



## Symposium on Biological Causes and Coping Strategies

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## The role of gut microbiota in the development of obesity in dogs‡

### Abstract

The gut microbiota has become the subject of extensive research in the last decades. Intensive research has revealed its role in several functions related to energy metabolism as well as its importance in both health and illness. An imbalance in gut microbiota composition results in dysbiosis, which has been associated with metabolic disorders such as obesity. In the latter context, the gut microbiota appears to mediate the impact of diet on the host body weight. There are many studies in rodents that show profound changes in the composition and metabolic function of the gut microbiota in subjects with obesity, indicating its influence on the development of the problem. Usually, the microbiota of obese dogs has a modified *Firmicutes: Bacteroidetes* ratio, which returns to normal values after dietary intervention with diet-induced weight loss. The role of the gut microbiota on the development of dog obesity is a recent topic of research, which has been complex due to most of the studies being carried out using commercial extruded diets, which differ in their nutritional composition, resulting in controversial findings. Nevertheless, it has been shown that macro-nutrients from the diet are the most relevant factors that can modulate the microbiota's composition in a particular way. Moreover, current studies indicate that a personalized nutritional intervention could modify the composition and function of gut microbiota in a positive manner, reducing the development of pathologies such as obesity.

**Keywords:** Obesity; Dogs; Intestinal microbiota; Nutritional disorders; Metabolic disorders; Environmental factors.

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## Introduction

According to the World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive accumulation of body fat that may impair health.<sup>(1)</sup> Obesity is the main nutritional disorder not only in humans, but also in companion animals, and the incidence of this condition in them has increased significantly, being a serious concern in veterinary clinic.<sup>(2)</sup>

Obesity represents a greater risk for developing disturbances such as diabetes, heart disease, high blood pressure, orthopedic disease,<sup>(3)</sup> urinary tract disease,<sup>(4, 5)</sup> respiratory dysfunction<sup>(6)</sup> as well as certain types of cancers that in turn lead to a shorter life span and a decreased quality of life.<sup>(7, 8)</sup>

In humans as in dogs, obesity has a genetic component that can be influenced by several environmental factors, including diet, lifestyle, and health condition.<sup>(9)</sup> Moreover, in the last decades the role of gut microbiota on several metabolic functions such as weight gain,<sup>(10)</sup> energy harvest,<sup>(11)</sup> fat metabolism,<sup>(12)</sup> satiety regulation, systemic inflammation,<sup>(13)</sup> metabolic homeostasis, and nutrient absorption, have been uncovered.

## Dog gut microbiota

The scientific community is gaining a greater understanding of bacterial communities as well as the physiology of gut microbiota, in part due to the Human Microbiome Project.<sup>(14)</sup> In this sense, the mammals' gut microbiota harbors more than 100 billion microorganisms, including fungi, archaea, virus, and bacteria.<sup>(15)</sup> The latter represent more than 90 % of the microbiota, and belong to either the gram-positive *Firmicutes* (64 %) or the gram-negative *Bacteroidetes* (23 %),<sup>(16)</sup> being both the most abundant phyla in lean and obese individuals,<sup>(17)</sup> respectively. In humans as in animals, the gut microbiota plays a significant role in health and disease.<sup>(18)</sup> In healthy dogs, several studies have shown that fecal microbiota is co-dominated by *Fusobacterium*, *Bacteroidetes*, and *Firmicutes* phyla.<sup>(19, 20)</sup>

The major taxa belong to phylum *Firmicutes*, where *Clostridia*, *Bacilli*, and *Erysipelotrichi* are the most representative classes. The class *Clostridia* is largely dominated by three *Clostridium* clusters: IV (families *Ruminococcaceae*, *Faecalibacterium prausnitzii*), XI (family *Peptostreptococcaceae*), and XIVa (families *Lachnospiraceae*, *Blautia* spp).<sup>(21, 22)</sup> Also, class *Bacilli* are formed almost exclusively of the order *Lactobacillales*, dominated by the genera *Streptococcus* and *Lactobacillus*; whereas *Erysipelotrichi* mainly comprises the genera *Coprobacillus*, *Turicibacter*, and *Catenibacterium*.<sup>(23)</sup> The phylum *Bacteroidetes* comprises the genera *Prevotella*, *Bacteroides*, and *Megamonas*.<sup>(19, 23)</sup> In addition, the phylum *Fusobacteria* includes the *Fusobacterium* genus, which is increased in dogs with access to the outdoors.<sup>(24)</sup>

In humans, *Fusobacterium* is associated with gastrointestinal diseases, so the *Fusobacterium* genus could play a different role in the intestinal tract of dog.<sup>(22)</sup> Additionally, other phyla such as *Proteobacteria* and *Actinobacteria* are typically

colonizers of the small intestine and it is possible to find them in smaller numbers in fecal samples.

## Dysbiosis

An imbalance in gut microbiota results in dysbiosis, which is caused by different factors such as inflammatory processes, unhealthy eating habits, or sedentary life-style. Likewise, dysbiosis has been associated with metabolic syndrome, cardiovascular disease, immune disorders, and obesity.<sup>(25)</sup> In the obesity context, the gut microbiome mediates the impact of diet on host body weight.<sup>(26, 27)</sup> In this sense, microbial 16S rRNA gene sequencing of fecal samples from animal models and humans have underlined the importance of the gut microbiota composition on the amount of energy extracted from the diet and the risk of developing obesity.<sup>(19, 28)</sup>

Previous studies have shown that, at the phylum level, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria* are significantly different between obese and normal-weight individuals.<sup>(29)</sup> In obese people, the ratio of *Firmicutes*:*Bacteroidetes* decreased significantly. In fact, previous research has reported significant changes in the abundance of *Firmicutes* and *Bacteroidetes* in both obese humans and dogs compared with their lean counterparts.<sup>(30)</sup>

Other studies have reported that the reduction in *Bacteroidetes* with respect to *Firmicutes* in obese individuals can be reverted through diet-induced weight loss, which increases the abundance of phyla that predominate in lean individuals.<sup>(31)</sup> Moreover, the increase of *Firmicutes* has been thought to cause an increased efficiency in energy extraction from the diet, resulting in higher levels of short-chain fatty acids that are suspected of altering the metabolism of obese individuals.<sup>(26, 28)</sup> Other studies have confirmed the role of *Proteobacteria* in obesity by producing pro-inflammatory molecules such as lipopolysaccharides, helping to harvest energy, increase host fat storage<sup>(32)</sup> and promote inflammation.

## The role of gut microbiota in dog obesity

Experimental evidence has revealed that obesity has a microbial component. For instance, transplanted gut microbiota from normal mice into germ-free mice recipients resulted in an increased body fat without any increase in food consumption.<sup>(10)</sup> This evidence raised the possibility that the composition of the microbial community in the gut affects the amount of energy extracted from the diet. A short time later, it was observed that obese subjects had an enriched microbiota that expresses genes encoding enzymes involved in polysaccharide breakdown and metabolism of simple carbohydrates.<sup>(26)</sup> Further studies confirmed that gut microbiota participates in whole-body metabolism by affecting energy balance, glucose metabolism, and the low-grade inflammation associated with obesity and related metabolic disorders.<sup>(33)</sup>

The human microbiota consists of two groups of dominant beneficial bacteria: *Bacteroidetes* and *Firmicutes*. In this regard, it has been observed that obese people

have a lower relative proportion of *Bacteroidetes* compared with lean individuals.<sup>(28)</sup> Research has also documented differences in the gut microbiota composition between obese and lean dogs.<sup>(19)</sup> However, to date, there is limited information about the role of gut microbiota on the development of obesity in canines.

One of the first studies on this subject used high-throughput pyrosequencing and qPCR to compare the microbiota composition of fecal samples from obese (n = 21) and lean (n = 22) dogs, as well as of animals fed a high-fat commercial dry diet either *ad libitum* (n = 6) or restricted (n = 4) for six months.<sup>(34)</sup> No significant differences were found in the microbiota alpha diversity between obese and lean dogs. However, the abundance of *Actinobacteria* phylum, as well as *Clostridiales*, particularly *Roseburia*, were significantly greater in obese dogs. Conversely, the abundance of *Bacillales* and *Fusobacteriales* was lower in this group. Moreover, there were no differences at the *Firmicutes* level between the dogs fed *ad libitum* or restricted. Nevertheless, *Firmicutes* and the order *Clostridiales* increased throughout the course of the study, whereas the *Bacteroidetes* decreased in dogs under restricted feeding.

The findings in human gut microbiota and its implication in the development of obesity have allowed studies to improve the health and welfare of companion animals, where obesity has increased exponentially in recent times in domestic dogs. Hence, there is a growing interest in assessing the impact of gut microbiota on canine health.

## Modulation of gut microbiota

The intestinal microbiota can be modulated by different factors being the diet the most relevant.<sup>(35,36)</sup> It has been reported that differences in dietary macronutrients modulate the microbiome profile rather than the source origin of the ingredient composition.<sup>(37)</sup> For instance, diets with high content of vegetable fiber cause an increase in *Firmicutes* abundance and a reduction in *Fusobacteria* and *Proteobacteria* phyla.<sup>(20,38)</sup> Conversely, using plant-based protein sources did not seem to significantly alter the gut microbiota in dogs, compared with a traditional diet based on mixed animal and vegetable protein sources.<sup>(39,40)</sup> However, it has been reported that there is a positive correlation between the abundance of *Clostridiaceae* family and dietary protein content.<sup>(39)</sup>

A recent study in dogs showed that the production of short chain fatty acids (SCFA) is not exclusively dependent on the diet's carbohydrate content.<sup>(41)</sup> In this trial, there was no difference in the levels of acetate, butyrate, and propionate between high-fat/low-starch and low-fat/high-starch. In other experiments, the addition of minced meat to a conventional kibble diet resulted in a small increase in fecal butyrate and isovalerate.<sup>(42)</sup>

The effects of probiotics and prebiotics on gut microbiota have also been tested. In this regard, the evidence suggests that these nutritional interventions could modify the composition of gut microbiota in a positive manner, reducing the devel-

opment of pathologies associated with inflammation,<sup>(13)</sup> which is the base of most metabolic disorders including those associated to obesity.

In humans, dietary interventions with prebiotics and probiotics not only modulate the composition but also the functionality of gut microbiota.<sup>(43)</sup> The probiotics might have beneficial effects through the production of metabolites and antimicrobial peptides, which modify local microbiota and interact with the host immune system.<sup>(44)</sup> In dogs, the probiotics have been used for treating acute uncomplicated diarrhea and inflammatory bowel disease (IBD). Likewise, a dietary intervention with a symbiotic probiotic-prebiotic combination has shown to increase fecal family *Lactobacillaceae* after two weeks of treatment,<sup>(45)</sup> a time in which the most beneficial bacteria present were *Lactobacillus*, from the *Firmicutes* phylum.<sup>(46)</sup>

It is important to consider that there are still inconsistencies in several nutritional interventions regarding microbiota modulation. Therefore, in dog research, the results from several studies should be taken into consideration, since most of them have been carried out using commercial extruded diets differing in nutritional composition. Moreover, the reduced number of individuals used in the trials is another limitation of these studies, considering the existence of individual differences in the microbiome profiles.<sup>(47)</sup> The manipulation of intestinal microbiota via nutritional intervention<sup>(48,49)</sup> or even using antibiotics<sup>(50)</sup> could potentially provide an easily applicable complementary therapy for obesity, which should be deeply studied.

## Nutritional approach to obesity control

Different types of dietary interventions have been evaluated for treating or controlling dog obesity. However, they have shown modest positive effects in the short-term as well as poor results in long-term trials.<sup>(51,52)</sup> Nevertheless, the effect of weight loss on intestinal microbiota composition have been evaluated in several studies.<sup>(30,53,54)</sup>

Mori et al.<sup>(55)</sup> reported that a commercial weight-loss diet (high-protein, low-fat, low-carbohydrate, and high-fiber levels), resulted in a significantly decreased proportion of *Actinobacteria* and *Firmicutes* phyla, whereas *Fusobacteria* phylum increased compared with microbiota from dogs fed with an antiallergenic diet (medium-protein, high-fat, medium-carbohydrate, and low-fiber levels). Another study evaluated the effect of a restricted feeding during a 12-weeks period in obese dogs, using a prescription commercial dry diet (high-protein/high-fiber).<sup>(56)</sup> This study also evaluated the interaction of restricted feeding with exercise, weight-loss, and intestinal microbiota composition.

Results from the study described above showed that the exercise had no influence on total weight loss and gut microbiota. Overall, the most abundant genera in the microbiota were *Fusobacterium* (~20–50%) and *Bacteroides* (~15–35%). *Megamonas* genus abundance negatively correlated with a fast weight loss rate, which was considered as  $\geq 1$  % per week. Moreover, acetic and propionic acid concentrations decreased in dogs with a fast weight loss rate.

These findings suggest that weight loss rate may be related to gut microbiota composition, as well as the production of metabolites produced by *Megamonas* and *Ruminococcaceae*. In addition, the *Ruminococcaceae* relative abundance was lower at the end of the study in dogs with a fast weight loss rate. They concluded that microbiota that favors short chain fatty acid production may negatively affect the weight loss rate in dogs. Nevertheless, it is difficult to separate the effects of weight loss from those of energy restriction and macronutrient composition. In addition, it is important to mention that different subpopulations of obese individuals might respond differently to specific weight loss diets.<sup>(54, 57)</sup>

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## Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

## Author contributions

Conceptualization: MC Camacho-Rea, DA Cardoso-Carmona

Writing-review and editing: MC Camacho-Rea, DA Cardoso-Carmona

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