The effect of diet and adiposity on the secretion of incretin hormones in cats

THESIS

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Abstract

Degree of adiposity and dietary macronutrient composition affect the secretion of incretin hormones but little is known about their effect in cats. In this study, 7 overweight cats were fed a maintenance diet (MD) for 2 weeks followed by a restricted calorie control diet (RCD, lower fat, higher in carbohydrates and fiber). Cats were fed ad-libitum initially and then food was restricted to achieve 1-2% loss of body weight weekly (11 weeks). When lean, cats were fed MD for 2 additional weeks. A standardized meal test (SMT) using a third diet was performed after at least 7 days on each diet, before and after weight loss (4 SMT’s total). Glucose, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) concentrations were measured immediately before and over 6 hours after feeding the SMT. Area under the concentrations curve (AUC) was compared for GLP-1, GIP, and insulin using linear regression models. Post-prandial GIP\textsubscript{AUC} was affected by degree of adiposity (P=0.026), with cats in the leaner state secreting increased GIP\textsubscript{AUC} compared to the cats in the obese state. There was also an effect of diet on GIP\textsubscript{AUC}, with cats on RCD diet having a higher median GIP\textsubscript{AUC} than the cats on MD (P=0.02). This suggests that dietary macronutrient content is important in determining GIP responses not only acutely but also on a long-term basis. There was an effect of adiposity on baseline GLP-1, with cats in the obese state having higher GLP-1 concentrations than cats in the leaner state (p=0.02). There was no effect of diet or adiposity of GLP-1\textsubscript{AUC}. Further investigation is needed to
understand the specific nutrients that effect changes in GIP secretion, and specific hormonal changes that accompany weight loss of the maintenance of a lean body condition.
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Chapter 1: Enteroendocrine cells

The gastrointestinal tract secretes a variety of hormones that have important effects on gastrointestinal motility, systemic metabolism, and satiety. Incretins are hormones released from the gastrointestinal tract by enteroendocrine cells during ingestion of a meal. The term “incretin effect” describes the greater release of insulin that occurs when glucose is administered orally compared to IV [1, 2]. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are the major hormones involved in the incretin effect. Under normal physiologic conditions, the effect of GLP-1 and GIP are glucose-dependent and in healthy humans account for approximately 80% of total insulin secretion [3]. Incretin hormones have many other effects beyond enhancing insulin secretion and those effects will be discussed in more detail below.

Enteroendocrine cells [EEC] including L cells, K cells, and I cells (and many others), are scattered through the gastrointestinal epithelium. These specialized cells secrete a wide range of peptide hormones which control many physiological functions in and outside of the gastrointestinal tract, including digestive motility and glucose homeostasis [4]. Similar to other EEC, K and L are “open-type” cells that contain a slender apical process that extends into the lumen of the gut and carries their sensing machinery. Hormone secretion occurs on the broad basolateral side [5]. On their basolateral side, EEC are located close to the vasculature and neurons of the lamina propria [6], which
allows small quantities of hormones to reach neuronal targets at high concentrations as well as escape degradation in the extra-cellular space by quickly entering the vasculature. Close proximity to vasculature and nerve endings also allows neuronal and hormonal regulation of EEC. [7]. EEC act as sensors of luminal nutrient content, integrating information regarding composition of the diet and quantities of nutrients through the use of nutrient-specific G protein-coupled receptors [GPR] and other sensing mechanisms. Under normal physiologic states, incretin hormone concentrations increase in the blood shortly after luminal sensing of macronutrients in the intestinal tract following ingestion of food [8].

K cells, located primarily in the duodenum and proximal jejunum (in most mammals), secrete GIP. This peptide was originally named “gastric inhibitory polypeptide” because of its effect on slowing gastric emptying [9]. In people however the effect of GIP on gastric emptying is relatively minor and its name was updated to glucose-dependent insulinotropic peptide when the role of GIP in the incretin effect was discovered. Once released from the K cells, GIP binds to receptors in the pancreas, where it promotes glucose-dependent insulin secretion from β cells. In addition, GIP stimulates adipocyte fat deposition by increasing lipoprotein lipase activity in the presence of insulin [10-12]. Dietary fat and carbohydrates are the most potent stimulators of GIP secretion in most mammals[13].

Studies in rats, pigs, and people have shown that L cells are located primarily in the ileum and colon, with small numbers in the distal jejunum [14, 15]. L cells secrete three different enteroendocrine hormones: GLP-1, glucagon-like peptide 2 (GLP-2), and
peptide YY (PYY). L cells are a heterogenous cell population; in different locations in the intestines, L cells secrete and express different combinations of these hormones [16]. GLP-1 contributes to glucose homeostasis via multiple mechanisms, including through glucose-dependent potentiation of insulin release and glucagon inhibition as well as extra-pancreatic actions. In pancreatic β-cells, GLP-1 stimulates proliferation, inhibits apoptosis and increases insulin gene transcription. GLP-1 also inhibits gastric acid secretion and slows gastric emptying and motility, which helps miminizing post-prandial spikes in blood glucose. GLP-1 also regulates appetite and promotes satiety through stimulation of central pathways in the vagus nerve, hypothalamus, and brainstem [17, 18].

GLP-2 has a primary trophic effect on enterocytes through direct stimulation of enterocyte proliferation and inhibition of apoptosis [19]. GLP-2 also improves intestinal barrier function, and reduces gastric motility and acid secretion [20, 21]. PYY has diverse functions, including regulation of insulin secretion, inhibition of gastric acid secretion and gastric emptying, and inhibition of gallbladder contraction during the cephalic phase [22]. PYY also decreases appetite through interaction with the vagus nerve and the hypothalamus [23].

Initially, EEC cells were classified according to what was then thought to be the one hormone they secrete. However, recent research has revealed that many of these cells are able to secrete more than one hormone [4, 24-26]. A population of K/L cells that secrete both GIP and GLP-1 is present in rats, pigs, and people [27, 28]. In the mid-small intestine, between 55-75% of the K and L cells co-secrete both GIP and GLP-1, and are
referred to as K/L cells [27]. The main stimulus for secretion of hormones from K cells and L cells is the presence of nutrients in the lumen of the gut. The degree of GLP-1 and GIP stimulation by different nutrients is species-dependent [29]. While fat is the most potent stimulator of GIP in humans and dogs, carbohydrates are the most potent stimulator of GIP secretion in pigs and rodents [6]. Amino acids are weak stimulators of GIP secretion in humans but strong stimulators of GIP secretion in dogs and rats [29, 30]. In general, GLP-1 secretion is stimulated by ingested lipid, carbohydrate, and protein as well as by GIP [30-32]. There is also evidence that secretion of GLP-1 from L cells is regulated in part by stimulation of the enteric nervous system and the vagus nerve [33].

The specific intestinal distributions of EEC cells in various species helps to determine the importance of different nutrient stimuli for GLP-1 and GIP secretion [30, 34].

Although the EEC distribution is similar in humans, pigs, and dogs, it has been shown that domestic cats have a markedly different K and L cell distribution pattern compared with other mammalian species. In most mammals, K cells are most abundant in the duodenum and jejunum, and are not found distal to the ileum [27, 34]. In cats, however, K cells are roughly equally distributed in the duodenum, jejunum and ileum, and also are found in the cecum and colon [35]. This unique species difference in EEC distribution in cats suggests potential differences in physiology of EEC and incretin hormones. Domestic cats also have a different enteroendocrine response to macronutrients compared to what has been described in other species. Specifically, although glucose is a major stimulator of GIP secretion in humans, rodents, pigs, and dogs, glucose does not stimulate GIP secretion in cats [36].
Release and Degradation of Incretin Hormones

Once stimulated, K and L cells secrete peptides into the interstitial space. GLP-1 and GIP are released into the lamina propria and enter the capillary bed or lymphatics. Active GLP (1-42) and active GLP-1 (7-37 and 7-36 amide) are quickly degraded into their inactive forms by dipeptidylpeptidase IV (DPP-IV). DPP-IV is a serine protease that specifically cleaves dipeptides [6], and is found abundantly in the brush border [37]. Active GLP-1 is also degraded by the enzyme neural endopeptidase 24.11 (NEP-24.11), a membrane-bound zinc metallopeptidase. DPP-4 and NEP have a widespread distribution in tissues. In particular, DPP-IV is abundant in the brush border and on the endothelial cells that line the capillaries of the intestinal mucosa [37], which are positioned close to the sites of GIP and GLP-1 secretion. Approximately 50% of GLP-1 released into the capillaries is transformed to its inactive form, N-terminally truncated GLP-1 9-36 amide, before it reaches the portal vein [38]. GLP-1 is further degraded in the liver, which means that only 10-15% of secreted GLP-1 reaches the systemic circulation in its active form [38]. Because of this, it is recommended to measure total GLP-1 (both the active and inactive forms) in order to accurately estimate GLP-1 secretion [39, 40]. Measuring total GLP-1 provides a better estimation of GLP-1 activity, because the effect of GLP-1 (incretin effect and others) is mediated largely by its effect on the vagus nerve (in the intestines) and on the liver [39, 40]. Active GLP-1 has a relatively short half-life of 1-2
minutes due to its rapid degradation by NEP and DPP-IV [38]. The half-life of active GIP in plasma is also short (about 2 minutes in rodents and 5 minutes in humans) [6].

Receptor Targets and Binding Sites of Incretin Hormones

GLP-1 acts directly on its receptor GLP-1R, which is a G protein-coupled receptor (GPCR). GLP-1R is located both centrally and peripherally [41, 42] and is most abundant in lungs, brain, taste cells, pancreas, and the gastrointestinal tract. GLP-1R are also present in muscle and liver tissue, as well. There are two different signaling pathways activated by the GLP-1R. In the brain and pancreas, GLP-1 binds to its receptor and activates the cAMP pathway. In the pancreas, GLP-1R activation by GLP-1 leads to activation of cAMP, which causes elevated intracellular calcium, leading to exocytosis of insulin-containing granules [16]. In muscle and liver, GLP-1 activates the cAMP-independent pathway [37].

Effects of Incretin Hormones in the Pancreas

The primary functions of the incretin hormones include: stimulation of glucose-dependent insulin secretion, insulin biosynthesis, and regulation of β-cell mass [6]. GLP-1 and GIP act as ligands on the G protein-coupled receptors GIPR and GLP-1R, which are present on pancreatic β-cells. The activation of GLP-1R and GIPR leads to activation of adenylate cyclase and production of cAMP, which stimulates insulin secretion via
PKA-dependent phosphorylation of downstream targets and PKA-independent activation of Epac2. The cAMP signaling system is also responsible for two other main effects in the β-cell: (1) the synergistic effect of incretins with glucose to promote insulin gene transcription, mRNA stability, and biosynthesis (2) enhancement of the glucose-sensing apparatus by increasing the expression of its components: GLUT-2 and glucokinase. Through these mechanisms, incretin hormones are able to replenish β-cell stores, prevent exhaustion of β-cell reserves and increase the sensitivity of β-cells to glucose [6, 30].

GLP-1 and GIP play an important role in the expansion of β-cell mass. These hormones potentiate glucose-mediated proliferation and differentiation of β-cells by activating early gene expression encoding transcription factors that regulate islet cell proliferation and differentiation. In addition to having a direct role in the formation of β-cell mass, GLP-1 and GIP also protect β-cells from apoptosis [6, 30].

GIP increases glucagon secretion through activation of GIPR in pancreatic alpha cells, which results in increased intracellular cAMP and Ca\(^{2+}\). However, this effect on glucagon secretion is typically masked during hyperglycemia when GIP-stimulated insulin secretion decreases glucagon secretion [30].

In contrast to GIP, GLP-1 inhibits glucagon secretion, which reduces hepatic gluconeogenesis during times of hyperglycemia. During periods of hypoglycemia, GLP-1 loses its inhibitory effect on glucagon secretion, which helps to prevent worsening hypoglycemia [30].
Incretin hormone secretion is primarily regulated through luminal nutrient content [43-45]. GIP is secreted from intestinal K cells in response to nutrient ingestion, specifically, glucose and fat. This effect appears to be species-specific; fat is the most potent stimulator of GIP in humans, whereas carbohydrates are the most potent stimulators of GIP release in rodents and pigs [6]. In addition to the specific type of nutrients ingested, the rate of nutrient absorption also affects GIP stimulation. GIP secretion is decreased in humans with diseases that impair intestinal absorption [46].

In addition to nutrient intake, other factors, such as neuropeptides and inputs from the autonomic nervous system have also been shown to influence GIP secretion. There is mixed evidence regarding the influence on vagal nerve inputs on GIP secretion [47-49] with some citing a positive influence on GIP secretion, and some showing no effect. These conflicting reports are likely due to the diversity of experimental models used and the variety of nutrient stimuli used to stimulate GIP secretion. In addition, alterations in vagal nerve inputs (through the use of various forms of vagotomy) also affect other parameters which have been known to affect GIP secretion, including gastric emptying [50] and gastric acidity [51].

In addition to potential vagal nerve mediation, GIP secretion is also regulated by inputs from peptides such as neuropeptide Y (NPY) and gastric-related peptide (GRP). NPY is an amino acid neurotransmitter present in the central nervous system (primarily in the hypothalamus) and enteric nervous systems. NPY is a potent orixigenic peptide with a
preference for carbohydrate intake [52, 53]. Increased hypothalamic levels of NPY cause increased GIP secretion in a dose-dependent manner in dogs [54].

Gastric-related peptide (GRP), a peptide that stimulates gastrin release from the G cells of the stomach, has also been implicated in GIP secretion. In vitro studies in rats and dogs have shown that non-adrenergic nerves releasing GRP also stimulate GIP secretion in a dose-dependent manner [55-57].

Nutrient ingestion (particularly fat and carbohydrates) is the primary stimulator of GLP-1 section [6], but other factors, including endocrine signaling and neural input also play important roles. Studies in rats have shown an important role of vagal inputs in regulating GLP-1 secretion. In these experimental models, surgical vagotomy with disruption of vagal input to the gut showed a complete blockage of fat-stimulated GLP-1 secretion, whereas electrical stimulation of vagal inputs caused an increase in GLP-1 secretion [33]. GRP and GIP have also been shown to stimulate GLP-1 secretion [6].

Ghrelin, an orexigenic hormone that is crucial to regulation of whole body metabolism, appears to play a key role in the regulation of GLP-1 secretion [58]. The injection of ghrelin into mice enhances the glucose-stimulated secretion of ghrelin. In addition, ghrelin also appears to play a role in GLP-1 mediated glucose tolerance in obese mice. Importantly, ghrelin directly stimulated GLP-1 release from immortal L-cell lines (murine GLUTag, human NCI-H716) through an extracellular signal-related kinase 1/2-dependent pathway. These studies support ghrelin’s role in enhancing the GLP-1 secretory response to ingested nutrients [58] albeit providing only weak evidence for that, given the lack of evidence for this in vivo or in-vitro with normal EEC lines.
Leptin, the “satiety hormone”, plays a key role in energy balance by opposing the actions of ghrelin and inhibiting hunger signals in the hypothalamus. *In vitro*, the administration of leptin directly stimulates GLP-1 secretion (up to 250% compared to control) in both mouse and human L cell lines [59]. *In vivo*, direct administration of leptin has also been shown to stimulate GLP-1 secretion in both rats and mice [59]. This research also suggests that leptin resistance may account for the decreased levels of GLP-1 found in obese humans [59].

Other adipokines such as IL-6 have also been found to have effects on GLP-1. IL-6 is an important adipokine that appears to have mixed effects on insulin resistance and glucose homeostasis. Although it has been linked to obesity-related insulin resistance [60, 61], it has also been proposed to enhance nutrient availability and promote whole-body insulin sensitivity [62]. In mice, elevated IL-6 concentrations stimulate GLP-1 secretion from intestinal L cells and from pancreatic alpha cells, leading to improved insulin secretion and glycemia. [63]. (IL-6 increased GLP-1 production from alpha cells through increased proglucagon and prohormone convertase expression). Another adipokine, RANTES, which is upregulated in obesity [64, 65], was shown to reduce glucose-stimulated GLP-1 release in vitro and to reduce plasma levels of GLP-1 and GLP-2 in response to an oral glucose load in mice [66]. Leptin secretion is directly proportional to fat mass in mammals. Therefore, if is expected that obese mammals would have decreased GLP-1 secretion. IL-6 secretion is also increased in obesity. Therefore, the relationship of obesity to GLP-1 secretion is complex.
Because of their role in glucose homeostasis and metabolism, many research efforts have focused on elucidating the complex relationship between obesity, diabetes, and alterations in the incretin effect. Obese patients have a decreased GLP-1 secretion in response to meal-feeding compared to lean individuals [67-69]. In addition, GLP-1 secretion is decreased in insulin resistant and diabetic patients, [69, 70] and the overall decrease in GLP-1 secretion appears to be related to the degree of insulin resistance [71].

Although the direct cause of the attenuation in GLP-1 secretion is unknown, it may be related to an increase in plasma non-esterified fatty acids [67, 72]). However, more recent efforts have not shown this effect [68]. Obese subjects with decreased GLP-1 secretion have improvements in GLP-1 secretion following weight loss [68], which suggests that the secretion capacity of these cell populations are plastic and responsive to environmental manipulation such as weight loss.

While research studies consistently documented decreased GLP-1 secretion in obese and insulin-resistant subjects compared to lean subjects, the effect of obesity and insulin resistance on GIP secretion is not as clear. Some studies have identified normal GIP responses in obese subjects compared to lean subjects [68], others have described attenuated [73, 74] or even increased GIP secretion profiles in obese and insulin-resistant subjects [71, 75, 76]. This may be due to alterations in GIP function in obese patients. Type-2 diabetics demonstrated a 54% decrease in the insulinotropic activity of
GIP in patients with type-2 diabetes who were infused with supraphysiologic concentrations of GIP. These patients had normal effects of GLP-1 [77].

Dietary Manipulation and Incretin Hormones

In addition to changes in metabolic status, specific nutrients also impact gut hormone secretion. The main stimulus for secretion of GIP and GLP-1 from enteroendocrine cells is the presence of nutrients in the lumen of the gut, with different nutrients having variable potency for stimulating secretion of different hormones.

Outside of their immediate stimulatory effects on enteroendocrine hormone secretion, chronic dietary changes in fat and fiber have also been shown to affect EEC proliferation and the potential hormone secretion capacity. Obese-prone rats fed a high fat, high-calorie diet had significantly lower plasma GLP-1 levels, decreased protein levels of GLP-1 in the intestinal epithelium and reduced number of L cells in the distal ileum compared to obese-prone rats fed regular chow that contained 26% fewer calories/gram [78]. In people, chronic consumption of a high fat diet led to increased GIP secretion in response to glucose in one study [79], whereas an extremely calorie-restricted diet decreased GIP secretion [80]. Chronic consumption of high fat diet in obese hyperglycemic mice leads to significant hyperplasia of GIP-secreting K cells, while no change in K cell density was seen with a high carbohydrate diet [81]. Feeding of a high fat diet caused decreased EEC differentiation through downregulation of transcription factors, which resulted in reduced EEC number and gut hormone levels [82].
In addition to fat modification, dietary fiber modification has also been shown to affect GIP and GLP-1 secretion. Chronic fiber supplementation in humans has been shown to cause decreases in GIP secretion [83] and increased basal GLP-1 secretion in humans [84]. Specifically, fermentable fiber has also shown to be important in modulating intestinal proglucagon expression [85]. Studies in rats who received chronic oligofructose supplementation showed increases in the number of GLP-1-expressing L cells in the proximal colon [86], indicating that the changes seen with dietary manipulation are due in part to increases in the number of hormone-secreting cells in the intestine. Rats supplemented with fiber show significant upregulation in the proglucagon gene expression and secretion of GLP-1 compared with rats fed a fiber-free diet [87]. In fact, the type of fiber appears to be important. Replacing cellulose fiber with rhubarb fiber in a diet upregulated ileal proglucagon mRNA [85]. Although the mechanism by which fiber promotes GLP-1 production is currently unknown, it is hypothesized that it is due to effects of fiber on fermentation and liberation of short chain fatty acids and other metabolic products from bacteria in the gut [88].

Rationale and Hypothesis

A long-term evaluation of the effects of weight loss and diet on the effects of enteroendocrine hormones has not been performed to this date in cats. The purpose of this study was to determine the impacts of weight loss and long-term dietary manipulation on the enteroendocrine hormone secretion capacity of healthy cats. Our first
hypothesis was that continuous exposure to diets that vary in macronutrient composition would lead to a change in secretion of GLP-1, GIP, and PYY in response to a standardized nutrient stimulus. The second hypothesis was that changes in body condition would also cause a different response to a standardized nutrient stimulus, independent of dietary effect. Gaining a better understanding of the factors that regulate EEC cell proliferation will reveal new potential targets for treatment of gastrointestinal and endocrine disease.
Chapter 2: Materials and Methods

Animals

The study protocol was approved by The Ohio State University Institutional Animal Care and Use Committee. Seven purpose-bred cats were used in this study, including five castrated males and two spayed females. At the start of the study all cats were 4 years old and overweight. Body condition score (BCS) was 9/9 in 1 cat, 8/9 in 3 cats, and 7/9 in 3 cats [89]. Cat body weights and body fat percentages are presented in Table 2.

Cats were group-housed in AAALAC accredited facilities. All cats were acclimatized and socialized for more than a year before the start of experiments with environmental enrichment provided. Routine laboratory tests, including complete blood counts, serum chemistry, and urinalysis were performed at the beginning of the experiment and all cats were considered healthy except for being overweight.

Study design

This was a repeated measure design study. Each cat underwent four consecutive phases of dietary treatments in the following order: 1) Maintenance diet (Purina® Friskies® Classic Paté Mariner’s Catch®, Nestlé Purina PetCare, St. Louis, MO) fed *ad libitum* for at least 2 weeks from day 0-14 (ObMD) 2) Reduced calorie diet (Purina Veterinary Diets® OM Overweight Management® Feline Formula, Nestlé Purina PetCare, St. Louis, MO) fed *ad libitum* for 1 week from study day 15-21 (ObRCD ) 3) Reduced
calorie diet (OM Overweight Management®) with caloric restriction fed from study day 22-99 (LeanRCD) 4) Maintenance diet (Friskies® Classic Paté Mariner’s Catch,) fed *ad libitum* for two weeks from study day 100-114 (LeanMD). Body condition was monitored by measurement of body weight, BCS, and dual-energy x-ray absorptiometry (DXA). A standardized meal test (SMT) was performed at the end of each treatment period (4 SMT’s total). The study design is illustrated in Figure 1.

**Standardized Meal Testing**

Standardized meal tests (SMT) were performed after a 12 hour fast. Forty grams of a highly palatable diet (Hill's® Prescription Diet® a/d® Canine/Feline Canned, Hill's® Pet Nutrition, Topeka, KS) were offered for 10 minutes to each cat in separate cages. A separate diet was used for the SMT to control for the acute effects of dietary manipulation on incretin hormone secretion. After 10 minutes, any remaining food was removed and the amount of food remaining was recorded. Blood samples were collected from cats at -15, and 0 minutes prior to feeding and 1,2, 4, and 6 hours post-feeding. Blood samples (1.5 ml) were collected through a jugular vascular access port (VAP, AccessTM Technologies, Norfolk Medical Products Inc, Skokie, Illinois) that had been placed 8-12 months prior to the beginning of the study as previously described [90].
Body Condition Monitoring

Body weight was measured weekly, and body condition score (BCS) was assessed monthly by two of the investigators (VJP and AJR). All BCS presented are averages between the 2 assessors for each assessment.

Body fat percentage was assessed using DXA Scan (GE Lunar Prodigy with enCORE™ v.13.6, GE, Fairfield, CT) as previously described [91]. DXA scans were performed at the following time points: at the end of ObMD, at the end of LeanRCD, and at the end of LeanMD. Data are not reported for the DXA scan at the end of LeanRCD because these did not differ significantly from the scan at the end of the LeanMD period (p >0.05). Scans were performed under sedation with intramuscular injections of dexmedetomidine (10mcg/kg) (Dexdomitor, Zoetis, Kalamazoo, MI) and butorphanol (0.2 mg/kg) (Torbugesic, Zoetis, Kalamazoo, MI). Sedation was reversed with a dexmedetomidine-equivalent volume of intramuscularly administered atipamezole (Antisedan, Zoetis, Kalamazoo, MI).

Feeding

Cats were fed twice daily in separate cages and were allowed an allotted time period to finish meals (2 hours for the morning meal, 14 hours for the evening meal). A set amount of food was offered, and at the end of the feeding period, the residual amount left was recorded. For the ObMD, ObRCD, and LeanMD phases, food was offered *ad libitum*. During the LeanRCD period of controlled weight loss (study day 22-99), food consumption was converted to a caloric intake estimation, then was adjusted weekly.
based on a previously described dietary management strategy to achieve weight loss with a rate of approximately 1-2% of body weight per week [89].

Blood collection and Storage

Blood was collected through vascular access ports (VAPs), that were surgically implanted in a previous study [92]. Samples were collected into chilled glass ethylenediaminetetraacetic acid [EDTA] tubes and were immediately placed on ice. Samples were centrifuged within 6 hours of collection (4°C, 2,271g for 15 minutes), the plasma was separated and then stored at -20°C. All samples were analyzed in one batch at the end of the study.

Glucose and Hormone Measurements

Blood glucose concentrations were measured with a hand-held point-of-care glucose monitor (AlphaTRAK2 Blood Glucose Monitoring System, Abbott Laboratories, Abbott Park, IL). Samples were tested in duplicate for all hormone assays. Insulin concentrations were measured with a feline insulin ELISA that has been previously validated in cats (Mercordia AB, Uppsala, Sweden) [36].

Total GLP-1 concentrations were measured with a GLP-1 ELISA (Multi-Species GLP-1 Total ELISA kit, Millipore Corporation, Billerica, MA, USA) that we validated for use in cats. According the manufacturer, this assay has a range of 4.1 – 1000 pM and a sensitivity of 1.5 pM. It has 100% cross reactivity with active GLP-1 (7-36) and (9-36) and does not cross react with GLP-2, GIP, glucagon or oxyntomodulin. Linear regression for expected versus observed results in serial dilutions was $R^2 = 0.999$, slope = 0.9647x
and a Y intercept = -2.1398 (Figure 2). For samples with low-medium concentration inter-assay CV (standards 2-3): N=4, Mean (range) = 9.1 (2.2-15.6). For samples with medium concentration (standard 4): N=4, Mean(range) = 16.9 (5.0-23.2). Intra-assay CV was also calculated. For samples with low-medium concentration (11.63-17.82 pM): N=8, Mean(range) = 4.2 (0.41-6.6). For samples with medium concentration (24.19-66.13): N=8, Mean(range) = 3.5 (1.87-5.4). For samples with high concentration (181.8, 518.19): N=2, CV= 2.2, 1.6.

Total GIP was measured with a human GIP ELISA kit (Human Total GIP ELISA kit, Millipore Corporation, Billerica, MA, USA) that was previously validated for use in cats [36]. This assay has a range of 8.2–2000 pg/mL and a sensitivity of 8.2 pg/mL. The manufacturer of this assay reports an inter-assay CV 1.8–6.1%, and an intra-assay CV of 3.0–8.8.

PYY was measured using a human ELISA kit but the assay could not be validated because of insufficient sensitivity and therefore the PYY data are not shown.

Statistical Analysis

Descriptive statistical analysis was performed using commercially available computer software (GraphPad Prism; GraphPad Software Inc, CA, USA).

Baseline concentrations for all hormone data were calculated for each timepoint by averaging the values at -15 minutes and 0 minutes. Area under the curve (AUC) was calculated for GLP-1 (GLP-1$_{AUC}$), GIP (GIP$_{AUC}$), insulin (Insulin$_{AUC}$), and glucose for all cats and all treatments using the trapezoidal method in GraphPad Prism. Maximally
stimulated GLP-1 (GLP-1\textsubscript{ST}), GIP (GIP\textsubscript{ST}) and insulin (Insulin\textsubscript{ST}) concentrations were calculated by subtracting the baseline hormone concentration from the maximum hormone concentration measured. Body fat mass was calculated as the product of body weight (kg) and body fat percentage, \( [\text{BFM (kg)} = \text{BW (kg)} \times \text{BF\%}] \). Descriptive statistics on body weight, body condition score, and body fat percentage were calculated and presented in Table 2. The Wilcoxon signed rank test was used to compare differences in these variables during the obese phase versus during the lean phase. Spearman’s correl 

Analytical statistics were performed using R statistical software (R version 3.1.3 -- "Smooth Sidewalk" © 2015 The R Foundation for Statistical Computing). Spearman’s rank correlation coefficient was used to screen for relationships between predictor variables: body fat percentage and body fat mass, and different formulations of response variables: hormone and glucose measurements without accounting for repeated measures from the same cat or diets. A Bonferroni correction was used to reduce the likelihood of discovering false correlations, and a P value threshold for significance was set at <0.002. 

Linear regression models were used to evaluate the effect of diet and adiposity on hormone measurements, and to control for repeated measures in cats, daily differences, and the combined effects of diet and adiposity. For each hormone, models were selected purposefully beginning with the predictor and response variable combination that had the most significant correlation in a model that also included fixed effects for day of sampling, diet and cat. Non-significant predictors were dropped until the overall fit of the model (assessed via adjusted R-squared and ANOVA) was compromised or the effects of the other predictors changed significantly. To this candidate best-fit model,
interaction terms were added, and alternate measures of adiposity were tried and the resulting models were compared to the original candidate using adjusted R-squared and ANOVA. Because of interest in additional hormone measures, additional linear regression models were fit using the same purposeful backwards stepwise selection method.

![Study Design](image)

Figure 1. Study Design. Obese cats were fed a maintenance diet (MD) from day 0-14 (ObMD; weeks 1-2), followed by a period of *ad libutum* feeding of a reduced calorie diet (RCD) from day 15-21 (ObRCD; week 3). Caloric restriction occurred over a period of 11 weeks to create controlled weight loss from study day 22-99 (LeanRCD; week 4-14). Cats were returned to the maintenance diet from day 100-114 (LeanMD; weeks 15-16). Black circles represent the SMT timepoints. Dietary manipulations and study timepoints are described on the X axis and mean body weight of the cats is displayed on the Y axis.
Figure 2. GLP-1 dilutional parallelism in feline plasma.

<table>
<thead>
<tr>
<th></th>
<th>MD Purina OM</th>
<th>RCD Friskies Classic Pate Mariner’s Catch (can)</th>
<th>SMT Hill’s a/d (can)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcal/kg</td>
<td>835</td>
<td>1,113</td>
<td>1,151</td>
</tr>
<tr>
<td>Protein (g/100 kcal)</td>
<td>13.2</td>
<td>10.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Fat (g/100 kcal)</td>
<td>3.8</td>
<td>6.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Carbohydrates (g/100 kcal)</td>
<td>6.1</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Crude fiber (g/100 kcal)</td>
<td>3.0</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 1. Macronutrient and calorie of the maintenance diet (MD), restricted calorie diet (RCD), and standardized meal test (SMT) diet.
Chapter 3: Results

Five neutered males and 2 spayed females were included in the study. All cats were overweight at the start of the study. Body weight (BW), body fat percentage (BF%, measured by DXA), body condition score (BCS), and calculated body fat mass (BFM = BW x BF%) at the beginning and end of the controlled weight loss period are presented in Table 2 and Figures 3-5. There was a significant decrease in BW, BF%, BFM, and BCS after the period of weight loss (P=0.02).
### Table 2. Mean ± Standard Deviation, Median (Range) Body Weight, Body Condition Score, and Body Fat Mass before and after the period of controlled weight loss. Mean and median values within a row with unlike superscript letters\(^{(a,b)}\) were significantly different (P =0.02).

At the end of the controlled weight loss period, cat weight decreased by (mean ± SD; median [range]) 1.3 kg ± 0.3; 1.2 (0.9-1.9). BCS decreased by 2-3 points (median = 2). Body fat percentage decreased by (mean ± SD; median [range]) 21.1% ± 5.3; 21(19-21).
28.8) from baseline, and body fat mass [mean ± SD; median; range] decreased by 1.5 kg ± 0.4; 1.4 (1.1-2.1).

Figure 3. Individual body weights of cats before and after the period of controlled weight loss. There was a significant decrease in body weight following the weight loss period (P=0.0156). The asterisk (*) indicates statistical significance.
Figure 4. Individual body fat percentages of cats before and after the period of controlled weight loss. There was a significant decrease in body fat percentage following the weight loss period (P=0.02). The asterisk (*) indicates statistical significance.

Figure 5. Individual body fat mass (kg) of cats before and after the period of controlled weight loss. There was a significant decrease in body fat mass (kg) following the weight loss period (P=0.02). The asterisk (*) indicates statistical significance.
Figure 5: Continued

weight loss. Body fat mass = body weight (kg) x percent body fat. There was a significant
decrease in body fat mass following the weight loss period (P=0.02). The asterisk (*)
indicates statistical significance.

Baseline (the mean of times -15 and 0 min) and post-prandial BG did not differ
between time points. Insulin:Glucose ratio correlated with body weight (r = 0.641 [95%
CI = 0.2885 – 0.8405], P < 0.01). Baseline insulin concentrations did not differ
significantly between time points, but the cats on the restricted calorie diet showed lower
stimulated insulin concentrations compared with the maintenance diet (P=0.05, Figure 6).

![Figure 6](image.png)

Figure 6. Effect of Diet on Stimulated Insulin (InsulinST) Concentrations.
Figure 6: Continued

Insulin_{ST} was calculated by subtracting the baseline insulin concentration from the maximum insulin concentration measured. MD represents the maintenance diet, and RCD represents the restricted calorie diet. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values. For linear regression modeling, data from each cat for each diet (MD, RCD) were pooled. MD represents pooled data from both timepoints on the maintenance diet (ObMD and LeanMD). RCD represents pooled data from both timepoints on the restricted calorie diet (ObRCD and LeanRCD). There was a higher stimulated insulin concentration on the maintenance diet compared to the restricted calorie diet (P=0.05). The asterisk (*) indicates statistical significance.

In the lean state, baseline GLP-1 concentrations were lower than in the obese state (P=0.02, Figure 7). There was a moderate negative correlation between body fat mass and the stimulated GLP-1 (\( \rho = -0.483 \), P=0.03) and the GLP-1_{AUC} did not differ between time points. There was no effect of long-term exposure to diet on baseline, stimulated or GLP-1 AUC concentrations.
Figure 7. Effect of Body Condition on Baseline GLP-1. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values. For linear regression modeling, all data from each cat’s body condition (obese, lean) were pooled. Obese represents pooled data from both timepoints in the obese state (ObMD and ObRCD). Lean represents pooled data from both timepoints in the lean state (LeanMD and LeanRCD). Cats in the obese body condition had a higher baseline GLP-1 than the cats in the lean condition (P=0.02). The asterisk (*) indicates statistical significance.

There was a moderate positive correlation between body fat mass and baseline GIP ($\rho =0.524$, $P=0.03$). When controlling for cat-specific effects, there was a significant effect of adiposity on GIP$_{AUC}$ ($p=0.03$), with the cats in the leaner state secreting more GIP than the cats in the obese state (Figure 8).
Figure 8. Effect of Body Condition on GIP\textsubscript{AUC}. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values.

For linear regression modeling, all data from each cat’s body condition (obese, lean) were pooled. Obese represents pooled data from both timepoints in the obese state (ObMD and ObRCD). Lean represents pooled data from both timepoints in the lean state (LeanMD and LeanRCD). Cats in the lean body condition had a higher GIP\textsubscript{AUC} than the cats in the obese condition (P=0.03). The asterisk (*) indicates statistical significance.

On the restricted calorie diet, cats tended to have lower baseline GIP concentrations than on the maintenance diet (P=0.08, Figure 9).
Figure 9. Effect of Diet on Baseline GIP. MD represents the maintenance diet, and RCD represents the restricted calorie diet. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values. For linear regression modeling, all data from each cat for each diet (MD, RCD) were pooled. MD represents pooled data from both timepoints on the maintenance diet (ObMD and LeanMD). RCD represents pooled data from both timepoints on the restricted calorie diet (ObRCD and LeanRCD). Cats on the maintenance diet tended to have a higher baseline GIP than the cats on the restricted calorie control diet (P=0.08).

In contrast, stimulated GIP concentrations tended to be higher on the restricted calorie diet than on the maintenance diet (P=0.08, Figure 10).
Figure 10. Effect of Diet on Stimulated GIP (GIP<sub>ST</sub>). MD represents the maintenance diet, and RCD represents the restricted calorie diet. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values. For linear regression modeling, all data from each cat for each diet (MD, RCD) were pooled. MD represents pooled data from both timepoints on the maintenance diet (ObMD and LeanMD). RCD represents pooled data from both timepoints on the restricted calorie diet (ObRCD and LeanRCD). Cats on the restricted calorie diet tended to have a higher stimulated GIP AUC than the cats on the maintenance diet (P=0.08).

There was also a similar effect of diet on the GIP<sub>AUC</sub> (P=0.02) with the cats on the restricted calorie diet having a higher median GIP<sub>AUC</sub> than the cats on the maintenance diet (Figure 11).
Figure 11. Effect of Diet on GIP<sub>AUC</sub> Secretion. MD represents the maintenance diet, and RCD represents the restricted calorie diet. Boxes represent the interquartile range, the bar represents median. The whiskers represent the minimum and maximum values. For linear regression modeling, data from each cat for each diet (MD, RCD) were pooled. MD represents pooled data from both timepoints on the maintenance diet (ObMD and LeanMD). RCD represents pooled data from both timepoints on the restricted calorie diet (ObRCD and LeanRCD). Cats on the restricted calorie diet had a higher GIP<sub>AUC</sub> than the cats on the maintenance diet (P=0.02). The asterisk (*) indicates statistical significance.
<table>
<thead>
<tr>
<th>GIP (pg/mL)</th>
<th>ObMD</th>
<th>ObRCD</th>
<th>LeanRCD</th>
<th>LeanMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>Mean ± SD Median (range)</td>
<td>12 ± 9 13 (0-25)</td>
<td>9 ± 6 8 (2-17)</td>
<td>8 ± 7 7 (0-20)</td>
</tr>
<tr>
<td><strong>GIP&lt;sub&gt;ST&lt;/sub&gt;</strong></td>
<td>Mean ± SD Median (range)</td>
<td>206 ± 98 181 (93-377)</td>
<td>355 ± 145 311 (177-523)</td>
<td>457 ± 241 335 (271-687)</td>
</tr>
<tr>
<td><strong>GIP&lt;sub&gt;AUC&lt;/sub&gt;</strong></td>
<td>Mean ± SD Median (range)</td>
<td>44,209 ± 23,290 41,618 (22,869-85,121)&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>63,244 ± 22,299 61,178 (32,871-93,658)&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>100,656 ± 54,490 83,571 (51,923-193,283)&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 3. Mean ± Standard Deviation (SD), Median (Range) Baseline GIP, GIP<sub>ST</sub>, and GIP<sub>AUC</sub> for cats in each of the 4 timepoints: ObMD, ObRCD, LeanRCD, and LeanMD.

Continued
Table 3: Continued

ObMD represents the obese cats on the maintenance diet, ObRCD represents the obese cats on the restricted calorie diet, LeanRCD represents the lean cats on the restricted calorie diet, and LeanMD represents the lean cats on the maintenance diet. For linear regression modeling, all data from cats for each treatment variable (Ob, Lean, MD, RCD) were pooled. Identical superscripts within a row represent pooled data that together is significantly different than pooled data from treatment groups with different superscripts (P < 0.05).
### Table 4.

Mean ± Standard Deviation, Median (Range) Baseline Insulin, Insulin\textsubscript{ST}, and Insulin\textsubscript{AUC} for cats in each of the 4 timepoints: ObMD, ObRCD, LeanRCD, and LeanMD.

<table>
<thead>
<tr>
<th>Insulin (ng/L)</th>
<th>ObMD</th>
<th>ObRCD</th>
<th>LeanRCD</th>
<th>LeanMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>Mean ± SD Median (range)</td>
<td>116 ± 120 82 (18-341)</td>
<td>157 ± 73 138 (86-241)</td>
<td>67±27 62 (35-116)</td>
</tr>
<tr>
<td><strong>Insulin\textsubscript{ST}</strong></td>
<td>Mean ± SD Median (range)</td>
<td>260 ± 286 163 (42-671)\textsuperscript{a}</td>
<td>202 ± 203 107 (54-554)\textsuperscript{b}</td>
<td>126 ± 51 142 (57-184) \textsuperscript{b}</td>
</tr>
<tr>
<td><strong>Insulin\textsubscript{AUC}</strong></td>
<td>Mean ± SD Median (range)</td>
<td>36,321 ± 24,076 32,224 (12,760-73,878)</td>
<td>53,410 ± 10,320 54,139 (38,943-65,093)</td>
<td>37,872 ± 11,156 36,202 (25,573-56,144)</td>
</tr>
</tbody>
</table>

Continued
Table 4: Continued

ObMD represents the obese cats on the maintenance diet, ObRCD represents the obese cats on the restricted calorie diet, LeanRCD represents the lean cats on the restricted calorie diet, and LeanMD represents the lean cats on the maintenance diet. For linear regression modeling, all data from cats for each treatment variable (Ob, Lean, MD, RCD) were pooled. Identical superscripts within a row represent pooled data that together is significantly different than pooled data from treatment groups with different (P < 0.05)
<table>
<thead>
<tr>
<th>GLP-1 (pM)</th>
<th>ObMD</th>
<th>ObRCD</th>
<th>LeanRCD</th>
<th>LeanMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>Mean ± SD</td>
<td>Median (range)</td>
<td>Mean ± SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>GLP-1ST</td>
<td>15±6</td>
<td>14 (7-22)</td>
<td>16 ± 9</td>
<td>13 (8-29)</td>
</tr>
<tr>
<td>GLP-1AUC</td>
<td>19 ± 12</td>
<td>14 (6-24)</td>
<td>23 ± 12</td>
<td>22 (5-39)</td>
</tr>
<tr>
<td>GLP-1AUC</td>
<td>8,190 ± 2,737</td>
<td>8,8-4 (4,741-12,458)</td>
<td>8,660 ± 3,224</td>
<td>9,191 (3,513-12,106)</td>
</tr>
</tbody>
</table>

Table 5. Mean ± Standard Deviation, Median (Range) Baseline GLP-1, GLP-1ST, and GLP-1AUC for cats in each of the 4 timepoints: ObMD, ObRCD, LeanRCD, and LeanMD.

Continued
Table 5: Continued

ObMD represents the obese cats on the maintenance diet, ObRCD represents the obese cats on the restricted calorie diet, LeanRCD represents the lean cats on the restricted calorie diet, and LeanMD represents the lean cats on the maintenance diet. For linear regression modeling, all data from cats for each treatment variable (Ob, Lean, MD, RCD) were pooled. Identical superscripts within a row represent pooled data that together is significantly different than pooled data from treatment groups with different superscripts (P <0.05)
Chapter 4: Discussion

In humans and animal models, both obesity and diet impact blood glucose, insulin, and enteroendocrine hormone secretion. Specifically, different nutrients have different effects on the enteroendocrine cells in terms of acute stimulation of hormone secretion. In this study we demonstrated for the first time in cats that long-term exposure to different diets alters the enteroendocrine response to a fixed stimulus. Over days, and compared to a maintenance diet, feeding a reduced calorie diet was associated with higher fasting GIP concentrations and increased GIP secretion after a standardized meal test. One of our primary goals was to evaluate if long-term diet exposure could modify the magnitude of response of enteroendocrine cells to a fixed stimulus, regardless of which specific nutrients contribute to this alteration. We therefore selected two study diets that are different in multiple aspects in order to maximize the effect. Compared to the maintenance diet, the reduced calorie diet contained 20% more protein, 42% less fat, 2.4 times more carbohydrates, and 30 times more fiber on a per weight basis than the maintenance diet (Table 1). In humans and mice, chronic exposure (at least 5 weeks) to a high fat diet results in increased GIP secretion [79, 81]. Dietary fiber also appears to impact GIP secretion, and may be related to the type of fiber (soluble versus insoluble, fermentable versus nonfermentable) and the duration of exposure. Short-term soluble
fiber supplementation in humans has been associated with decreased GIP secretion [93], while short-term supplementation with insoluble [94] and mixed [95] fiber sources have been shown to enhance GIP secretion. Long-term supplementation with wheat bran (an insoluble fiber) caused a progressive decrease in GIP secretion in response to the test meal in healthy humans [83]. Because of our study design, the long-term effect of specific dietary factors on GIP secretion in cats could not be determined and it is important to emphasize that these factors may be different than what has been described in other species. It has already been shown before that the distribution of enteroendocrine cells in cats as well as their acute responses to stimuli differ from those of other mammals. Unlike other mammals, GIP-secreting K cells in cats are abundant throughout the intestines, including in high concentrations in the ileum and still with significant presence in the colon [35]. Also in contrast to other mammals, oral glucose does not stimulate GIP secretion in cats [36]. Therefore, while the present study generated some interesting hypotheses regarding potential factors that contribute to long-term regulation of GIP secretion, it will be unwarranted to extrapolate from data in other mammals on which one of these hypotheses is more likely and should be examined first. Further research is needed to elucidate the specific dietary factors that influence GIP secretion in cats.

Only one other study to date has examined the effect of adiposity on GIP secretion in cats [96]. This previous study evaluated GIP secretion in lean cats, overweight cats, and overweight diabetic cats and found no significant differences in baseline and stimulated GIP secretion between overweight cats and lean cats. This
seemingly conflicts with our findings however, our study followed a repeated measures design that examined a single group of cats that was originally obese and then underwent a period of weight loss, whereas the previous study [96] examined different groups of cats with stable body conditions. A stable lean or obese body condition likely represents an entirely different physiologic state than a newly-lean body formed as a result of a recent period of weight loss and calorie restriction [68, 97]. Another potential reason for differences between our study and the previous study is the fact the previous study [96] did not provide a standardized diet to all cats throughout the study, and instead used different diets for the initial baseline measurements and for the follow up timepoints. Failing to adequately control for diet and failing to maintain a consistent diet throughout the study could have confounded the pattern of GIP secretion.

Studies in people regarding the impact of obesity on GIP report conflicting results. Some identified normal GIP responses in obese compared to lean subjects [68], others have identified decreased [73] or increased [98] secretion profiles in obese patients. One potential reason for these inconsistencies may be related to the size of the nutrient stimulus used to induce GIP secretion. One study found that the exaggerated GIP response in obesity appears to be related to the ingestion of a high-calorie meal, and that this exaggerated GIP secretion pattern does not occur when the test subjects are given a lower calorie stimulus meal [99]. In addition, these most studies have focused on comparing GIP secretion between lean and obese individuals, as opposed to measuring changes that may occur as a result of weight loss, as was performed in our study. Human studies examining the specific effect of weight loss on changes in GIP secretion have also
yielded mixed results [68, 97]. One study found that obese subjects who underwent a 6 month period of weight loss had a decrease in total GIP secretion in response to a meal stimulus test [68]. Although participants in this study were instructed to adhere to a low calorie diet, this study didn’t control specifically for the type of diet or macronutrient content, which have been known to alter GIP secretion [81, 83]. Another group performed a similar study evaluating differences in hormones before weight loss, after an 8 week period of weight loss, and after 52 weeks of weight loss maintenance [97]. GIP secretion was increased by 36% after 8 weeks of weight loss, but then returned to the baseline levels at 52 weeks [97]. Combining the results of these 2 human studies and consistent with the results of our study, perhaps food restriction itself triggers increased GIP responses but the lean state in itself has no effect (or an opposite effect). In our study, we examined changes in enteroendocrine hormone secretion immediately after 11 weeks of weight loss and while food intake was still restricted. At that time, stimulation of GIP secretion by a standard meal was greater in the lean state than in the obese state. This trend was opposite when looking at baseline GIP, with a positive correlation between body fat mass and baseline GIP secretion. It is possible that the changes we found were related to the initial phase of weight loss or food restriction, and that over a longer time period (or when in a maintenance phase when caloric intake is appropriate for the new lean state) the results would be different. On the other hand, metabolic adaptation (weight loss resulting in a slowing of the metabolic rate that is greater than what would be expected based on the change in body composition alone) persists in people for years in people [100]. Further investigation would need to be performed in cats to understand
differences in GIP secretion that may occur during that he maintenance phase of weight loss as opposed to the acute weight loss phase.

In our study, there was no effect of diet on GLP-1 concentrations and there was no effect of adiposity on overall post-prandial GLP-1 secretion. However, fasting GLP-1 concentrations were lower in lean vs. obese. These findings contrast with the findings of another study that found decreased overall GLP-1 secretion in obese vs. lean cats after oral glucose [101]. We did find a negative correlation of peak stimulated GLP-1 with body fat mass which is overall in line with the previous study. There are several potential explanations for the discrepancy between the two studies. In a repeated-measures design, our study compared differences before and after weight loss, as opposed to examining two different groups of cats (lean and obese) with stable body condition at a single time point [101]. In people, GLP-1 secretion was lower in obese before and after weight loss compared to lean subjects, although weight loss improved the response [68, 97]. Another difference between our study and the study by Hoenig et al. was in the measurement of GLP-1. Hoenig et al measured active GLP-1 (7-36), while we measured total (active and inactive) GLP-1 (7-36, 9-36). Total GLP-1 represents all GLP-1 that was secreted from the gut (and its entire biological effect including paracrine and vagal-mediated) while active GLP-1 represents only a small fraction (about 15%) of the total that has direct effect on the pancreas. Importantly, the proportion of active GLP-1 is not fixed and can be altered in different disease states. For example, human diabetics have decreased active GLP-1, but normal total GLP-1 concentrations [98].
Our study also differs from the Hoenig et al. study in the use of a meal stimulus as opposed to an oral glucose stimulus [101]. It is possible that of glucose alone stimulates GLP-1 secretion from a subset of L cells that are regulated differently than the entire population of L cells that are stimulate by a whole meal. If that is the case, it is likely that these different subsets of cells would be regulated differently and affected differently by obesity. In our study, we used a standard meal stimulus (Hill's® Prescription Diet® a/d®) to control for the acute effects of diet on enteroendocrine hormone secretion. This diet was chosen because of its high palatability, which allowed the cats to consume it consistently and quickly. A final reason for the differences between these two studies may involve the fact that in the study by Hoenig et al., [101] immediately following glucose-administration 25% of the cats developed acute diarrhea and another 10% vomited. No adverse effects occurred in our study. Acute diarrhea has been shown to increase enteroendocrine hormone secretion in humans [46] and the effect of vomiting has not been tested. Similarly to our study, another study that examined client-owned cats found no differences in GLP-1 secretion between overweight cats and lean cats, although there was a difference between overweight cats and diabetes [96].

Baseline and post-prandial blood glucose did not differ with dietary modifications, or with changes in adiposity in our study. The effect of diet on pre and post-prandial blood glucose in cats has been previously examined. Specific dietary modifications, including the feeding of high carbohydrate [102, 103] and high fat diets [104] are associated with increased pre-and post-prandial blood glucose concentrations compared to control diets. However, some of these studies failed to control for differences in other macronutrients
(such as fat and protein), possibly confounding the results [102, 103]. Obesity has also been associated with increased pre-and post-prandial blood glucose levels [104] but in that study diet was not adequately controlled [104].

In order to evaluate insulin resistance, we measured insulin concentrations and calculated insulin/glucose ratios at all time points. While fasting and post-prandial insulin concentrations did not differ between lean and obese, there was a moderate positive correlation between the insulin:glucose ratio and body weight, suggesting that adiposity was associated with decreased insulin sensitivity as expected. Previous studies have shown that weight gain is associated with decreasing insulin sensitivity as measured by intravenous [105] and oral [101] glucose tolerance testing. One explanation for the lack of difference in absolute insulin concentrations between lean and obese in our study is the high inter- and intra-biological variability of insulin concentrations [104]. In addition, although the cats did undergo a marked weight loss, in some the changes in adiposity may not have been large enough to cause a significant change in insulin sensitivity. One of the cats in our study remained overweight despite losing 20% of his original body weight. Finally, insulin resistance can occur independent of obesity, and lean individuals with insulin resistance have been identified in both humans [106] and in cats [105]. Given the small numbers of cats in the current study, these individual differences may have made it difficult to find differences in insulin sensitivity between time points.

Our study had several limitations. Considering our small sample size, a Type II error could explain the lack of effect of diet and weight loss on post-prandial GLP-1 secretion, insulin secretion, or blood glucose levels. Also, we did not report on changes in fecal
microbiota or adipokines which play important roles in the regulation of EEC secretion [59, 88, 107-109]. Our study is the first to establish that long-term dietary exposure alters GIP secretion response to a fixed stimulus in cats, however, cats were evaluated after variable exposure times to a diet (at least two weeks). This variability in exposure time was a compromise that was intended to prevent unwanted change in body weight. For example, in the obese state, if we had prolonged the period of ad lib feeding of the weight loss diet in order to make it equivalent to the exposure to the maintenance diet, we could have had significant weight loss confounding our results. Although previous research has shown that enteroendocrine cells turnover occurs every 5-7 days [110] it is possible that two weeks were insufficient time to maximize the effect of diet on EEC secretion, and that equalizing exposure time across all treatments would have shown a more pronounced effect.

Another limitation to our study involves the fact that we did not use histopathology to quantify enteroendocrine cells. Several studies in rodents have evaluated the effects of dietary manipulation on EEC distribution, and have found mixed effects [81, 82, 111]. In mice, a high fat diet led to increased density of K cells [81] and L cells [111]. In contrast, a study in rats [82] found that a high fat diet caused decreased EEC numbers (through decreased differentiation from stem cells). Although the effect of high fat diet differed in mice and rats, the mechanism of the effect was similar, mediated by a change in numbers of cells. Whether or not other mechanisms are involved is yet unknown. For example, differences in secretory responses could be related to alterations in sensitivity to stimuli or the capacity of individual cells to secrete with alteration in
hormone storage. Combining the study of EEC secretion with evaluation of adipokines, fecal microbiota, and evaluation of histopathologic changes will help to provide more complete insight to the complex mechanisms that impact EEC proliferation and hormone secretion.

In conclusion, the feeding of a low fat, high fiber, high carbohydrate diet was associated with increased GIP secretion in cats. A leaner body condition was also associated with increased GIP secretion. Further investigation is needed to understand the specific nutrients that effect changes in GIP secretion, and specific hormonal changes that accompany weight loss or the maintenance of a lean body condition. Gaining a better understanding of the factors that influence enteroendocrine hormone secretion in cats may help guide more targeted therapies for the prevention and treatment of metabolic disorders such as obesity and diabetes.
References


