JIVS Journal of Istanbul Veterinary Sciences

Pet microbiota and its relationship with obesity

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Review Article
Volume: 8, Issue: 2
August, 2024
Pages: 131-139

Article History
Received: 04.05.2024
Accepted: 04.06.2024
Available online:
17.06.2024

ABSTRACT

The incidence of obesity in pets appears to be increasing in line with the increasing incidence of obesity in humans, and leads to decreased life expectancy. Obesity, which is considered a multifactorial disease caused by excessive adiposity, leads to a decrease in quality of life and serious health problems. It is known that there is an increase in the incidence of respiratory disorders, cardiological disorders, metabolic and endocrine problems, orthopedic diseases and some types of cancer in obese cats and dogs. There are many factors in the formation of obesity. One of these factors is the balance of the microbiota in gut. Many studies have shown that the microbiota affects critical steps in the formation of obesity and there are strong relationships between dietary content, microbiota, and obesity. In particular, high-fat diets are known to increase microbiome composition in terms of gram-negative bacterial strains and trigger dysbiosis. Again, in cases where dysbiosis occurs, the levels of volatile fatty acids also vary and lead to undesirable results through hormonal mechanisms. This condition, which causes hyperphagia, hypertriglyceridemia and insulin resistance, increases the incidence of obesity and diabetes mellitus. The ratio of Firmicutes and Bacteroidetes, which are among the largest phylae of the microbiota, shows serious differences when compared in underweight and obese animals. In this article, these relationships between microbiota and obesity are reviewed.

Keywords: obesity, pet nutrition, microbiota, prebiotic, probiotic

DOI: https://doi.org/10.30704/http-www-jivs-net.1478463
To cite this article: Kukirik, M., & Demirel, G. (2024). Pet microbiota and its relationship with obesity. ***Journal of Istanbul Veterinary Sciences, 8*(2), 131-139.** **Abbreviated Title*:* J. İstanbul vet. sci.**

Introduction

Obesity constitutes an important health problem in pets as well as in humans in developed countries. Most researchers agree that the incidence of obesity in the pet population, as in humans, is increasing and leading to a decrease in quality of life. A study conducted in 11 European countries reveals that 30-70% of pet dogs and 20-50% of dog owners are overweight (Muñoz-Prieto et al., 2018). Recent studies have shown that the prevalence of obesity in dogs in developed countries varies between 25% and 44% (Pegram et al., 2021). In the United Kingdom, obesity was the seventh most common disease reported in dogs in 2014, and in 2021 obesity was the third most common disease, after periodontal disease and otitis externa (O'Neill et al., 2021). According to the results of the Pet Obesity Research published by the Association for the Prevention of Pet Obesity in 2022, it was reported that 59% of dogs and 61% of cats were classified as overweight or obese (Anonymous, 2022). Energy imbalance is the main reason for the development of obesity in cats and dogs. In this context, excessive dietary intake or insufficient energy

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use leads to a state of positive energy balance, resulting in excess fat storage in the body (Kopelman, 2000). However, some pets may be classified as overweight due to markedly reduced energy expenditure due to neutering and reduced activity levels even if there is no increase in energy intake. Cats are generally regarded as being overweight when their body weight is at least 10-20%, obese 20% above ideal weight (Roudebush et al., 2008a).

Obesity alters the metabolic and endocrine function of adipose tissue, resulting in increased release of fatty acids, hormones, and pro-inflammatory molecules that contribute to obesity-related complications (Okada et al., 2017). Obesity-associated diseases include diabetes, insulin resistance, lipid profile abnormalities, orthopedic diseases, cardiorespiratory diseases, neoplasia, and shortened life expectancy (Roudebush et al., 2008b). In a lifelong study conducted with 48 Labrador Retriever dogs, it was found that the median life expectancy was significantly higher in the experimental group fed 25% fewer calories compared to the control group. In addition, it was determined that the onset of chronic health problems such as osteoarthritis was later in the experimental group consumed less calories (Kealy et al., 2002). In a study conducted with 1457 cats, it was revealed that obese or overweight cats were 3.9 times more likely to develop diabetes mellitus and 4.9 times more likely to develop lameness complaints than thin and lean cats (Scarlett & Donoghue, 1998).

Many factors are involved in the development of obesity, including inadequate lifestyle, neuronal and hormonal mechanisms, imbalance between energy intake and expenditure, and genetic and epigenetic factors. White adipose tissue is the most well-known type of fat where triglycerides are stored and lipids are mobilized for systemic use when other tissues need energy. Although once thought of as a passive fuel depot, white adipose tissue is now an active endocrine organ that provides feedback from the adipose tissue and communicates with the brain and peripheral tissues by secreting a wide variety of hormones and protein factors called adipokines, including leptin (Trayhurn, 2005).

Microbiota in cats and dogs

The gut microbiota can be defined as the total population of microbial species living in the digestive tract. From birth, a diverse community of microorganisms, including bacteriophages, bacteria, archaea, viruses, and eukaryotic microorganisms, use the digestive system as a host. This community of microorganisms makes an important contribution to the digestion and utilization of foods in the gastrointestinal tract. The mammalian gastrointestinal microbiota is diverse and complex, consisting of at least hundreds, perhaps thousands, of interdependent or competing species that are often poorly characterized (Ley et al., 2008). Microbial density varies greatly throughout the gut. While the stomach and small intestine contain a small number of bacteria, the large intestine has a much more dense population. Bacterial components are the largest in this population and provide basic digestive functions such as fermentation of fibers (Sender et al., 2016). In one study, molecular analysis using 16S rRNA sequencing revealed that Firmicutes, Fusobacteria, Bacteroidetes and Proteobacteria commonly reside in the gastrointestinal tract of dogs (Handl et al., 2011). According to studies conducted in dog feces, it has been observed that there is a core microbiota consisting of Firmicutes, Bacteroidetes and Fusobacteria (Hand et al., 2013). These phyla include bacteria belonging to the Clostridia and Bacilli classes, such as Faecalibacterium, which produce short chain fatty acids (SCFA), and Lactobacillus, which have probiotic properties by producing lactic acid (Pilla & Suchodolski, 2021a). In another study, it was shown that in the gastrointestinal tract of cats, Firmicutes and Proteobacteria, with the predominance of the Bacteroidetes phylum, are common, while archaea, fungi and viruses are found in much lower amounts (Tun et al., 2012). The dominant bacterial phyla in cat feces are Firmicutes, Bacteroidetes and Proteobacteria, respectively (Barry et al., 2012). High amounts of obligate anaerobic bacteria in the feces of cats are considered abnormal in dogs and humans (Johnston et al., 2001). While bacterial diversity in cats varies less between individuals, it is known that individual microbiota diversity in dogs is higher (Handl et al., 2011). The bacterial load, especially in the large intestine, is closely related to energy homeostasis, fat metabolism and obesity. The gut microbiota is also thought to play a role in regulating food intake by affecting metabolic function and hormones that stimulate areas of the brain associated with eating behavior (Hildebrandt et al., 2009).

The most important mediators that enable the microbiota to exert its effects on host health are bacterial metabolites such as tryptophan metabolites, SCFA and secondary bile acids (Lavelle & Sokol, 2020). SCFAs, especially acetate, propionate and butyrate, are products of bacterial fermentation of dietary fibers. It has been shown that acetate and propionate are mainly produced by the Bacteroidetes phylum, while butyrate is predominantly produced by the

Firmicutes phylum (Ismail et al., 2011). SCFAs contribute to intestinal health by acting as energy substrates for colonic epithelial cells, maintaining epithelial barrier integrity, regulating energy metabolism, and providing an anti-inflammatory effect (Koh et al., 2016). In addition, by regulating the satiety signal and intestinal movements, they lower the intestinal pH and provide the formation of an antimicrobial environment against pH-sensitive enteropathogens (Cherrington et al., 1991, Rowland et al., 2018). In addition to being an energy source for colonocytes in mice (Hartstra et al., 2015), butyrate has been shown to be effective against diet-mediated obesity without causing hypophagia (Lin et al., 2012a). It is known that propionate contributes to gluconeogenesis (Lin et al., 2012b), reduces cholesterol synthesis and increases leptin gene expression (Harris et al., 2012).

One of the ways that the microbiota exerts its effects is through lipopolysaccharides (LPS). LPS, an endotoxin found in the cell membrane of gram-negative bacteria, provides the immunomodulation activity of the microbiota. They are powerful activators of the inflammatory response and cause the inflammatory response to occur even in small amounts (de Vos et al., 2022). LPSs that activate Toll-like receptor 4 (TLR-4) induce antigen-presenting cell activation in this way. Thus, by establishing a connection between innate immunity and acquired immune response, a response to microbial factors occurs and signalling cascades for damaged tissue repair are activated (Poltorak et al., 1998). One of the microbiota's contributions to digestion is through secondary bile acids. Primary bile acids that escape the enterohepatic bile cycle undergo modifications such as deconjugation and dehydroxylation by bacteria in the intestine (de Vos et al., 2022). As a result, secondary bile acids are formed, changing the bioavailability and bioactivity of the bile cycle and their effects on the metabolic pathways in which they are involved (de Aguiar Vallim et al., 2013).

It is extremely important for the microbiota to be in an individual-specific order and balance to maintain general health. Imbalance within the microbiota is called dysbiosis and potentially leads to the formation of pathologies (Carding et al., 2015). Dysbiosis has been linked to inflammatory bowel disease, acute diarrhea, autoimmune diseases, various cancers, and obesity in humans (Rojo et al., 2017). The state of dysbiosis is thought to promote adiposity and lead to obesity through several different mechanisms. These include changes in satiety signals in the brain, regulation of hormones originating from the gastrointestinal tract, and affecting lipid metabolism in white adipose tissue and liver (Tremaroli & Bäckhed, 2012).

Dietary content and connection to microbiota

Gastrointestinal microbiota may vary depending on diet, eating habits and nutritional content. The bacterial population, which may vary depending on the substrate present in the intestinal tract, is primarily affected by the nutritional compositions that make up the diet. While changes in bacterial taxa require major changes in macronutrients in the diet, changes in micronutrients are required for bacterial metabolites and microbiota function changes (Pilla & Suchodolski, 2021b). While cats are obligate carnivores whose diets must be high in protein, dogs are metabolically omnivorous and can digest and metabolize higher amounts of carbohydrates than cats (Deng & Swanson, 2015). This difference in digestive systems is one of the factors that shape the diversity in the microbiota of cats and dogs. Microbiota changes depending on nutrients; It leads to a decrease in the synthesis of SCFAs, which protect the intestinal epithelial barrier integrity, reduce inflammation and increase the expression of hormones that suppress hunger. In addition, adipocyte expression is inhibited, resulting in dyslipidemia. It is argued that this situation will result in conditions such as low-grade chronic inflammation and obesity (Amabebe et al., 2020a). In this context, one of the most important factors affecting diversity is protein:carbohydrate ratio. In a study comparing the effects of a diet containing medium levels of protein and carbohydrates (MPMC) and two diets containing high levels of protein and low levels of carbohydrates (HPLC) on the microbiota in kittens, it was observed that the number of Actinobacteria in the feces was higher and the number of Fusobacteria was lower in kittens fed MPMC. Fecal Fusobacterium count was found to be highest in kittens fed by HPLC (Hooda et al., 2013). In another study conducted in 8 healthy adult cats, the effects of dietary protein levels on the microbiota were examined. While the bacterial similarity index was 66.7% in the group fed with a medium-level protein diet, this value was 40.6% in the group fed with a high-level protein diet. It was observed that Bifidobacterium populations were higher in cats fed a diet containing moderate protein than in the other group, while Clostridium perfringens populations were higher in the group fed a diet containing high levels of protein. The results of this study indicate that the amount of protein in the diet can cause dramatic changes in microbiota, and the necessity of probiotic/ prebiotic supplements should be considered in cats fed high protein diets (Lubbs et al., 2009). In a similar study conducted with canine subjects, it was shown that C. perfringens increased while the Bifidobacterium phylum decreased (Zentek et al., 2003). Similar studies have also been conducted on humans. In human subjects undergoing diet trials such as high protein-low carbohydrate or high protein-medium carbohydrate, fecal SCFA and butyrate concentrations have been shown to decrease in the group with reduced carbohydrate levels (Duncan et al., 2008). Supporting the results of this study, in another study, it was observed that fecal output decreased in the obese group on a high-fat diet, and the total SCFA amount, butyrate concentrations and Bifidobacteria count in the stool were lower in subjects fed diets containing different levels of carbohydrates and fat. It is thought that changes in the microbiota may be related to various gastrointestinal diseases (Brinkworth et al., 2009).

Diets containing high fat-carbohydrate make the microbiota rich in bacteria associated with pathogenicity such as Firmicutes (Clostridium), Prevotella and Methanobrevibacter, while beneficial bacteria such as Bacteroides, Bifidobacterium, Lactobacillus and Akkermansia are lacking (Amabebe et al., 2020b). Firmicutes:Bacteroidetes ratio is used to evaluate the increased amount of body fat and susceptibility to obesity (Dreyer & Liebl, 2018). It is thought that a microbiota composition that supports obesity may increase fatness by obtaining more energy from diet (Turnbaugh et al., 2008). It was observed that the amount of the Actinobacteria phylum increased while the amount of the Bacteroidetes phylum decreased significantly in a study investigating the effects of a diet containing moderate protein and high fiber on the microbiota of 8 neutered male obese cats with limited feeding. Likewise, it was concluded that Prevotella bacteria decreased significantly with weight loss (Pallotto et al., 2018). In a study in which a commercial weight loss diet was applied to 22 obese cats for 24 weeks, in the first sampled microbiota composition was observed to be the most dominant phylum Firmicutes, followed by Bacteroidetes. After restricted feeding with a commercial diet containing high protein and high fiber content, it was observed that there were decreases in 7 bacterial species belonging to the Firmicutes phylum. Additionally, decreases in concentrations of fecal metabolites including SCFA, branched-chain fatty acids, phenol, and indoles have been reported. As a result of this study, it is understood that restricted feeding along with dietary content may have an effect on the microbiota due to the decreasing amount of substrate for the colonic microbiota (Opetz et al., 2023). One of the effects of diet content on obesity and general health status is through LPSs. High fat content in the diet and uncontrolled increases in body weight cause high intestinal permeability, increasing the circulating plasma LPS level. Thus, the metabolic endotoxemia situation is occurred (Cani et al., 2007).

It is known that probiotic supplements given with the diet have positive effects on health. A decrease in the number of Clostridium spp. and Enterococcus faecalis in the feces was observed in 15 cats in which Lactobacillus acidophilus supplement was used. In addition, while plasma endotoxin concentrations decreased, an increase in phagocytic capacity was demonstrated in peripheral granulocytes. These results are evidence that probiotic supplements added to the diet create positive systemic changes in addition to immunomodulatory effects (Marshall-Jones et al., 2006).

Relationship between microbiota and obesity

High-fat diets alter the composition of the microbiome in a way that increases the proliferation of gram-negative bacterial strains, namely Bacteroidetes. This causes increased production of LPS, a component of the Gram-negative cell membrane, and increased intestinal permeability. High levels of LPS and SCFA production activate TLR-4, which selectively binds LPS and induces low-grade inflammation that plays a role in the development of obesity (Graham et al., 2015). TLR-4 activation also results in the regulation of inflammatory pathways that contribute to insulin resistance and increased adiposity (Petrich et al., 2004). A study using rats fed a high-fat diet found an increase in acetate conversion rate and glucose-stimulated insulin secretion proportional to total calories consumed. It was concluded that increased acetate concentrations originate from the gut microbiota and drive parasympathetic nervous system activation, resulting in pancreatic β-cell stimulation and increased secretion of ghrelin, an appetite-stimulating hormone (Perry et al., 2016).

A study on the relationship between obesity and microbiota was also conducted using twin human individuals and mice. Fecal samples from human twins discordant for obesity were transplanted into germ-free mice. In a study conducted on mice, microbiota of obese individuals transported to lean control groups and the results showed that there was a significant increase in adiposity without an increase in food consumption. This study showed that obesity can be transmitted through microbiota modulation (Ridaura et al., 2013). In another study involving 10 obese and 10 normal weight dogs, microbiome analysis was performed in feces. It was understood that the most abundant phyla in both groups were Firmicutes and Bacteroidetes, followed by Fusobacteria, Proteobacteria and Actinobacteria. However, when the two groups were compared, significant differences were observed in the microbiome composition. While a higher number of Firmicutes was observed in obese dogs compared to normal dogs, Bacteroidetes was found in lower numbers. The ratio of Firmicutes to Bacteroidetes was significantly lower in normal weight dogs compared to obese dogs (Thomson et al., 2022). In another study conducted by Moinard et al. (2020), it was shown that the Firmicutes:Bacteroidetes ratio increased in dogs on a high-fat diet, and this was accompanied by a decrease in insulin sensitivity and changes in epithelial permeability (Moinard et al., 2020). It was also observed that this level decreased in dogs with weight loss. Considering the studies conducted on dogs, it appears that changes in the Firmicutes:Bacteroidetes ratio contribute to the development and maintenance of obesity in dogs, in line with humans and other animals. Martínez-Cuesta et al. (2021) it has been suggested that the change in this ratio leads to the induction of specific metabolic pathways involved in SCFA production and, as a result, causes an increase in fat tissue in the individual (Martínez-Cuesta et al., 2021).

In another study on the effect of obesity on intestinal microbiota diversity, the composition of the fecal microbiota was evaluated in 22 lean and 21 obese domestic dogs, as well as five research dogs fed ad libitum and four research dogs as a lean control group. As a result, Firmicutes, Fusobacteria and Actinobacteria were the dominant bacterial phyla, and Actinobacteria phylum and Roseburia genus were significantly more abundant in obese domestic dogs. In research dogs, it was observed that the order Clostridiales increased significantly under ad libitum feeding (Handl et al., 2013).

Another study was conducted to investigate the anti-obesity and hypocholesterolemic effects of Bifidobacteria animalis DY-64, a lactic acid bacterium isolated from the human intestine, with 40 male Sprague-Dawley rats. The rats were divided into four groups, respectively, and four rations were created with control diet, B. animalis supplement added to the control diet, high-fat-high-cholesterol diet, and B. animalis supplement added to the high-fat-high-cholesterol diet, and a feeding protocol was applied for 4 weeks. At the end of the experiment, when the group fed with a high-fat-high-cholesterol diet was compared with the group fed with a diet supplemented with B. animalis; It was understood that the increase in body weight, liver and fat tissue weights was greater than the former. It was observed that serum total cholesterol, LDL-cholesterol and leptin levels, which were significantly higher in the group fed with a high-fat-high-cholesterol diet than in the control group, decreased in the fatty diet group supplemented with B. animalis. These results indicate that B. animalis DY-64, isolated from human intestine, exerts hypocholesterolemic effects by reducing serum and liver cholesterol levels and plays a role in preventing obesity caused by high-fat diet (Choi et al., 2013). Similarly rats fed a high-fat diet and a control diet were supplemented with L. rhamnosus for 13 weeks. Then, body weights, insulin sensitivity, and expression of genes related to glucose and lipid metabolism were examined. As a result, a decrease in weight gain and increased insulin sensitivity were observed in the group fed with a high-fat diet, while no change was observed in the group fed with a control diet. Significant adiponectin production had been also reported. These results suggest that supplementing the diet with L. rhamnosus improves insulin sensitivity and reduces lipid accumulation by stimulating adiponectin secretion (S.-W. Kim et al., 2013). An attempt has also been made to establish a link between body condition and intestinal diversity in relation to obesity. In this context, a study conducted with 24 healthy 2-year-old Beagle dogs focused on this connection. When fecal analyses were performed in dogs fed a commercial diet based on metabolic properties, it was understood that this main microbiota in the intestine was distributed differently according to body condition score. In subjects with high body condition scores, Firmicutes was the most common bacterial species, followed by Bacteroidetes, Fusobacteria, Proteobacteria and Actinobacteria. It was observed that taxonomic diversity was higher when the body condition score was 3 but decreased when the score increased to 4-6. Fusobacteria species were observed to increase especially at condition score 6 and higher. Thus, the idea that microbiome diversity may have an opposite relationship with fitness has emerged (Chun et al., 2020). In a study conducted in Italy, 16 dogs with a body condition score of 7 and above out of 9 formed the obese group, while 15 dogs with a body condition score of 4 and 5 out of 9 formed the control group. The average overall weight loss percentage after calorie restrictions with the commercial obesity diet was 12.9% of the initial body weight. At the end of the study, total protein, C-reactive protein, haptoglobin and reactive oxygen compounds in the blood were higher in the obese group than in the lean group. In the feces, biogenic amines such as putrescine, cadaverine, spermine and spermidine were found to be more in the obese group and the amount of Firmicutes decreased and the amount of Bacteriodes increased in the obese group. This study ultimately provided evidence that obese dogs suffer from a subclinical inflammatory state characterized by higher levels of certain inflammatory markers and an accompanying higher total antioxidant capacity (Vecchiato et al., 2023).

It is known that there are significant microbiota changes in obese cats compared to thin cats (Kieler et al., 2016). According to the results of the research, Fusobacteria was found more in thin cats and Actinobacteria in obese cats, but the difference was not significant (Li & Pan, 2020). In another study conducted with cats, different result was obtained from the results observed in humans and rodents. Significant decreases were observed in the microbiome of obese cats compared to normal cats, which suggests a dysbiosis state in obese cats. The most common phylum in obese cats was Bacteroidetes with a rate of 40.9%, while Firmicutes came in second with 27.9%. It was concluded that the Firmicutes/ Bacteroidetes ratio was significantly low (Ma et al., 2022). Result suggests that, unlike humans and rodents, high Bifidobacterium levels may be associated with obesity. Accordingly, probiotic and prebiotic supplements to be used for weight management should be well designed. Consistent with the results of the aforementioned study, an another study showed that the Firmicutes phylum was found at higher levels in neutered thin cats than in the neutered obese group, while the Bacteroidetes phylum was significantly lower (Fischer et al., 2017).

The results of a study conducted with mice on the effects of SCFAs is also pointed out to a microbiota effect. In the study investigating the mechanism behind the anorectic effect of prebiotic fibers, the relationship of acetate, with appetite control was emphasized. Two groups of rats fed a high-fat diet supplemented with highly digestible inulin and a high-fat diet supplemented with low-digestible cellulose were subjected to various analyses after 8 weeks. The results showed that mice fed the inulin-supplemented diet gained significantly less weight and consume less food than the other group. It was determined that there was a significant increase in acetate in the colonic content (Frost et al., 2014). In another study, mice were supplemented with prebiotics and synbiotics from birth until the 42nd day and were then placed on a high-fat diet for 8 weeks. The results showed that adult insulin sensitivity and dyslipidemia improved and the most significant changes in gene expression were in the ileum, where Bifidobacterium is concentrated and authors concluded that early-life synbiotics protect mice from excessive fat accumulation caused by a high-fat diet (Mischke et al., 2018).

Conclusion

Obesity is a common metabolic dysfunction disease that is highly associated with the homeostasis of the gut microbiota. Evaluating the changes in the intestinal microbiota of obese pets and the physiological consequences resulting from these changes has an important place in the universal fight against obesity. The intestinal microbiota paves the way for obesity by regulating energy absorption, central appetite, fat storage and triggering chronic inflammation. Similarly, there are also strong connections between dysbiosis and obesity. At the phylum level, the increased Firmicutes/Bacteroidetes ratio is thought to be an important feature of the intestinal microbiota in obesity. The relationships between the composition and diversity of the intestinal microbiota and metabolic diseases such as obesity also emerge as a target for the prevention and treatment of these diseases. Considering all these, more studies are needed to elucidate the relationship between obesity and microbiota.

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