest circulating concentration being GLP-1 (7-36), also known as the
COOH-terminally amidated form. The other GLP-1 form, GLP-1 (7-37), is glycine extended and is found circulating in smaller amounts [28]. Characterization of the forms of GLP-1 in cats has not, to date, been reported.

Endogenous GLP-1 is quickly degraded in the body by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP-4). GLP-1 is degraded to GLP-1 (9-36)NH₂ and GLP-1 (9-37) by removal of the first two N-terminal amino acids, a process that takes approximately 60 to 120 seconds [22]. DPP-4 is found in multiple tissues and cell types including: the kidney, lung, adrenal gland, liver, intestine, spleen, testis, pancreas, CNS, lymphocytes and macrophages. DPP-4 is abundant in endothelial cells, so much so that at least half of the GLP-1 that enters the portal system is degraded before it enters the systemic circulation [15]. GLP-1 (9-36) has no insulinotropic effect and is eliminated by the kidneys (63). In addition to being degraded by DPP-4, GLP-1 is also degraded by a zinc metallopeptidase (neutral endopeptidase 24.11 or NEP-24.11), high levels of which are found in the kidney [29]. Studies have shown that the elimination rates for GLP-1 and its metabolites are similar in human patients with and without T2DM [30].

Incretins and Glycemic Regulation

The ultimate goal of glucose homeostasis is to ensure constant and sufficient delivery of energy to the brain in face of fluctuations in energy supply to the body as a whole.

Normal regulation of blood glucose encompasses control of food intake (including hunger and satiety), control of digestion and absorption of ingested carbohydrates, regulation of energy storage in the form of glycogen in the liver and muscles and fat in
adipose tissue, regulation of glucose production and release from the liver, and regulation of glucose delivery to peripheral tissues. After digestion and absorption, glucose is released from enterocytes into the portal venous system. As the portal blood traverses the hepatic sinus, glucose is transported into hepatocytes by the non-insulin dependent glucose transporter-2 (GLUT2). Glucose is then trapped inside the hepatocyte by phosphorylation into glucose-6-phosphate, a process that is facilitated by the enzyme hexokinase. In hepatocytes, insulin and glucagon have opposite effects that are mediated by cAMP. Insulin decreases cAMP in hepatocytes while glucagon increases cAMP. Decreased cAMP concentrations leads to activation of hexokinase, increasing the likelihood of trapping glucose in the liver. Decreasing cAMP also leads to decreased activity of Glycogen Phosphorylase (thus inhibiting glycogen breakdown), inhibition of Fructose 2-6 Bisphosphatase and stimulation of Phosphofructokinase-1 (thus promoting glycolysis and inhibiting gluconeogenesis). Insulin and glucagon do not affect GLUT2 which serves to transport glucose into hepatocytes.

Insulin secretion is primarily regulated by blood glucose concentrations. Other stimuli and modifiers of insulin secretion include nutrients (e.g. amino acids and free fatty acids), gastrointestinal hormones (including gastrin, cholecystokinin, and secretin), and neuropeptides [including acetylcholine (parasympathetic activation), and adrenaline (either β-adrenergic stimulation or alpha-adrenergic inhibition)] [31].

Of special interest are the pathways in which incretin hormones potentiate insulin secretion from pancreatic β-cells. Following facilitated diffusion through the GLUT2
transporter, glucose is phosphorylated by glucokinase to glucose-6-phosphate and enters glycolysis and then oxidation in mitochondria. This leads to an increase in the ATP to ADP ratio. This change in the ATP:ADP ratio decreases the open probability of $K_{\text{ATP}}$ channels leading to decreased leakage of potassium and membrane depolarization. Depolarization of the membrane leads to opening of voltage-gated calcium channels (VGCC) and, in slight delay, to opening of voltage-gated potassium ($K_v$) channels. Calcium enters through the calcium channels into the $\beta$ cells down its concentration gradient. The increase in intracellular calcium leads to release of insulin. The signal is terminated by leakage of potassium through the $K_v$ channels. Intracellular calcium is also increased by $K_{\text{ATP}}$ channel-independent mechanisms which are augmented in the presence of GLP-1. Binding of GLP-1 to its receptor on the $\beta$ cell results in activation of adenylyl cyclase (AC). Activation of AC leads to increased cAMP which activates the Epac and PKA (protein kinase A) pathways. PKA inhibits the $K_v$ and $K_{\text{ATP}}$ channels on the cell membrane resulting in greater membrane depolarization and influx of calcium through VGCC. Intracellular calcium stores [in the endoplasmic reticulum (ER)] release calcium in response to increased concentrations of intracellular calcium secondary to opening of VGCC but also in response to increased IP3 (mediated by PKA) and in response to the Epac signal which stimulates the Ryanodine Receptor (RyR) in the ER. Finally, stimulation of the Epac and PKA pathways also directly facilitates exocytosis of the insulin-containing vesicles. These effects of GLP-1 in the pancreatic beta cells lead to glucose-dependent stimulation of insulin secretion and increased sensitivity of the beta cells to
glucose. GLP-1 also increases *insulin* gene transcription and biosynthesis through both PKA and PKA-independent pathways [32], and stabilizes β-cell insulin stores by increasing mRNA stability[33].

Protection from apoptosis for β-cells is important in the setting of T2DM where toxicities from increased cytokines and lipids, in addition to hyperglycemia, can lead to increased β-cell death [34]. GLP-1 directly supports β-cell health by both increasing β-cell mass through stimulation of proliferation and neogenesis of beta cells (which includes differentiation of pancreatic ductal cell stem cells), as well as inhibition of apoptosis [34]. GLP-1 acts by multiple pathways to stimulate β-cell proliferation and survival including the PKA (through cAMP), and PI-3K/PKB pathways. The latter results in increased expression of PDX-1, (pancreatic and duodenal homeobox 1, also known as insulin promoter factor 1) [34]. Pdx1 is a transcription factor that is highly conserved across species, and that is necessary for normal pancreatic development and β-cell maturation [35]. Pdx1 targets many genes, including those for proinsulin and glucokinase. In addition to increasing expression of Pdx1, GLP-1 also enhances binding of the Pdx1 to the insulin gene promoter and stimulates differentiation of exocrine pancreatic cell lines toward a β-cell phenotype. Pdx1 also plays a role in repressing glucagon secretion from α-cells [35]. Another pathway by which GLP-1 promotes β-cell proliferation and improved function is through calcium/calcineurin/NFAT (nuclear factor of activated T-cells) signaling [36]. GLP-1 activates β-catenin/T-cell factor-like 2 (TCF7L2)-dependent Wnt signaling in yet another pathway which promotes β-cell
proliferation. In addition, TCF7L2 plays a role in insulin gene expression as well as insulin secretion in mature β-cells [37], and has been identified as an important T2DM risk allele in people [38]. Interestingly, TCF7L2 also mediates GLP-1 secretion from L cells.

Glucagon is the primary counter-regulatory hormone to insulin, and is responsible for promoting glycogenolysis, gluconeogenesis (as explained above), and release of glucose from the liver in order to prevent hypoglycemia in the fasted state. Glucagon is secreted by the α-cells of the pancreas. After secretion, glucagon is carried in the blood via the portal system to bind to glucagon receptors on the hepatocytes. Binding of glucagon to its G protein-coupled receptor leads to increased conversion of ATP to cAMP (counteracting the effects of insulin in the hepatocyte as explained above). Importantly, glucagon stimulates the activity of glucose-6 phosphatase, which releases the phosphate and enables glucose to exit the hepatocytes. Stimulation of release of glucagon from α-cells occurs primarily in response to hypoglycemia, but also in response to release of epinephrine and in the presence of increased blood concentrations of arginine, alanine, acetylcholine and cholecystokinin [31]. Glucagon secretion is also stimulated by GIP although this effect of GIP is balanced by its effect on augmenting insulin secretion. The release of glucagon is inhibited by insulin, somatostatin from the δ-cells of the pancreas, and increased fatty acids and keto acids in the blood. Through its effects on insulin secretion, GLP-1 plays an important role in the suppression of glucagon secretion. Importantly, the suppressive effect of GLP-1 on glucagon secretion is lost during hypoglycemia [34, 39].
GLP-1 reduces food intake and increases satiety in healthy people [40, 41] and in patients with T2DM [42]. The methods by which it does so are multifactorial, with both signals of satiety and delayed gastric emptying contributing to decreased intake. GLP-1 decreases gastric acid secretion and slows the rate of gastric emptying [43, 44]. GLP-1 participates in the “ileal brake” which is a feedback mechanism in which the presence of nutrients within the ileum decreases gastric motility and emptying [41, 44, 45]. The above effects lead to slowing of introduction of food into the intestinal tract and thereby reduction of postprandial blood glucose (PBG) excursions. The delay in gastric emptying may be the most important contribution of GLP-1 in controlling PBG (more important than potentiation of insulin secretion) [46]. These effects seem to be mediated through peripheral stimulation of the vagus nerve which relays sensory information to the brain [15]. GLP-1 also inhibits food intake in rodents, and given long-term, inhibits weight gain through reduction of meal size when given centrally or peripherally [47–50]. GLP-1 receptors have been located in the vagus nerve, as well as the central nervous system, especially the brainstem and hypothalamus [51, 52]. When not bound to larger proteins, GLP-1 can cross the blood-brain barrier. Interestingly, a synthetic conjugate of albumin and exendin-4 (a GLP-1 analog), which was too large to cross the blood-brain barrier was shown to retain its GLP-1 effects. Specifically, when this synthetic protein was injected peripherally into Glpr −/− mice central nervous system stimulation still occurred including decreased gastric emptying, decreased food intake, and weight loss thereby continuing to give credence to the ability of GLP-1 to stimulate...
these physiologic changes through peripheral stimulation[53]. Glpr -/- mice are also noted to have fasting hyperglycemia [54]. While GLP-1’s effects are mainly attributed to controlling PBG, fasting hyperglycemia in these knockout mice implied that it may have more of a contribution to FBG than previously recognized. This effect was confirmed in a human study in which infusion of GLP-1 in subjects with T2DM resulted in near normalization of FBG and PBG [55].

GLP-1 receptors are 7-transmembrane-spanning, heterotrimeric G-protein-coupled receptors, and can be found in multiple tissues including: α-cells, β-cells, δ-cells, lung, heart, kidney, stomach, intestine, pituitary, skin, vagus nerve, and the CNS [15]. Of particular interest are GLP-1Rs found in pancreatic ductal cells and thyroid cells [56, 57]. Down-regulation of GLP-1 receptors occurs with glucocorticoids therapy as well as high glucose, activation of PKC, and GLP-1, and may be up-regulated with treatment with DPP-4 inhibitors.

Reduced Incretin Effect in T2DM

Augmentation of insulin secretion through GLP-1 binding to its receptor on the pancreatic β-cell contributes at least 50-70% of insulin secretion following a meal [15], outlining the importance of maintaining, regaining, or effectively replacing the incretin effect in T2DM [34]. The incretin effect is impaired in T2DM in humans, and GLP-1 concentration is lower in people with obesity, T2DM, insulin resistance, and glucose intolerance [58-61]. This loss of incretin effect is mainly documented in studies in which restoration of incretin levels and actions are documented following improved glycemic
control with anti-diabetic drugs such as metformin or other biguanides [62], as well as insulin therapy [63]. Metformin is currently considered the first line therapy for T2DM in people in which lifestyle change alone is ineffective. It is an oral antidiabetic drug of the biguanides class which increases insulin sensitivity and reduces gluconeogenesis [64]. While metformin’s ability to restore incretin levels were noted over a decade ago, recent studies have demonstrated that metformin does so in part by increasing GLP-1 concentrations through stimulation of the expression of glucagon in addition to enhancing secretion of GLP-1 from L cells [65]. The strong effect of body mass index on GLP-1 response has been shown in many large studies [59, 61, 66-68], and decreased GLP-1 response to oral glucose in the oral glucose tolerance test (OGTT) was demonstrated in ten healthy males with drug-induced insulin resistance, a high-calorie diet, and relative physical inactivity [69]. Supraphysiologic doses of GLP-1 have been demonstrated to be effective in restoring the incretin effect in patients with T2DM [70, 71]. As the effects and release of GLP-1 are glucose-dependent, the risk of clinically relevant hypoglycemia with use of GLP-1R agonists is low [72].

American Diabetes Association (ADA) Therapeutic Goals

The ADA plays a pivotal role in standardizing recommendations for diagnosis, therapy and therapeutic goals of T2DM in people. The AMA 2014 guidelines for therapeutic goals will be briefly summarized as they are directly pertinent to interpreting the results of clinical studies on the use of incretin-based therapies in people, which will allow for some extrapolation and direction to studying incretin-based therapy in cats.
Monitoring of T2DM in people is largely based on two methods of monitoring, Hemoglobin A1c concentrations (HbA1c) and self-monitoring of blood glucose (SMBG).

In clinical trials assessing response to incretin-based therapy, significant decreases in HbA1c are reported. Specifically, these decreases resulted in a significant number of patients reaching the AMA therapeutic goal of ≤6.5% (in patients at low risk for hypoglycemic events) or <7% for the remainder of the diabetic population. HbA1c represents the average amount of glycated hemoglobin in a red blood cell over the last three months. The percentage of glycated hemoglobin corresponds with the average concentration of blood glucose. For example, a HbA1c of 6% corresponds with an average blood glucose of 126 mg/dl, and a HbA1c of 7% corresponds with an average blood glucose of 154 mg/dl [73]. With this background knowledge, the evidence of success of incretin-based therapies for the treatment of T2DM in people will be reviewed.

Incretin-based Therapy in Humans with T2DM

In humans there are currently two therapeutic modalities aimed at reestablishing the incretin effect: GLP-1R agonists and DPP-4 inhibitors. Initial studies looking at incretin therapy utilized native GLP-1 and found that continuous subcutaneous administration of GLP-1 in patients with T2DM resulted in significant reductions in fasting and postprandial glucose, fructosamine, and hemoglobin A1c, as well as weight loss, inhibition of gastric emptying, and reduced levels of free fatty acids [55]. GLP-1R agonists that are altered in such a way to prevent rapid degradation by DPP-4 were
developed in order to circumvent the need for continuous subcutaneous administration of native GLP-1. The three GLP-1R agonists currently approved for use in people today are exenatide (Byetta®; Eli Lilly & Co, Indianapolis, IN, USA), exenatide extended-release (Bydureon®; Eli Lilly & Co, Indianapolis, IN, USA), and liraglutide (Victoza®, Novo Nordisk, Copenhagen, Denmark).

Alternatively, therapy can be directed at decreasing available DPP-4 thereby extending duration of action of endogenous GLP-1. These drugs are called DPP-4 inhibitors, or gliptins, the first of which was sitagliptin (Januvia®, Merck & Co., Whitehouse Station, NJ, USA) which was approved by the FDA in 2006. Other drugs in this class that have been approved by the FDA include: saxagliptin (Onglyza®, Bristol-Myers Squibb Co., Princeton, NJ, USA), linagliptin (Tradjenta®, Eli Lilly & Co, Indianapolis, IN, USA), and alogliptin (Nesina®, Takeda Pharmaceutical Company, Osaka, Japan). Advantages of DPP-4 inhibitor therapy (sitagliptin as an example) includes oral bioavailability of the drug (rather than injectable) [74], effectiveness at decreasing plasma glucose and improving HbA1c by as much as 0.5%, as well as decreasing fasting plasma glucose when used as monotherapy [75, 76] (or more when used in combination with other antidiabetic drugs [77-80]), and increasing postprandial GLP-1 levels [75]. A major disadvantage of DPP-4 inhibitors is that they are known to decrease secretion of endogenous GLP-1, thereby losing the β-cell protective effect of GLP-1 as well as the glucose-lowering ability of GLP-1 [81]. In addition, they do not seem to delay gastric emptying, and their main effects appear to be mediated through control of FBG [82-85].
The gliptins are, in general, considered weight neutral drugs [86]. One theory as to why the gliptins are weight neutral while GLP-1R agonists promote weight loss is that supraphysiologic blood concentrations of GLP-1 are needed in order to have the noted effects of GLP-1R agonists including delayed gastric emptying and increased satiety.

Fasting, endogenous GLP-1 concentrations average 10 pmol/L [87]. Gliptins, by inhibiting DPP-4 and preventing degradation of GLP-1, thereby allowing GLP-1 levels to remain at postprandial levels which are approximately two to three fold fasting levels [88, 89]. In contrast, a 0.6 mg dose of liraglutide given to an 80 kg man would result in GLP-1 levels of approximately 6000 pmol/L (range 3000-9000 pmol/L) [90]. Note that this is the starting dose of liraglutide given for one week prior to titration upward of the dose, as the beneficial effects of improved glycemic control and weight loss are not observed until an average daily dose of liraglutide of 1.2 mg or 1.8 mg [91], and a dose of approximately 1 mg to healthy adults resulted in a mean GLP-1 level of 9000 pmol/L (range 6000-12000 pmol/L). Thus, it seems likely that supraphysiologic doses are necessary to optimize glycemic control and contribute to weight loss.

Liraglutide Pharmacodynamics in People

Liraglutide (Victoza®; Novo Nordisk, Copenhagen, Denmark) is a human GLP-1 analog that is administered subcutaneously in people once daily, at any time of the day. Liraglutide shares a 97% sequence identity with endogenous GLP-1. Liraglutide differs from endogenous GLP-1 by the addition of a C-16 fatty acid (palmitoyl acid) which is attached to lysine at position 26 via a glutamine spacer. Liraglutide also differs from...
native GLP-1 in position 34 in which lysine is substituted by arginine. Liraglutide is known by both its chemical structure (Arg^{34}Lys^{26}-(N-ε-(γ-Glu(N-α-hexadecanoyl)))-GLP-1(7-37)) and the shortened NN2211 [92, 93]. Endogenous GLP-1(7-36)amide has a half-life of 1.5 minutes when administered intravenously, and 1.5 hours when administered subcutaneously [93]. Liraglutide given subcutaneously has a half-life of 11-13 hours in people, with maximum plasma concentration reached after 10 to 14 hours [90, 93, 94]. This prolonged absorption is due to liraglutide’s increased self-association into oligomers, reduced susceptibility to degradation by DPP-4 and reversible binding to serum albumin, making the drug suitable for once daily administration [94]. Liraglutide is completely degraded within the body within multiple organs and tissues by DPP-4 and NEP; there is no single organ of elimination [95].

**Liraglutide Clinical Trials in People**

The results of phase 3 randomized, controlled clinical trials for liraglutide were reported from 2009 to 2010, and included the six Liraglutide Effect and Action in Diabetes (LEAD) studies [96-101]. In these studies, the efficacy and safety of liraglutide was studied over 26 to 52 weeks and included 5796 patients. Liraglutide was evaluated as a monotherapy, as well as with concurrent therapies including oral antidiabetic drugs, and was compared to therapy with exenatide, insulin glargine, sitagliptin, glimepiridine, in addition to combination therapy with glimepiridine, metformin and rosiglitazone. When the results of these trials are examined in combination, 35-58% of patients met the ADA target for HbA1c of <7% when given liraglutide at 1.2 mg/day, and 42-54% when given
liraglutide at 1.8 mg/day. FBG was reduced in all studies with the greatest reduction in FBG occurring in the LEAD-4 trial (liraglutide 1.8 mg with metformin and rosiglitazone) where the FBG was reduced by 43.2 mg/dl from baseline. When used as a monotherapy (LEAD-3), FBG was reduced by 25.6 mg/dl from baseline. When liraglutide therapy is compared to twice daily exenatide (LEAD-6) a greater reduction is observed in liraglutide therapy (FBG decreased by 28.98 mg/dl with liraglutide 1.8 mg/day, and by 10.8 mg/dl with exenatide 10 µg BID). Weight loss was observed when liraglutide was used as a monotherapy (-2.05 kg with 1.2 mg/day, and -2.45 kg with 1.8 mg/day). When liraglutide therapy is compared with insulin glargine therapy, liraglutide therapy resulted in a mean weight loss of -1.8 kg while insulin glargine resulted in a weight gain of 1.6 kg (LEAD-5).

Reports of other benefits of liraglutide therapy including slowing of the progression of diabetic nephropathy and improvement in cardiovascular health exist. In 2013, Imamura, et al. reported 23 patients with at least a ten year history of DM and under treatment for diabetic nephropathy who were treated for twelve months with liraglutide. Decreased proteinuria and a slowing of the rate of decline of the estimated glomerular filtration rate were found, in addition to the anticipated 0.5% improvement in mean HbA1c [102]. The LEAD trials also revealed that liraglutide consistently causes a small decrease in systolic blood pressure (range amongst trials was -2.1mmHg in LEAD-3 to -6.7mmHg in LEAD-4). While these small decreases in systolic blood pressure may seem insignificant, it was shown by Patel, et al. in the ADVANCE trial that a decrease as
small as -5.6mmHg could result in a reduction in the risk of death from cardiovascular disease by 18% [103].

*Actual and Potential Side Effects of Liraglutide*

The most common side effects with liraglutide use are gastrointestinal in origin, most commonly transient nausea and vomiting which was observed in 4.5-40% of liraglutide-treated patients in the LEAD trials. In the patient population experiencing these side effects, 90% of patients relayed that the effects were transient and dissipated after the first 4 weeks of therapy [96-101]. The group experiencing nausea did not experience more weight loss than those without gastrointestinal effects; therefore weight loss attributed to liraglutide therapy is not dependent upon experiencing nausea.

Hypoglycemia in liraglutide-treated patients was rare, with only 7/2953 patients experiencing major hypoglycemic events. Six of the 7 reported patients were also taking a sulfonylurea (insulin secretagogue). Sulfonylurea is relatively commonly associated with major hypoglycemic events, even as sole therapy. The seventh patient was receiving an insulin infusion while in-hospital. Injection site-related conditions were reported in more than 5% of the patients in the LEAD-3 trial and LEAD-6 trial and were reported with the use of liraglutide (13% at 1.2mg, and 17% at 1.8mg) and glimepiridine (15%) in LEAD-3, and liraglutide 1.8mg (8.9%) and exenatide (9.1%) in LEAD-6. Drop-out rates ranged from 9-35% for liraglutide, 11-39% for active comparator, and 16-39% for placebo [94].
There is no single organ responsible for degradation of liraglutide, and the kidney is the primary organ of elimination of liraglutide. Although renal impairment can lead to some accumulation of the drug, it is unlikely to result in significant changes to the pharmacodynamics of liraglutide. Studies in people with mild renal impairment confirm this, with the only significant difference between groups with renal impairment and without being a slightly increased likelihood to stop liraglutide use due to gastrointestinal side effects, as well as a small but significant decrease in serum creatinine [104, 105]. However, given the insufficient evidence to show that liraglutide clearance continues to be normal in people with moderate and severe renal impairment, the FDA continues to warn the prescriber to use caution with administration of liraglutide in people with renal impairment [94, 106]. A similar warning from the FDA occurs in relation to use of liraglutide with hepatic impairment due to lack of evidence of safety in this condition. Despite this, at least one study shows that liraglutide was well-tolerated in people with hepatic impairment, and in fact, that liraglutide exposure may be decreased with increasing severity of hepatic impairment [107]. People with T2DM undergoing therapy with liraglutide may be at risk for acute kidney injury, however this risk does not exceed the risk of AKI associated with diabetes in general [108].

Meta-analysis of 25 clinical studies, including the LEAD studies above, showed no increased risk of thyroid or pancreatic cancer or acute pancreatitis observed with treatment with liraglutide. An FDA report in 2013 sparked concern when it was reported
that acute pancreatitis and pancreatic cancer were more common amongst patients receiving GLP1R agonist or DPP-4 inhibitor therapy (specifically exenatide and sitagliptin therapies). Since that time, conflicting reports have been published as to the long-term effects of incretin therapy on the pancreas, with some groups reporting pancreatic ductal cell metaplasia and increased GLP1Rs found in neoplastic lesions within the pancreas, and others showing no increased risk of pancreatitis nor pancreatic neoplasia with long term therapy [109]. Most recently, two separate studies have found a distinct lack of concerning pancreatic lesions associated with animal models and in vitro testing of pancreatic neoplastic cell lines. In fact, the latter study found that liraglutide actually decreases proliferation and promotes apoptosis of human pancreatic cancer cells, inhibits growth of implanted tumors in vivo in a mouse model, and that tumor size was inversely correlated with GLP-1R expression [57]. In a 52 week study of liraglutide therapy in Cynomolgus monkeys, while there was an increase in pancreas weight in female monkeys, histopathology revealed no evidence of abnormalities and proliferation rate was similar between groups [110]. As of the time of publication, the FDA warns against the use of liraglutide in patients with a history of pancreatitis, with no mention of increased risk of pancreatic cancer (Victoza® product insert). Thyroid C-cell tumors are increased in certain rodent lines treated with liraglutide. Numerous studies have yet to find evidence of increased risk of thyroid C-cell tumors in people treated with liraglutide. No increase in calcitonin (a marker of thyroid C-cell tumors that was increased in rodent models) nor increases in numbers of C-cells has been noted in
nonhuman primate studies [56]. Similarly, there was no significant increase in calcitonin in people treated with liraglutide for 104 weeks amongst liraglutide doses or groups. Currently, liraglutide therapy is considered contraindicated in people with a history or familial history of medullary thyroid carcinoma or multiple endocrine neoplasia type 2 (Victoza® product insert).

Liraglutide Therapy in Type 1 Diabetes Mellitus

Liraglutide has been used therapeutically in people with Type 1 diabetes mellitus (T1DM), although no large, controlled studies had been performed in this group at the time of writing. While potentiation of insulin secretion may not be possible depending on the stage of disease and β-cell mass reserve in this population of diabetics, there continues to be potential added benefit to GLP-1R agonist therapy, including preservation of remaining β-cell mass through reduced apoptosis and promotion of neogenesis, reduced gluconeogenesis, promotion of weight loss, and possibly improved glycemic control [13].

Oda, et al. described the use of liraglutide in a small group of healthy dogs and dogs with T1DM [111]. This study supported the presence of an incretin effect in dogs, as was previously demonstrated [112, 113]. Treatment with 15µg/kg of liraglutide in T1DM dogs did result in improved glycemic control, although increased insulin concentrations could not be documented.
Incretin-based Therapies in Cats

The incretin effect in cats is smaller than the effect described in other species. In one study in which oral and IV administration of glucose were compared [114], there was no significant difference in insulin secretion but BG concentrations were higher when glucose was administered intravenously, implying some degree of incretin effect. In this study, glucose did not stimulate GIP secretion (in contrast to its effect in other mammals) but it did stimulate GLP-1 secretion. GLP-1 concentrations and insulin concentrations correlated well after glucose stimulation as well as after lipid and amino acid stimulation.

The use of exenatide in healthy cats has been evaluated by two separate groups [115, 116]. Exenatide was quickly absorbed after a single SQ injection of 1µg/kg and using hyperglycemic clamps it was shown that it enhances glucose-dependent insulin secretion but glucose tolerance was not improved. The standard dose of twice-daily exenatide in people is 5µg to 10µg per dose [116]. In an average 80kg man, this would result in a dose range of to 0.0625µg/kg to 0.125µg/kg; therefore a dosage of 1µg/kg in a 5kg cat would be approximately 6.25 to 12.5 times the average human dose. In both studies in cats a dose of 1µg/kg was well tolerated, even when injected twice daily for 28 days. This chronic exenatide use led to significant weight loss in healthy cats of 7.0 +/- 4.9% (from 4.78 +/- 1.5kg to 4.48 +/- 1.5kg), with no evidence of vomiting or inappetence, and no clinical episodes of hypoglycemia.
We recently reported the preliminary results of a study on a long-acting formulation of exenatide in healthy cats. Three weeks after a single subcutaneous injection FBG was decreased, glucose tolerance improved, insulin concentrations increased, and median glucagon concentrations decreased. No changes in insulin concentrations were found, and no side effects noted throughout the study[117].

The use of DPP-4 inhibitors has also been described in healthy cats by Furrer, et al. This group utilized 12 healthy research cats to test the experimental, injectable DPP-4 inhibitor NVP-DPP728. The investigators used intravenous glucose tolerance tests and a meal response test to evaluate insulin and glucagon levels following administration of the drug. They found that glucagon levels were significantly decreased in all tests, that insulin output was increased in the intravenous glucose tolerance test, and concluded that further investigation in the use of DPP-4 inhibitors in diabetic cats was warranted [118]. Finally, an abstract presented at the European College of Veterinary Internal Medicine compared the use of short-acting exenatide, long-acting exenatide, and the DPP-4 inhibitor sitagliptin in 9 healthy cats (3 cats in each treatment group). Gastrointestinal side effects were noted in 2 cats in each group. The investigators found that while plasma insulin levels were increased in all groups, the short-acting exenatide group experienced the most significant increases. They also concluded that all three drugs were safe to administer to healthy cats, and that further investigation with each drug in diabetic animals was indicated [119].
Chapter 3: Pharmacology of the GLP-1 Analog Liraglutide in Healthy Cats

Introduction

Feline diabetes mellitus (DM) is a close model for type 2 diabetes mellitus (T2DM) in people. These diseases are characterized by insulin resistance, relative insulin deficiency, decreased β-cell mass and islet amyloid depositions [3, 4].

Despite technological advances in insulin formulations and monitoring, insulin therapy is still commonly associated with inadequate glycemic control leading to short and long term complications such as hypoglycemia, weight gain, and vascular diseases. Incretin-based therapy for T2DM in was first introduced in 2005 and has quickly become a widely used adjunctive therapy for T2DM in people. The American Diabetes Association recommends use of a glucagon-like peptide-1 receptor (GLP-1R) agonist as one of five additional therapeutic drugs to consider when lifestyle change and metformin fail to achieve good glycemic control in patients with T2DM [14]. Benefits of incretin therapy for T2DM in people include restoration of glucose sensitivity of pancreatic β-cells, induction of glucose-dependent stimulation of insulin secretion while simultaneously suppressing glucagon secretion, inhibition of β-cell apoptosis and increased β-cell
neogenesis, as well as slowing gastric emptying rate and enhancing satiety peripherally and centrally. These benefits result in decreased risk of treatment-associated hypoglycemia, improved beta-cell function, and weight loss [120-122]. Recently, non-glucose-dependent benefits in cardiovascular health in people have been recognized, and research continues into possible neuropathic effects of incretin hormones [15]. The active forms of endogenous GLP-1 in humans include GLP-1(7-37) and GLP-1(7-36). Both forms are quickly degraded (within 1 – 2 minutes) in the body by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4) to inactive metabolites. Liraglutide is a synthetic GLP-1R agonist which differs from endogenous GLP-1 by the substitution of lysine for arginine at position 34, and the attachment of a C-16 fatty acid (palmitoyl acid) at position 26. These changes in molecular structure allow liraglutide to bind to interstitial albumin and avoid metabolic degradation, lending the drug a half-life of 13 hours in humans after subcutaneous injection [94].

The purpose of this study was to investigate the pharmacokinetics and pharmacodynamics of liraglutide in healthy laboratory cats. We hypothesized that liraglutide would result in a glucose-dependent stimulation of insulin secretion, inhibition of glucagon secretion and improved glucose tolerance.
Materials and Methods

Animals

All animal use was approved by The Ohio State University Institutional Animal Care and Use Committee. Eight young, healthy, purpose-bred laboratory cats were used in this study, including six castrated males and two spayed females. All cats were three years of age. Median initial body weight was 4.8 kg (range 4.7 –7.0 kg). Five of the cats were overweight (body condition score was 5/9 in 3 cats, 6/9 in 2 cats, 7/9 in 1 cat, and 8/9 in 2 cats). Cats were group-housed in AAALAC accredited facilities. All cats were acclimatized and socialized for 4 weeks before the start of experiments with environmental enrichment provided. Cats were fed a dry commercial cat food (IAMS Proactive Health Original with Chicken) by twice-daily timed-feedings. Daily physical examinations were performed and body weight was monitored at least weekly. Body weight was stable in all cats during the acclimatization period. Routine laboratory tests (including complete blood counts, serum biochemistry, total thyroxine, coagulation profile, and urinalysis were performed on days -1, 15, and 28 of the experiment.

Study Design

A repeated-measures study design was used for the pharmacodynamics aspect of the study. Cats were maintained in a fasting state for 14 hours prior to each experiment. Hyperglycemic clamps (see below) were performed on days 0 (HGC) and 14 (LgHGC). Subcutaneous injections of liraglutide (Victoza® 18mg/3ml multi-dose pen, Novo
Nordisk, Copenhagen, Denmark) were administered once daily with the use of a 32-gauge hypodermic needle that was attached to the prefilled injection pen, as directed by the manufacturer. The injection was administered in a previously shaved area on the cranial dorsum. The pen was set to deliver a fixed dose of 0.6 mg (mean dose 0.112±0.019 mg/kg). Liraglutide was injected in each cat on days 1, and 8 through 14 of the study. The LgHGC was performed two hours after the final liraglutide injection.

**Hyperglycemic Clamp Procedure**

Blood glucose concentrations (BG) were measured 90 minutes prior to each clamp procedure to ensure that hyperglycemia was not present. Blood glucose was measured at -15 and 0 minutes and averaged to obtain baseline fasting BG concentrations. Blood glucose was then measured every 5 minutes for 90 minutes with a hand-held point-of-care glucose meter (AlphaTRAX 2; Abbott Animal Health, Abbott Park, IL, USA) [123]. During the procedure an intravenous dextrose infusion was administered at an increasing rate over the first 30 minutes (adjustment period) to achieve hyperglycemia at a target of 225 mg/dl (range 200-250 mg/dl). The infusion rate was determined based on the 5-min BG measurements. The final 60 minutes of the infusion was considered the actual hyperglycemic clamp during which the infusion rate was adjusted as necessary to maintain target BG, and samples were collected for measurement of insulin and glucagon concentrations at baseline (-15 and 0 min) and 30, 45, 60, 75 and 90 minutes. For dextrose intravenous infusion, a 50% dextrose solution (50% Dextrose USP; VET
ONE, MWI, Boise, ID, USA) was diluted with saline to a 20% solution and administered using a syringe pump. Dextrose was infused through a cephalic catheter while blood samples for glucose and hormones measurements were collected via jugular catheters.

**GLP-1 Pharmacokinetics Study**

The pharmacokinetics of liraglutide was evaluated after a single subcutaneous injection on day 1. Cats were maintained in a fasting state for 14 hours prior to the injection. Blood samples for liraglutide concentrations were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 84 hours.

**Liraglutide Side Effects**

In all cats, monitoring for potential liraglutide-related side effects included daily physical examinations, as well as close observation of general attitude, level of activity, quantification of food intake during timed feedings in individual cages, urination, and defecation throughout the experiment. On days -1, 15 and 28, a complete blood count, serum chemistry profile, coagulation profile, and urinalysis were repeated in all cats.

**Catheter Placement and Maintenance, Blood Collection and Storage**

Blood was collected through Vascular Access Ports (VAPs) which were surgically implanted as previously described [124] 2 to 28 days prior to the beginning of the study in 6 cats. In the other 2 cats, central intravenous catheters (MILA International, Inc., Erlanger, KY, USA) were placed 2 days prior to the beginning of the study as previously
described (16) and were used for blood collection on days 0-5; these catheters were subsequently replaced with VAPs on day 5. Cephalic catheters (Terumo Surflo® I.V. Catheter, 22 G X 1”; Terumo Corporation, Tokyo, Japan) were placed the day before each clamp procedure (-1 and 13) and removed at the end of the clamp. All catheters and VAPs were placed under sedation with intramuscular injections of dexmedetomidine (20 mcg/kg). Sedation was reversed with a dexmedetomidine-equivalent concentration of intramuscularly administered atipamezole. Butorphanol (0.2 mg/kg) was used as an analgesic for all catheter placements. Buprenorphine (0.02 mg/kg) was administered as an analgesic for VAP placement. Samples were collected into chilled EDTA tubes, centrifuged within 2 hours of collection (4°C, 4,000 rpm for 15 minutes), and then separated and placed in plain glass tubes. Plasma was stored at -20°C until analysis.

Continuous glucose monitors (CGM; Medtronic MiniMed® with SOF-SENSOR® Glucose Sensor, Northridge, CA, USA) were placed under sedation (see above) on day 7 for monitoring of hypoglycemia during daily consecutive liraglutide dosing. The CGMs were maintained with twice-daily calibrations as previously described [125] until the sensor failed (range 3 to 7 days after placement).
**Glucose and Hormone Measurements**

Blood glucose concentrations were measured with a hand-held point-of-care glucose monitor that was previously described for use in cats [123], as well as CGMs as described above. Samples were tested in duplicate for all hormone assays. Insulin concentrations were measured with a previously validated feline insulin ELISA (Mercodia AB, Uppsala, Sweden) [16].

Glucagon concentrations were measured with a high-sensitivity, high-specificity glucagon ELISA (Glucagon ELISA; Mercodia AB, Uppsala, Sweden) that was concurrently validated for use in cats. Linear regression results for expected versus observed results in serial dilutions were $R^2 = 0.9958$ ($P < 0.0001$), slope $= 0.896 \pm 0.02$ (CI $= 0.848 – 0.944$) and a Y intercept $= -0.36 \pm 0.57$ (Fig. 1). At lower concentrations of the standard curve (range 1.5 to 3 pmol/L); the average coefficient of variation (CV) was 7.0%. In the range of 3 – 8 pmol/L, the average CV was 4.6%, and in the range of 8 – 15 the average CV was 5.7%. Overall the intra-assay CV was 6.7% and the interassay CV was 8.1%. Recovery post spiking was at 86.8 – 102.0%. According to the manufacturer, the assay’s sensitivity is 1 pmol/L and it does not cross react with glucagon-related peptides including oxyntomodulin, glicentin, mini-glucagon, GLP-1, GLP-2 and GRPP.
Figure 1: Glucagon dilution parallelism in feline plasma.

The concentration of liraglutide was measured with an active GLP-1 ELISA (High Sensitivity GLP-1 Active Chemiluminescent ELISA kit, Millipore Corporation, Billerica, MA, USA) as previously described[90, 126]. In brief, samples were incubated for 4 hours at 37°C in order to completely degrade endogenous GLP-1 and then the manufacturer’s protocol was followed. This assay has a range of 0.14 pmol/L to 100 pmol/L. It has 100% cross reactivity with active GLP-1 (7-36), 72% cross-reactivity with active GLP-1 (7-37), and 0% cross-reactivity with inactive GLP-1 (9-36), GLP-2, GIP, glucagon, and oxyntomodulin. The manufacturer of this assay reports an intra-assay CV of 3-6% and an inter-assay CV of 10-13%. We found the intra-assay CV to be 13.1% for samples between < 3.73 pmol/L, (standards 1 to 4), 3.4% between 3.74 – 33.12 pmol/L, (standards 5 to 6) and 1.7% for samples >33.125 (standard 7 and above).
Statistical Analysis

Statistical analysis was performed using commercially available computer software (GraphPad Prism; GraphPad Software Inc, CA, USA and SPSS 14.0 for Mac; SPSS Inc 2005, Chicago, IL, SAS v.9.3, SAS Institute Inc., Cary, NC). All data were assessed for normal distribution using the Shapiro-Wilk test before applying parametric and nonparametric analysis where appropriate. The Shapiro-Wilk test was used to assess deviance from normal distribution of data. Mean ± SE are presented for normally distributed data. Data that were not normally distributed are presented as median and range.

Paired t-tests were used for comparison of blood glucose concentrations during HGC vs. LgHGC. A Wilcoxon matched-pairs signed rank test was used to compare TGI (total glucose infused) during HGC and LgHGC. The repeated measures of blood insulin and glucagon concentrations during the glucose clamp before (day 0) and after liraglutide injections (day 14) were then used as the outcome in statistical modeling. Separate analyses were run for insulin and glucagon. Statistical analyses were performed using PROC MIXED in Statistical Analysis Systems (SAS, v. 9.3, SAS Institute inc. Cary, NC, USA). The mean of the insulin and glucagon measurements at time -15 min and at time 0 min was calculated and considered as the baseline measurement (i.e., as time=0 value). First order autoregressive correlation structure, AR(1), was used to account for the correlated data structure due to the repeated measurements on individual cats. Time, treatment effect (measurements taken before and after the injection of liraglutide), and
blood glucose levels measured at the same time as insulin and glucagon concentrations were considered as potential explanatory variables in the model. Initially, univariate analyses were run by including them in the model individually. If associated with the outcome with $P < 0.20$ in the univariate analysis, variables were included in a full model simultaneously. Non-significant variables were dropped from the model one at a time, if $P > 0.05$. Fit of the models was evaluated by visual assessment of the residual plots.

Results

Pharmacodynamics

All but one cat had at least one episode of vomiting or diarrhea during liraglutide treatment. These events occurred most commonly on days 9-12 of the study (Fig. 2). Appetite was decreased in all cats. In one cat this progressed to complete anorexia on days 11 and 12 and the cat was withdrawn from the study on day 13. Weight loss was recorded in all 7 cats who completed the study at day 14 ($9 \pm 3\%; P=0.006$). No significant clinicopathologic changes were observed between days -1, 15, and 28.
Fig. 2: Adverse effects of liraglutide in 8 cats. Note that liraglutide (0.6 mg subcutaneous) was administered once on day 1 and then consecutively on days 8 through 14.

Baseline (the mean of times -15 and 0 min) BG, insulin, and glucagon concentrations did not differ significantly between HGC and LgHGC (Table 1). The mean of clamped BG concentrations (30 – 90 min) as well as the coefficient of variation of BG concentrations during that time did not differ significantly between HGC and LgHGC (Table 1 and Figure 3).
<table>
<thead>
<tr>
<th></th>
<th>HGC</th>
<th>LgHGC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG (mg/dl), baseline</td>
<td>91 ± 10.9 (80.5 – 106.0)</td>
<td>90.5 ± 10.9 (84 – 128.7)</td>
<td>0.49</td>
</tr>
<tr>
<td>Insulin (ng/L), baseline</td>
<td>166.1 ± 141.0 (14 – 412)</td>
<td>159.2 ± 83.1 (79 – 325)</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucagon (pmol/L), baseline</td>
<td>7.1 ± 4.8 (2.1 – 14.8)</td>
<td>5.7 ± 4.5 (3.1 – 12.6)</td>
<td>0.13</td>
</tr>
<tr>
<td>BG (mg/dl), clamped</td>
<td>228.8 ± 10.35 (214.3 – 247.7)</td>
<td>224.5 ± 9.29 (212.1 – 239.4)</td>
<td>0.0581</td>
</tr>
<tr>
<td>Insulin (ng/L), clamped</td>
<td>455.5 ± 115.8 (411 – 660)</td>
<td>760.8 ± 350.7 (449 – 1493)</td>
<td>0.0022*</td>
</tr>
<tr>
<td>Glucagon (pmol/L), clamped</td>
<td>0.66 ± 0.4 (0.0 – 1.0)</td>
<td>0.5 ± 0.4 (0.0 – 1.1)</td>
<td>0.0089*</td>
</tr>
</tbody>
</table>

**Table 1:** Paired comparisons of baseline BG, insulin, and glucagon concentrations [mean ± SD (range); time -15 and 0] and clamped BG, insulin and glucagon concentrations (time 30 to 90 minutes) during two hyperglycemic clamp procedures before (HGC) and following 7 consecutive days of liraglutide administration (LgHGC). * Denotes statistical significance P < 0.05.
Mean insulin concentrations were significantly increased in the LgHGC compared to the HGC \( (P = 0.0022) \). Higher insulin concentrations at baseline were associated with greater increases in insulin concentration during the LgHGC (but not during the HGC). Mean glucagon concentrations were decreased in the LgHGC compared to the HGC \( (P = 0.0089; \) figures 4 and 5). In 6/7 cats, TGI was higher during the LgHGC compared to HGC [median (range) of 1.61 (1.11 – 2.54) g/kg vs. 2.25 (1.64 – 3.10) g/kg]. In one cat the TGI was lower during the LgHGC compared to the HGC and this cat was identified as an
outlier (Grubb’s outlier test). After excluding this cat from TGI statistical analysis there was a trend toward an increased TGI in the LgHGC versus the HGC (P = 0.087; figure 6).

Figure 4: Insulin and glucagon concentrations (mean ± SD) in two hyperglycemic clamps, prior to liraglutide administration (HGC; circles) and following seven consecutive days of liraglutide administration (LgHGC; triangles).
Figure 5: A magnified view of glucagon concentrations (mean ± SD) in hyperglycemic clamps performed before (HGC; circles) and after (LgHGC; triangles) liraglutide administration.
Figure 6: Box plot showing a trend toward increased total amount of glucose infused observed during LgHGC compared to HGC.

Pharmacokinetics

Liraglutide concentrations were measured in 6 cats. The results are presented in figure 7. Liraglutide was first detected at the first sampling point (15 min post injection) and reached a peak ($T_{\text{max}}$) between 4 and 12 hours after injection. $C_{\text{max}}$ ranged from 89.073 pmol/L to $>104.987$ pmol/L (exceeding the upper limit of detection of the assay). By 48 hours, the mean concentration was no longer significantly different from baseline. GLP-1 was still detectable in the serum at the final time point (84 hours) in 5/6 cats. The half-life was estimated at approximately 12 hours.
Figure 7: Liraglutide concentrations (median and range) obtained over 84 hours following a single subcutaneous injection of 0.6 mg/cat of liraglutide. By 48 hours, the median concentrations were no longer significantly different from baseline values.

Discussion

Augmentation of insulin secretion through the incretin effect contributes at least 50-70% of insulin secretion following a meal [15], outlining the importance of maintaining, regaining, or effectively replacing the incretin effect in T2DM [34]. The incretin effect is impaired in T2DM in people, and GLP-1 concentrations are lower in people with obesity, T2DM, insulin resistance, and glucose intolerance [58-61]. GLP-1 is homologous amongst mammalian species [26] and GLP-1 mediates (at least in part) the incretin effect in cats as it does in people [114]. Here we studied the effect of the DPP-4 resistant GLP-1 analog liraglutide for the first time in cats. Our study confirms that in healthy cats, 7
consecutive days of liraglutide therapy result in glucose-dependent augmentation of insulin secretion and suppression of glucagon secretion with a trend towards increased TGI during a hyperglycemic clamp.

Exenatide is another DPP-4 resistant GLP-1R agonist that has been studied in cats [115, 116]. In contrast to our findings with liraglutide, in all three studies on exenatide, no side effects were observed despite using a dose of exenatide that is about 15 times higher than recommended in people. A similarly high dose in people typically results in nausea, vomiting and diarrhea. Similar side effects are commonly reported with liraglutide therapy in people and as with exenatide, these side effects are dose dependent. While these side effects are observed in a substantial number of patients, they typically subside over the first few weeks of therapy. In fact, a stepwise escalation of dosing appears to have reduced the frequency that these side effects are noted in people [94]. Gastrointestinal side effects in people are likely related to central and peripheral nervous system effects, as well as decreased gastric emptying [127]. Similarly, 7/8 cats in our study experienced at least one episode of vomiting or diarrhea. Decreased appetite was observed in all cats and in one cat this progressed to complete anorexia. Although the duration of consecutive daily liraglutide doses was only 7 days, the frequency of gastrointestinal-related episodes was noted to be subsiding by the fifth day after initiation of consecutive daily dosing. Continuous exposure to a GLP-1 agonist downregulates the effects of that agonist on gastric emptying, which in turn reduces the effect on postprandial glucose excursions [128]. This downregulation does not occur
with intermittent exposure even after many months (and years) of treatment. As a result, the overall effect of GLP-1 agonists on postprandial glucose excursion and gastric emptying are greater with intermittent exposure compared to continuous exposure. This is seen even when peak drug concentrations are higher during continuous versus intermittent exposure. Conversely, the effect on fasting glucose is decreased with intermittent compared to continuous exposure [128]. These findings explain the clinical observation in people wherein short-acting GLP-1 analogs that do not reach steady state concentrations (like exenatide) are not associated with a significant decrease in fasting blood glucose, and lead to a high frequency of vomiting and nausea. In contrast, these side effects are mostly abated when drug concentrations stabilize, even at high concentrations, with long-acting GLP-1 agonists after steady state is achieved. At the same time, fasting blood glucose is reduced in people when steady state is achieved. In this study we did not observe any decline in fasting blood glucose, however this might be related to the relatively short duration of treatment. In another study on exenatide extended release we performed after this study, increased exenatide concentrations were maintained for many weeks and fasting blood glucose decreased significantly[117].

Liraglutide is packaged in a prefilled injection pen, and the dose administered to the cats in this pilot study was chosen as the lowest deliverable dose (0.6 mg/dose) for the pen in order to facilitate accurate dosing. For the cats in our study, this resulted in a mean dosage of 0.112 ± 0.019 mg/kg. In comparison, when the same pen is used in an 80 kg person at the lowest dose, the person receives 0.0075 mg/kg. Thus, our cats received a
dose that was about 15 times higher than the starting dose in people. Given the dose-dependent nature of gastrointestinal signs observed in people in liraglutide therapy, it seems plausible that the gastrointestinal signs observed are related to the high doses used in these cats. Nonetheless, compared to the frequency and duration of gastrointestinal side effects in people, the side effects in cats were less frequent and subsided faster. In people, weight loss is observed during GLP-1 analog treatment even in those that do not experience gastrointestinal side effects [94]. In this study, weight loss was significant even after excluding one cat that developed complete anorexia. The average weight loss of 9% of body weight in two weeks exceeds the current recommendations for safe weight loss in cats of 0.5-2% of body weight per week [129]. Thus, if liraglutide is to be used as therapy for obesity in cats, its dose and/or frequency should be reduced.

No evidence of clinical hypoglycemia was observed in any of the study cats. Similar findings have been obtained in human studies in which clinical hypoglycemia was not observed in people undergoing liraglutide therapy, unless another antidiabetic therapy is used concurrently (i.e. insulin glargine or sulfonylureas) [94]. Liraglutide has also been studied in non-diabetic obese people as treatment for obesity. Importantly, hypoglycemia in these non-diabetics is not observed (similar to our non-diabetic cats) emphasizing that the effects of liraglutide are indeed glucose-dependent.

Fasting glucagon concentrations in our study are lower than previously reported in cats [118]. In these previous reports the glucagon assays were validated prior to the wide
recognition of cross reactivity between glucagon, GLP-1 and other glucagon gene products. Therefore, while these assays would be sensitive for detecting glucagon, they have decreased specificity and previous results likely represented cross-reaction with multiple glucagon gene products.

Examination of the difference between the means of the blood glucose concentrations during the hyperglycemic clamps before and after drug administration revealed a difference that approached statistical significance (P = 0.058). The large number of data points in this calculation is responsible for a high power for detection of even small differences that are likely not biologically significant. However, considering the possibility of a biologically important difference, increased average blood glucose during HGC is expected to result in increased insulin secretion and a false decrease in the apparent effect of liraglutide. Thus, our results reflect a “worst case scenario” and most likely the effect of liraglutide in causing augmentation of insulin secretion could be even greater.

The upper limit of the GLP-1 assay was frequently exceeded when measuring liraglutide plasma concentrations. This precluded our ability to determine a more accurate time to peak concentration, and may have been avoided with sample dilution.

Further studies are needed to evaluate the efficacy and tolerability of liraglutide in feline T2DM patients. Given the similarities between T2DM in cats and people, as well as the results of this study in healthy cats, liraglutide has the potential to both improve
glycemic control and promote weight loss, as seen in human diabetics administered this drug. Given the relatively long half-life and evidence in people that prolonged administration leads to higher sustained plasma concentrations, evaluation of decreasing frequency of drug administration to improve tolerability could be considered [90].

Our study has shown that liraglutide potentiates glucose-dependent insulin secretion and glucagon inhibition in healthy cats without affecting fasting blood glucose. Liraglutide administration was associated with decreased appetite and significant weight loss, although these effects appeared to be worse at the initiation of therapy and ameliorated as therapy continued. The maximum concentration of liraglutide in cats was observed between 4 and 12 hours, with an elimination half-life of approximately 12 hours making once daily dosing feasible. However, a decrease in dosing frequency may result in less severe weight loss and potential associated complications. Further investigation into the use of liraglutide in type 2 diabetic cats, and as a weight loss drug in overweight cats, is warranted.

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References


Marre, M., et al., Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). Diabet Med, 2009. 26(3): p. 268-78.


