



Microbiota, Enteroendocrine Cells and Weight Loss in Cats

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Abstract

Enteroendocrine cells (EEC) are the first-line sensors of the quality and quantity of dietary nutrients in the digestive system. EEC also sense and respond to many other compounds in the gut including bile acids and bacterial metabolic products. The information that is acquired through sensing of luminal content by EEC is relayed to other organs through a variety of hormones. These hormones affect the central nervous system, the pancreas, adipose tissue, and other organs. The EEC together with the central nervous system are an integral component in the regulation of systemic metabolism. Obesity is an epidemic in humans and animals, and in recent years, significant strides have been made in understanding the role of the gut in all facets of obesity from its development through its maintenance, resistance to weight loss, and difficulty maintaining a lean state after weight loss. In this lecture, we will discuss some of the aspects pertaining to the interaction between diet, gut microbiota, and EEC as it relates to obesity in general and to weight loss in obese cats in particular.

Gut Microbiota and Obesity

The gut is a natural environment for a diverse and dynamic microbial ecosystem, and its structure and functions are now a major target of research. The composition of gut microbiota varies after birth, but it becomes relatively stable when the animal matures and throughout adulthood.¹ These stable populations of commensals are maintained by the interaction of gut mucosa, the immune system, and the intestinal microbiota itself. This is despite constant exposure to a variety of antigens, food ingredients, and pathogenic bacteria. Interestingly, the gut microbiome of an animal is determined to a large extent by the evolutionary adaptation of the animal to a diet rather than other factors in the animal's immediate environment.^{2,3} However, dietary changes still can have a

Glossary of Abbreviations

BCS: Body Condition Score

DEXA: Dual-Energy X-Ray Absorptiometry

DM: Diabetes Mellitus

EEC: Enteroendocrine Cells

GIP: Glucose-Dependent Insulinotropic Peptide

GLP-1: Glucagon-Like Peptide-1

IMT: Intestinal Microbiota Transfer

LPS: Lipopolysaccharide

MC4R: Melanocortin-4 Receptor

SCFA: Short-Chain Fatty Acids

VAN: Vagal Afferent Neurons

profound effect on the microbiota. For example, shifting mice from a low-fat plant polysaccharide-rich diet to a high-fat, high-sugar diet caused a change in the microbiota that was detectable within 24 hours and stabilized within seven days.⁴

Accumulating data also suggests that intestinal microbiota are involved in a wide variety of autoimmune and metabolic

diseases throughout the body (e.g., multiple sclerosis, Crohn's disease, obesity, and diabetes). Intestinal microbiota transfer (IMT) from a healthy to a diseased patient has been proven effective in treating some of these conditions.¹ In some instances the native microbiota is a "healthy" one and should be restored by treatment (e.g., *Clostridium difficile* infections), but in others the native microbiota are part, if not the cause, of the pathological process.¹ When the intestinal microbiota is altered, such as during therapy directly in IMT or indirectly in dietary treatment of obesity, the recovery of the native microbiota after treatment cessation could be an important factor in the long-term success of therapy.

As in people, feline obesity is becoming increasingly prevalent.⁵ Obesity in people is associated with phylum-level changes in the microbiota, reduced bacterial diversity, and altered representation of bacterial genes and metabolic pathways. Studies of lean and obese mice suggest that the gut microbiota affect energy balance by influencing the efficiency of calorie harvest from the diet and how this harvested energy is used and stored.⁴ It has been demonstrated that transplantation of gut microbiota from mice with an obese phenotype to germ-free mice leads to development of an obese phenotype in the recipients.^{6,7} In mice, a large proportion of the energy that is extracted from the diet comes from absorption of bacterial fermentation products in the colon. This is unlikely to be the case in cats; however, colonic bacteria could affect systemic metabolism through fecal metabolomic changes. For example, it was recently demonstrated in cats that propionate,

a product of bacterial metabolism in the colon, acts as a gluconeogenic substrate in lean and overweight cats when absorbed from the colon.⁸

Improving energy extraction from the diet is only one mechanism by which gut microbiota could alter systemic metabolism and affect obesity. Possibly even more important are the effects of microbiota on the regulatory systems of the host, such as EEC and the nervous system. Enteroendocrine cells and the peripheral terminals of vagal afferent neurons (VAN) are positioned within the gut mucosa to respond to nutrients, bacterial products, and bile acids. EEC and VAN integrate this input and together control local and systemic responses, such as satiety, insulin sensitivity, and secretion, and more. Bacteria can affect EEC and VAN directly through their metabolic products, alteration in bile acids composition, and release of cell components such as lipopolysaccharide (LPS).

Plasma concentrations of LPS, a breakdown product of gram-negative bacteria, is increased in obese people and in rodents fed a high-fat diet. Obesity also is considered a state of chronic low-grade inflammation. Obesity may be the cause of increased absorption of LPS from the gut, which then leads to, or perpetuates, chronic inflammation. Alternatively, chronic exposure of LPS from the gut may be the cause of obesity. The latter has been supported by numerous observations. Knocking out the LPS receptor (TLR₄) in mice confers resistance to development of obesity when feeding a high-fat diet.^{9,10} A more recent study showed that chronic exposure to bacterial LPS induces leptin resistance in vagal afferents and promotes hyperphagia.¹¹

Enteroendocrine Hormones and Obesity

EEC secrete a variety of peptide hormones that participate in the control of intestinal function, such as motility and secretion, and also affect remote organs including the brain, liver, pancreas, and adipose tissue. Some of these hormones are directly linked to the development of obesity. Glucose-dependent insulintropic peptide (GIP) is a peptide hormone that is secreted from EEC (K cells) and is a major incretin hormone (i.e., it increases the sensitivity of pancreatic beta cells to the effects of glucose). GIP also increases the sensitivity of adipose tissue to the effect of insulin, thus promoting obesity. Glucagon-like peptide-1 (GLP-1) is secreted from EEC (L cells), and also is an incretin hormone. In contrast to GIP, GLP-1 decreases the tendency for weight gain. GLP-1 receptors are expressed in several brain-stem nuclei involved in appetite regulation.¹² GLP-1 suppresses appetite and energy intake in normal weight and obese individuals. In animal models of obesity that include miniature pigs and rodents, GLP-1 analogs reduce feeding frequency and meal size.¹² GLP-1 also promotes satiety and weight loss through decreasing the rate of gastric emptying. GLP-1 is so effective in promoting weight loss that recently the long-acting GLP-1 analog liraglutide was approved by the Food and Drug Administration for treatment of obesity in people.¹³

Unlike long-acting analogs, however, native GLP-1 requires either a postprandial state or an ongoing meal to induce satiation.¹⁴

Prolonged fasting attenuates the satiating effects of GLP-1. GLP-1 receptors are constitutively expressed in VAN, but under fasting conditions, they are mostly located in the cytoplasm, rendering the effect of GLP-1 insignificant in the fasting state. In a postprandial state, there is a 42% increase in GLP-1R's at the plasma membrane of VAN, rendering them responsive to the effect of GLP-1.¹⁵ This phenotypic shift in GLP-1R expression in VAN is mediated by the effect of ghrelin. Ghrelin is an orexigenic hormone that is secreted from EEC in the stomach (A-like cells) during the fasting state. In the hypothalamus, ghrelin stimulates appetite, body weight gain, and adiposity.¹⁴ Leptin has opposite effects to ghrelin in the hypothalamus. Leptin secretion from adipocytes is proportional to their total mass, but leptin also is increased postprandially. Obesity develops in individuals who are leptin-resistant, but interestingly, leptin resistance in the hypothalamus occurs after the onset of the obese phenotype.¹⁴ In contrast, leptin resistance in VAN leads to weight gain and polyphagia, and precedes the obese phenotype.¹⁶

Leptin interacts with GLP-1 to induce satiation at the level of the hypothalamus and peripherally, in part, by stimulating GLP-1 release from L cells.^{14,17} It would be expected then that in obese individuals, GLP-1 would be increased as a result of increasing leptin stimulation, unless leptin resistance occurs at the level of L cells or if other obesity-related factors suppress GLP-1 secretion. In people and in cats, there are conflicting reports on GLP-1 responses in obesity with some showing lower GLP-1 plasma concentrations in obese versus lean, while others show the opposite.^{18,19}

The melanocortin-4 receptor (MC₄R) is expressed in the hypothalamus and is responsible for mediating the feeling of satiety in situations of positive energy balance.²⁰ In human obesity and diabetes, MC₄R is a major susceptibility gene. This receptor may be the link between diabetes, obesity, and abnormalities in the incretin effect. Recently, it was discovered that MC₄R also is expressed in intestinal L cells and mediates the secretion of GLP-1.²¹ Interestingly, in cats, polymorphism in the MC₄R gene was demonstrated in obese diabetics but not in obese non-diabetics.²⁰ It could be speculated that cats with a polymorphism in this gene are predisposed to both obesity and reduced incretin effect making them more likely to develop diabetes mellitus (DM). In cats that do not have this polymorphism, obesity is caused by other factors, and with an intact incretin effect, they are able to compensate for insulin resistance and do not develop DM.

Microbiota and Enteroendocrine Hormone Interactions in Obesity and Weight Loss in Cats

Degree of adiposity, dietary macronutrient composition, and gut microbiota affect the secretion of enteroendocrine

hormones, and those, in turn, affect food intake and weight gain. Little is known about these interactions in cats. The feline gut metagenome has been described in a small number of overweight cats.^{22,23} Barry, et al., found that several phyla within the cat's fecal microbiome were affected by fiber content at 30 days after a change in diet. This study, however, did not assess a possible correlation with body weight or the rate of conversion back into the original phyla distribution after the original diet was resumed.²² We have studied gut microbiota, fecal metabolomics, and incretin hormones before and after weight loss in purpose-bred cats.^{24,25} Obese cats were initially fed a maintenance diet (MT),^a then switched to a weight-loss diet (WL)^b without calorie restriction. After a week on the WL diet, caloric restriction was initiated to achieve 1 to 2% weight loss per week. After 12 weeks of weight loss, the cats were switched back to the MT diet, while maintaining stable body weight and body condition score (BCS). Fecal samples for microbiota and short-chain fatty acid (SCFA) profiles were collected during four study conditions: Obese/MT diet, Obese/WL diet, Lean/WL diet, and Lean/MT diet. A standardized meal test (SMT) using a third diet also was performed at each of the four conditions. For each condition, the SMT was performed after fecal samples were collected so that the third diet did not affect microbiota assessment. In each SMT, glucose, insulin, GLP-1, and GIP concentrations were measured immediately before and over six hours after feeding. Body weight, BCS, muscle condition score, and body fat percentage using dual-energy X-ray absorptiometry (DEXA) were assessed throughout the study. Some of the results of these study are discussed below.

The bulk of fecal microbiota typically is comprised of two main phyla, *Bacteroidetes* and *Firmicutes*, with a predominance of *Bacteroidetes* noted in lean individuals.^{26,27} In some studies of diet-induced obesity, this ratio shifts to a greater prevalence of *Firmicutes*.²⁶ In our cat study, microbiota and metabolomic profiles revealed changes associated with diet and BCS consistent with reports of diet-induced obesity in murine and human studies. One of the more interesting changes was the increase in the butyrate-generating *Faecalibacterium prausnitzii* while on the WL diet. This bacterium also is increased in human type II diabetics.²⁸ However, we did not find an overall increase in butyric acid production while on the WL diet. We did find that in lean and obese cats there was an increase in propionic acid while fed the WL diet compared to the MT diet. In obese cats fed the WL diet, production of isobutyric acid, isovaleric acid, and valeric acid was decreased compared to when fed the MT diet. When comparing lean to obese states, when fed the MT diet, the lean cats had decreased production of acetic acid, butyric acid, isobutyric acid, and isovaleric acid compared to when they were obese.

Surprisingly, total postprandial GIP secretion in lean cats was increased compared to obese cats. Also unexpected

was the finding that total postprandial GIP secretion was increased on the WL diet compared to cats on the MT diet. This was unexpected because this diet is lower in fat and higher in carbohydrates, a profile that with acute stimulation in cats is associated with reduced GIP secretion.²⁹ Perhaps the increase in GIP secretion on the WL diet was the result of an increase in propionate production by microbiota, signaling K cells to increase secretion. We also found that compared to lean cats, obese cats had higher fasting GLP-1 concentrations, but there was no effect of diet or adiposity on postprandial secretion of GLP-1.

Overall, our data suggests that the microbiota and their fermentation products are altered in cats not just as a result of diet but also independently, as a result of obesity. Our results also suggest that in cats, as observed in other species,³⁰ long-term exposure to certain components of a diet could affect the capacity of EEC to respond to acute stimulation. Likely, this altered capacity to respond to stimuli is the result of changes in the numbers or distribution of EEC. These changes could be a direct effect of the diet or could be mediated by microbiota and their metabolic products. These represent potential novel targets for treatment of obesity and maintenance of the lean state. For example, an effort could be made to reduce the proliferation of GIP-secreting cells by designing weight loss and maintenance diets that are low in carbohydrates. Interestingly, based on SCFA profiles, the microbiota of the obese phenotype on the maintenance diet was not completely restored when the cats were lean and then refed the maintenance diet. This could suggest that gut microbiota in obese cats are opportunistic and enabled by a diet causing the obese phenotype and that dietary treatment of obesity may result in recovery of the cat's "lean" microbiota, which could then stabilize and resist recurrence of obesity. More research is needed to identify the specific role of microbiota in cats and their metabolic products that affects EEC. Further studies also are warranted to determine the impact of altering microbiota populations in cats with inflammatory diseases and other metabolic disorders.

Conclusion

Induction of weight loss can be achieved by reducing caloric intake. However, maintaining weight loss over time or decreasing the tendency to gain weight is difficult. Multiple factors are at play in this process, including gut microbiota, EEC, the nervous system, and the interaction between them. Enteroendocrine cells, by nature of their physical location and capacity to integrate a variety of inputs and send a variety of messages, are the mediators between gut microbiota and the host. In cats, the capacity to secrete GIP increases with weight loss and high-fiber, high-carbohydrate diet. This increased capacity to secrete GIP may make it more difficult to maintain lean-body weight in cats because of increased insulin sensitivity in adipose tissue. The role of

GLP-1 in food intake and the therapeutic potential of microbial metabolic products in increasing GLP-responses and decreasing GIP responses requires further investigation.

Footnotes

^a Purina® Friskies® Classic Paté Mariner's Catch, Nestlé Purina PetCare, St. Louis, MO

^b Purina® Pro Plan® Veterinary Diets® OM Overweight Management® Feline Formula, Nestlé Purina PetCare, St. Louis, MO

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