Differences in the composition and predicted functions of the intestinal microbiome of obese and normal weight adult dogs

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ABSTRACT

Obesity is a multifactorial nutritional disorder highly prevalent in dogs, observed in developed and developing countries. It is estimated that over 40% of the canine population suffers from obesity, which manifests in an increased risk of chronic osteoarticular, metabolic, and cardiovascular diseases. The intestinal microbiome of obese animals shows increases in the abundance of certain members capable of extracting energy from complex polysaccharides. The objective of this study was to compare the composition and predicted function of the intestinal microbiome of Chilean obese and normal weight adult dogs. Twenty clinically healthy dogs were classified according to their body condition score (BCS) as obese ($n = 10$) or normal weight ($n = 10$). DNA was extracted from stool samples, followed by next-generation sequencing of the 16S rRNA V3–V4 region and bioinformatics analysis targeting microbiome composition and function. Significant differences were observed between these groups at the phylum level, with anincrease in Firmicutes and a decrease in Bacteroidetes in obese dogs. Microbiome compositions of these animals correlated with their BCS, and obese dogs showed enrichment in pathways related to transport, chemotaxis, and flagellar assembly. These results highlight the differences in the gut microbiome between normal weight and obese dogs and prompt further research to improve animal health by modulating the gut microbiome.

Subjects Microbiology, Molecular Biology, Veterinary Medicine
Keywords Obesity, Gut microbiome, Canines, Microbiome function

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INTRODUCTION

The gut microbiome represents an extensive catalog of microorganisms residing in the gut of animals (*Marchesi & Ravel, 2015*). Its composition is usually dominated by bacteria, with contributions of fungi, protozoa, and viruses (*Vemuri et al., 2020*). The gut microbiome has been directly or indirectly associated with host health in humans and other mammals such as dogs (*Suchodolski, 2011*; *Alessandri et al., 2019*). It has an impact on gut homeostasis, host metabolism, nutrient absorption, immune responses, and neurodevelopment, among

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others (*Suchodolski et al., 2012*; *Heintz-Buschart & Wilmes, 2018*; *Alessandri et al., 2020*; *Siddiqui, Akbar & Khan, 2021*).

The gut microbiome of healthy dogs is co-dominated by three phyla: Fusobacterium, Bacteroidetes, and Firmicutes (*Middelbos et al., 2010*; *Hand et al., 2013*; *Chun Ju et al., 2020*), with a lower proportion of Proteobacteria and Actinobacteria (*Barko et al., 2017*; *Salas-Mani et al., 2018*; *Alessandri et al., 2020*). In contrast to humans and other animal microbiomes, Fusobacteria is abundant in the gut of healthy dogs (*Song et al., 2013*; *Vital et al., 2015*; *Bermingham et al., 2017*).

Although the composition of the gut microbiota is stable during adult life, it is widely variable among humans (*Guard et al., 2017*). This microbial stability is also expected in dogs but only observed in the short term (*Pilla & Suchodolski, 2020*). Factors such as diet, drugs, and age are among the most important factors shaping the gut microbiome in dogs (*Chandler et al., 2017*; *Gupta, Paul & Dutta, 2017*; *Kim et al., 2017*; *Li et al., 2017*; *Montoya-Alonso et al., 2017*). A loss of microbiome homeostasis, or dysbiosis, has been linked to certain diseases such as inflammatory bowel diseases and metabolic disorders, among others (*Pilla & Suchodolski, 2020*). This alteration has been shown to be a consequence of the loss of key species or overgrowth of toxigenic microorganisms such as enterotoxigenic *Bacteroides fragilis* (*Chandler et al., 2017*; *Gavazza et al., 2018*; *Craven & Washabau, 2019*).

Obesity in dogs is a multifactorial disorder, with a prevalence greater than 40% in developed countries (*Mao, Xia & Chen, 2013*; *Montoya-Alonso et al., 2017*; *Forster et al., 2018*). Obesity is defined as the excessive accumulation of adipose tissue in the body, usually due to excessive food intake or the inadequate use of energy, causing a positive energy balance (*Khera et al., 2019*). Consequently, obese dogs suffer from a decrease in quality and life expectancy and an increased risk of developing diseases such as diabetes mellitus (DM), dyslipidemia, and cardiovascular disease, among others (*Marshall et al., 2009*; *Clark & Hoenig, 2016*; *Chandler et al., 2017*; *Bjørnvada et al., 2019*). At least in the last 50 years, the prevalence of DM has increased in dogs (*Guptill, Glickman & Glickman, 2003*; *Heeley et al., 2020*).

It has been observed that the relative abundance of Firmicutes and Bacteroidetes is altered in obese human subjects with an overrepresentation of Firmicutes, compared to lean subjects (*Kasai et al., 2015*; *Haro et al., 2016*; *Coelho et al., 2018*). Interestingly, in dogs this change in relative abundance can be observed in Firmicutes, Bacteroidetes, or Fusobacteria (*Bermudez Sanchez et al., 2020*). These taxonomic differences between normal weight and obese animals can contribute to the development and perpetuation of obesity (*Li et al., 2017*; *Bermudez Sanchez et al., 2020*). Proposed mechanisms include fat storage, regulation of energy metabolism, extraction of energy from short-chain fatty acids, increased low-grade inflammation, and impaired bile acid metabolism (*Khan et al., 2016*; *Kieler et al., 2017*; *Xu et al., 2017*; *Garcia-Mazcorro et al., 2020*).

Dogs, being domestic carnivores, take advantage of meat-based diets, and diet has a major influence on the composition of the gut microbiota (*Wernimont et al., 2020*). For instance, high fiber diets lead to an increase in the relative abundance of Firmicutes and a decrease in *Fusobacterium* and *Proteobacterium* (*Bermudez Sanchez et al., 2020*). A high-fat and low-carbohydrate diet enriches genera related to fat digestion, such as *Allobaculum*

and *Parasutterella* (*Kilburn et al., 2020*). Partial weight loss can be achieved after dietary changes (*Xu et al., 2017*; *Coelho et al., 2018*; *Apper et al., 2020*).

The energy balance in animals is at a delicate equilibrium between energy consumption and expenditure (*Stubbs & Tolkamp, 2006*). The gut microbiota mediates changes in energy storage, in some cases leading to pathophysiological consequences in the short, medium, or long term (*Ley et al., 2006*). Few studies have addressed the impact of obesity in the canine gut microbiota, and the microbiome functions that could be altered in these animals are not well known. The goal of this study was to compare the composition of the intestinal microbiota in a group of obese and normal-weight dogs and predict what metabolic functions could be enriched or reduced in their microbiomes.

METHODS

Subjects and inclusion criteria

This study was approved by the Bioethics Committee at the Veterinary Clinic Los Avellanos (Approval Certificate HCVLA-008). The study was performed at the same clinic, located in Independencia, Santiago, Metropolitan Region, Chile. Samples were collected during November 2020. Twenty dogs aged between 2 and 8 years old were sampled (Table S1). Animals were of any breed or sex and fed commercial diets (pellets) from different brands (Table S2). Inclusion criteria were for individuals who presented a normal clinical examination, physiological parameters (temperature, heart, and breathing rate), and no signs of gastrointestinal disease. Animals did not receive antibiotics or probiotics at least three months before the beginning of the study. All dogs had been spayed or neutered before the study.

All dogs were subjected to a complete clinic examination by a veterinarian. According to their body condition, ten normal weight dogs and ten obese dogs were enrolled. The body condition score (BCS) was determined based on a nine-point scale (*German et al., 2009*; *Chun et al., 2019*), based on palpation and visual inspection of the ribs, waist, bony prominences, the base of the tail, and abdomen. A one-unit increase in BCS corresponds to an approximate 10% increase in body weight (*German et al., 2009*; *Chun et al., 2019*). Animals with BCS values between 4–5 were considered normal weight, and dogs with BCS 8–9 were considered obese. Information regarding breed, age, and sex was obtained directly from each owner (Table 1).

Analysis of the gut microbiome

Stool samples were collected immediately after defecation and stored at −80 °C until processing. After thawed, 150 mg of each sample were used for total DNA extraction (Quick-DNA Fecal/Soil Microbe Miniprep Kit, Zymo Research, Irvine, CA, USA) using a Disruptor Genie device (Scientific Industries, USA). Fecal DNA samples were diluted to 20 ng/μl in nuclease-free water (NanoDrop 2000c; Thermo Fisher Scientific, Waltham, MA, USA). DNA samples were submitted for Illumina MiSeq sequencing to the DNA Sequencing Services at Molecular Research (MR-DNA, USA). The variable region of the 16S rRNA V3–V4 gene was amplified using primers 341F and 785R (*Klindworth et al., 2013*), adding a barcode in the forward primer. The reaction was run for 30 cycles using

Table 1 Animal data.

Code	Age (years)	Breed	Sex	Weight (kg)	Body condition score
1-N	5	crossbreed	F	31.8	5
2-N	5	crossbreed	F	31.7	5
3-N	3	crossbreed	M	13.3	5
4-N	4	labrador retriever	M	25	5
5-N	4	crossbreed	M	26	5
6-N	7	crossbreed	M	28	5
7-N	3	crossbreed	F	17.5	5
8-N	5	cocker spaniel	F	12	5
9-N	3	crossbreed	M	13.5	5
10-N	2	crossbreed	F	21.5	5
Average	**4.1 ± 1.4**			**22.75 ± 7.2**	**5 ± 0**
1-O	5	crossbreed	M	49	9
2-O	3	crossbreed	M	15	8
3-O	3	crossbreed	M	17.3	9
4-O	2	crossbreed	M	23	9
5-O	8	crossbreed	M	30.4	9
6-O	5	crossbreed	M	17	8
7-O	3	crossbreed	M	14.1	8
8-O	10	german shepherd	M	42	9
9-O	10	great dane	M	55	9
10-O	8	crossbreed	M	20.4	8
Average	**5.7 ± 3.1**			**33.5 ± 17.3**	**8.6 ± 0.5**

Notes.
F, Female; M, Male.

the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA). After amplification, the PCR products were verified on a 2% agarose gel. Several samples were pooled and purified using calibrated Ampure XP microspheres (Agencourt Bioscience Corporation, Beverly, MA, USA). The pooled and purified pooled PCR products were used to prepare a DNA library using the TruSeq DNA LT Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. Sequencing was performed using the MiSeq platform (Illumina, USA).

Bioinformatics analyses

The raw DNA sequences provided by the external service were analyzed employing the QIIME version 1.8.0 open-source bioinformatics tool (*Caporaso et al., 2010*). Each sequence sample was demultiplexed into individual files, and barcodes were removed from the 5′-end of each read (via demultiplex_fasta.py script). The processed sequences were uploaded to the European Nucleotide Archive under the project code PRJEB38793. Individual reads were assigned to bacterial taxonomy employing the DADA2 v1.10 R package (*Callahan et al., 2016*), following a modified procedure. Briefly, sequences were quality-filtered to remove undetermined base callings and trimmed down to 220 nucleotides before estimating

the sequencing error model. The model was used to infer Amplicon Sequence Variants (ASV) (*Callahan, McMurdie & Holmes, 2017*) and those variants used to assign bacterial taxonomy with a Naïve Bayesian classifier (*Wang et al., 2007*) and the SILVA database version 132 (*Quast et al., 2013*; *Yilmaz et al., 2014*). The ASV abundance table was utilized to infer the abundance of metabolic functions and pathways with the PICRUSt2 python package (*Douglas et al., 2020*). Briefly, the PICRUSt2 software reconstructs a metabolism, first aligning an ASV to a reference tree that allows the selection of a reference genome and prediction of the gene content per ASV. Then, PICRUSt2 infers the abundance of metabolic functions and pathways employing the abundance of each ASV in a sample and the selected reference genome. Microbiome composition at the phylum level was assessed with the Shannon diversity index and the weighted UniFrac method (*Lozupone et al., 2011*) employing the scikit-bio python package (http://scikit-bio.org/). The weighted UniFrac was statistically assessed employing ANOSIM and PERMANOVA, using the scikit-bio software. Univariate analyses of the differences in the relative abundance of phyla, family, and genera were assessed with the non-parametric Mann–Whitney *U*-test (*Mann & Whitney, 1947*) and the DESeq2 R package (*Lin & Peddada, 2020*). Finally, multivariate analysis of the differences in the abundance of taxa, metabolic functions, and pathways was assessed with the Linear Discriminant Analysis (LDA) Effect Size (LEfSe) method (*Segata et al., 2011*). The LefSe method was performed employing the Galaxy server (*Afgan et al., 2018*) at https://huttenhower.sph.harvard.edu/galaxy/). In the case of the metabolic functions, the abundance of the KEGG orthologs (KO) and KEGG pathways were clustered and analyzed in a sample basis, and later, the contribution of each taxon at the genus levels and treatment was assessed only for the significant effect sizes of LDA (absolute value of the log10 LDA greater than 2) employing the Pearson Correlation Coefficient. Significance level for all statistical analysis was p-value <0.05.

RESULTS

This work analyzed the gut microbiome of ten obese (O) and ten normal (N) weight dogs, according to their BCS. The characteristics of the animals are presented in Table 1. Both groups were statistically similar in age and weight (Mann–Whitney *U*-test $p \approx 0.14$ and $p \approx 0.09$, respectively).

After 16S rRNA sequencing of fecal samples, each sample contained between 100 and 400 ASVs (Fig. 1A). Rarefaction curves showed saturation indicating the sequencing depth was appropriate to describe the microbial composition. Alpha diversity using the Shannon Index, measuring the number of species and their abundances in each sample, was significantly different between both groups (N: 1.55 ± 0.15, O: 1.32 ± 0.20; Mann–Whitney *U*-test p-value ≈ 0.014; Fig. 1B).

Microbiome compositions in both groups were analyzed using the Weighed Unifrac beta diversity method. A PCoA plot of their compositions showed clustering of normal weight animals separated from obese dogs (Fig. 1C). The statistical assessment showed the beta diversity between obese and normal dogs was statistically different (ANOSIM R ≈ 0.179, p-value $= 0.01$; PERMANOVA pseudo-F ≈ 6.125, p-value $= 0.009$).

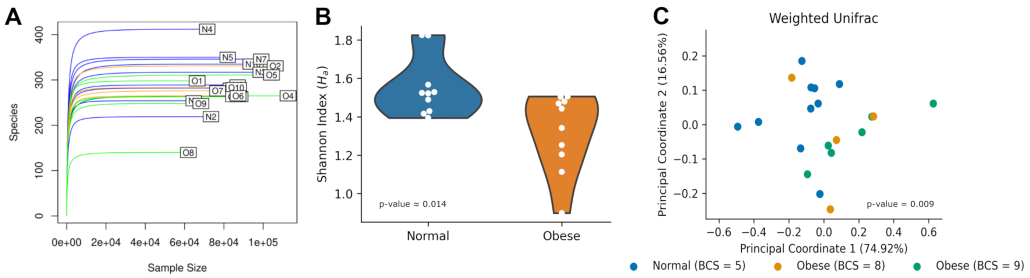

Figure 1 Microbiome diversity among normal and obese weight dogs. (A) Rarefaction curve. Number of identified Amplicon Sequence Variants (ASVs) as a function of the number of sequenced 16S amplicons. Blue lines identify samples of normal weight dogs, orange lines identify samples of obese dogs with a BCS of 8, and green lines identify samples of obese dogs with a BCS of 9. (B) Shannon index for each group. The violin plot shows all indexes and an estimation of the probability distribution of the data. (C) Principal Coordinate Analysis of the weighted UniFrac index for each sample. Each dot represents the UniFrac index, the proportion of relative abundance, and the similarity of phyla between the two samples. Indexes for normal weight dogs are shown in blue, while indexes for obese dogs are shown in orange (BCS= 8) and green (BCS = 9).

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In both groups, the most abundant phyla were Firmicutes and Bacteroidetes, followed by Fusobacteria, Proteobacteria, and Actinobacteria (Fig. 2). Both groups presented significant differences in their microbiome composition at the phylum level. Compared to normal weight dogs, obese animals had a higher relative abundance of Firmicutes and lower abundance of Bacteroidetes (Mann–Whitney *U*-test *p*-value $\approx$ 0.014 and 0.011 respectively; Fig. 2A). Similarly, a LEfSe analysis at the phylum level showed significant enrichment of Firmicutes in obese dogs and significant enrichment of Bacteroidetes, Deferribacteres, and Tenericutes in normal weight dogs (Fig. 2C). Furthermore, the ratio Firmicutes to Bacteroidetes was significantly lower in normal weight dogs compared to obese dogs (0.28 $\pm$ 0.16 *vs.* 0.53 $\pm$ 0.21 respectively, *p*-value $\approx$ 0.004; Fig. 2D).

At the genus level, samples in both groups were dominated by *Blautia*, *Bacteroides*, and *Peptoclostridium* (Fig. 3). Among these, significant differences in both groups were found in *Peptoclostridium* (DESeq2, adjusted *p*-value $\approx$ 0.048) and *Bacteroides* (DESeq2, *p*-value $\approx$ 0.048). In general, obese dogs had an increase in the relative abundance of *Peptoclostridium* and a decrease in *Bacteroides* genera (Fig. 3).

Finally, using PICRUSt we predicted the abundance of major putative metabolic pathways in the gut microbiome of these animals and compared their total representation in both groups employing the LEfSe method (Fig. 4). A LEfSe analysis was first performed to determine genera enriched in both groups. We observed that *Peptoclostridium* was increased in obese animals, and several other genera were decreased (including Ruminococcaceae, Oscillibacter, and Parasutterella; Fig. S1).

Interestingly, obese animals showed an enrichment in KEGG pathways and orthologs related to motility (chemotaxis proteins K03406, flagellar assembly), as well as transport functions and two-component systems (Figs. 4A and 4B). On the contrary, normal weight animals showed a deployment in general biosynthetic pathways (terpenoids, folate, lipopolysaccharide, Fig. 4A), as well as hexosaminidases (Fig. 4B).

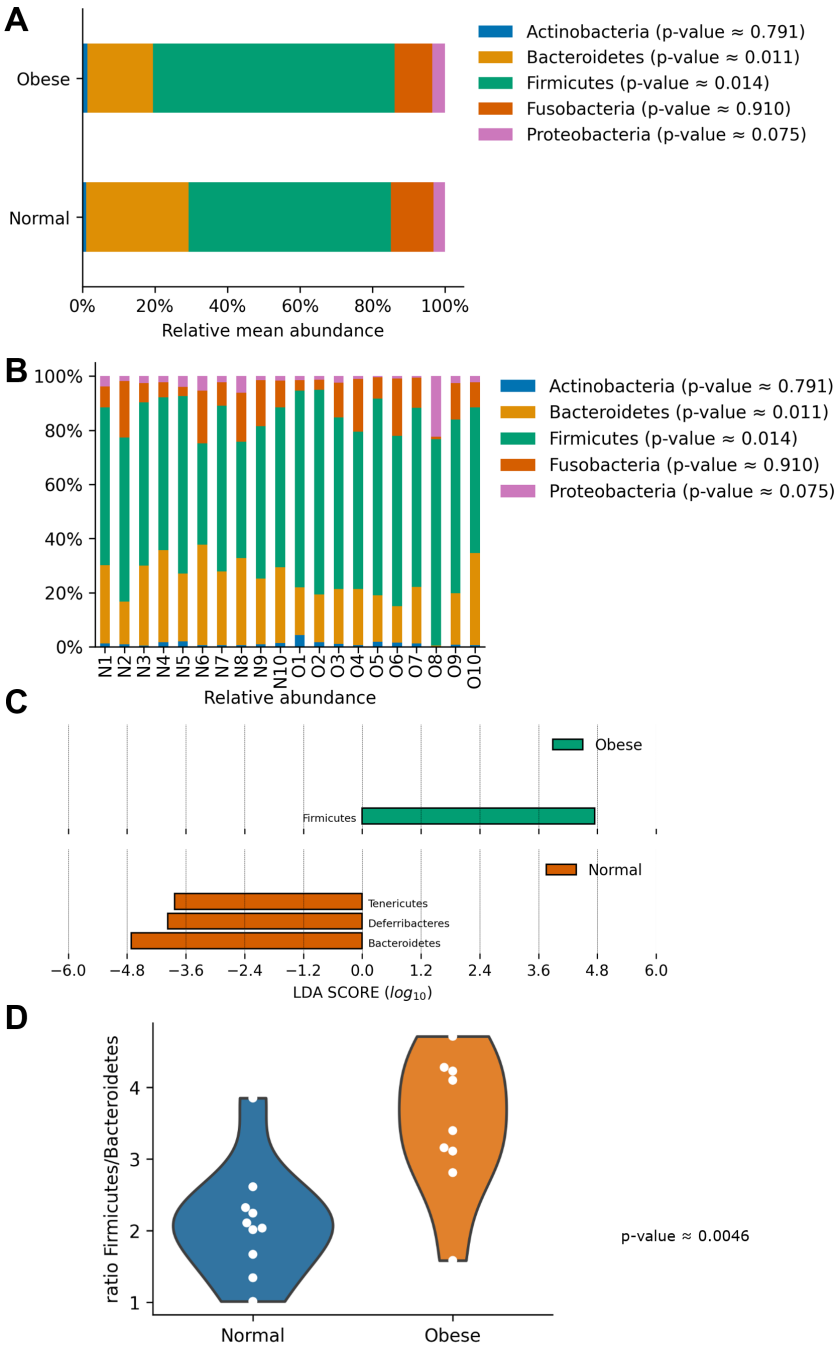

Figure 2 **Relative abundance of representative taxa in dog gut microbiota at the phylum level.** Relative abundance of representative taxa in dog gut microbiota at the phylum level. The figure shows the average proportion of Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Fusobacteria (A) and each microbiome composition for each animal (B). *P*-values were obtained with the non-parametric *U*-test to determine differences. (C) Linear Discriminant Analysis Effect Size at the phylum level to identify major phyla enriched in obese and normal weight dogs. (D) Plot of the ratio Firmicutes/Bacteroides in both groups.

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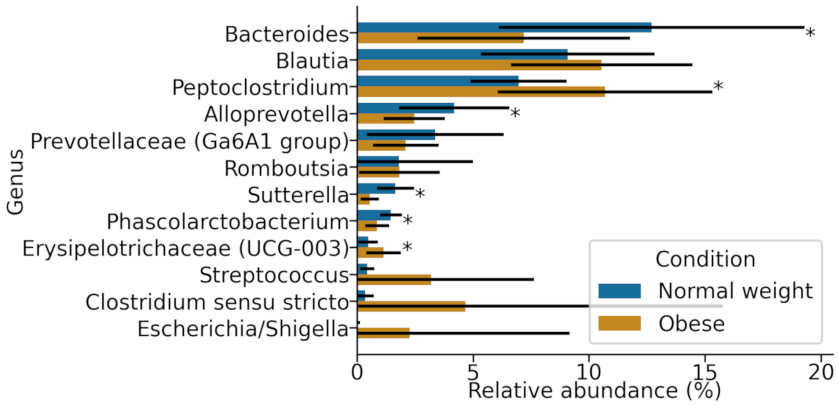

Figure 3 **Most abundant genera in both obese and normal weight dogs.** Data show the mean and standard deviation across all animals in both groups. Asterisks indicate p-value < 0.05.

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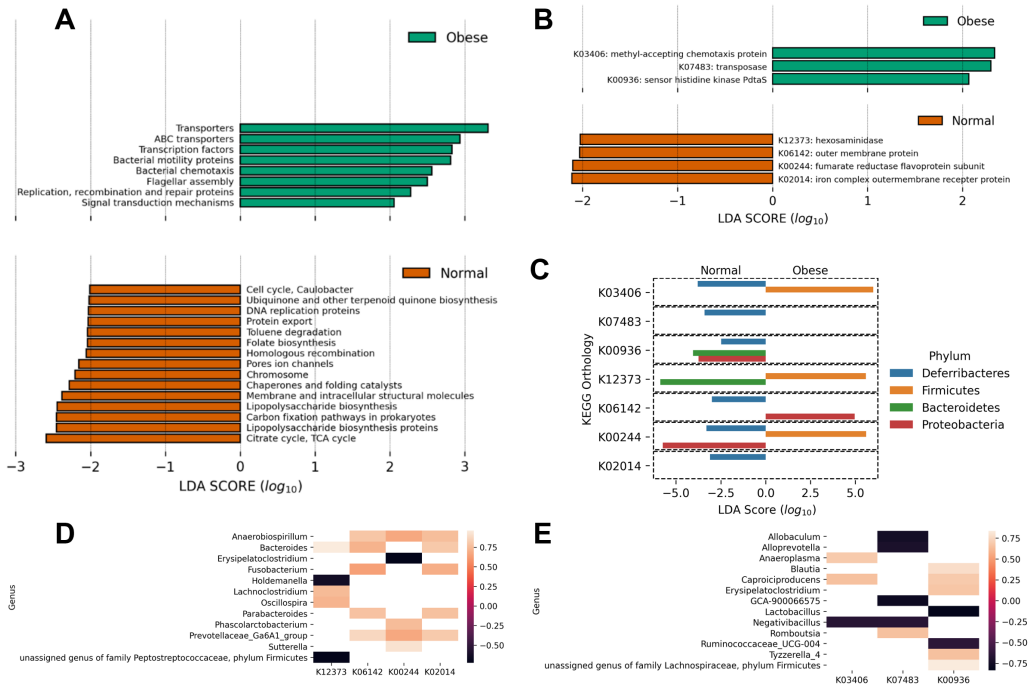

Figure 4 **Predicted metabolic functions in the microbiome of obese and normal weight dogs.** (A and B) Linear Discriminant Analysis Effect Size of the PICRUSt predicted metabolic functions abundance in obese animals. The top and bottom figures show the metabolic functions which abundance change most likely categorize the subjects in the study in normal weight (red) or obese (green). (A) KEGG pathways; (B) KEGG orthologs. (C) Determination of phyla contributing to the significant KEGG orthologs in (B) per-group basis. A LEfSe analysis per KEGG ortholog was applied to the abundances of metabolic functions per phylum, determining with phylum categorize the subjects in the study in normal weight or obese. (D and E) Pearson correlation analysis comparing the relative abundance of genera to the relative abundance of significant metabolic functions. (D) Significant correlations in normal weight dogs. (E) Significant correlations in obese dogs. KEGG orthologs are listed in (B).

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To detail the individual contribution of any microorganism to enriched or depleted metabolic pathways, the LEfSE analysis of the abundance of the KEGG orthologs at the phylum level was performed (Fig. 4C). The analysis revealed that the higher relative abundance of Firmicutes in obese dogs contributed to increases in chemotaxis proteins (p-value $\approx$ 0.005), hexosaminidase activity (p-value $\approx$ 0.016), and fumarate reductase activity (p-value $\approx$ 0.0005). These processes are related to respiration, motility, and degradation of host glycans. In addition, the analysis revealed that Bacteroidetes abundance in normal weight animals was responsible for the abundance of sensor histidine kinase function in obese dogs (p-value $\approx$ 0.003) and to hexosaminidase activity in normal weight dogs (p-value $\approx$ 0.016). This analysis was also performed at the genus level (Fig. 4D). *Anaerobiospirillum*, *Bacteroides,* and *Prevotellaceae* relative abundance correlated positively with 3 of 4 enriched KEGG orthologs in normal weight dogs (K06142, K00244, K02014; Fig. 4D). Similarly, *Caproiciproducens* relative abundance correlated positively with 2 of 3 identified KEGG orthologs by LEfSe in obese dogs (K03406, K00936; Fig. 4E). On the contrary, *Allobaculum*, *Alloprevotella*, *Lactobacillus*, *Negativibacillus,* and *Ruminococcaceae* relative abundance correlated negatively with the abundance of the three identified KEGG orthologs (Fig. 4E).

DISCUSSION

The gut microbiome has emerged as a factor shaping metabolic responses in animals, including canines (*Bermudez Sanchez et al., 2021*). In this study, we observed a significant decrease in Bacteroidetes and an increase in Firmicutes in obese dogs (Figs. 2 and 4). Bacteroidetes, together with Firmicutes, is one of the most abundant phyla in the canine intestinal microbiome, both in obese and normal weight dogs. A tendency of Bacteroidetes to decrease and Firmicutes to increase in obese dogs has been observed previously (*Suchodolski, 2016*). Interestingly, the ratio Firmicutes/Bacteroidetes has been shown to increase in dogs undergoing a high-fat diet accompanied by a reduction in insulin sensitivity and alterations in epithelial permeability (*Moinard et al., 2020*). This ratio has been shown to decrease in dogs under weight loss or inflammatory bowel disease (IBD) (*Barko et al., 2017*; *Li et al., 2017*; *Bermudez Sanchez et al., 2020*; *Moinard et al., 2020*). Notably, most of these studies have been reported in US and European countries, but only a few in other countries. In general, the evidence indicates a similar trend of increasing the Firmicutes to Bacteroidetes ratio in obese animals in different countries (*Handl et al., 2013*; *Li et al., 2017*; *Montoya-Alonso et al., 2017*; *Bermudez Sanchez et al., 2020*). However, further studies and proper statistical comparisons are required to determine the effect of geography on the gut microbiota and obesity in dogs. The Firmicutes/Bacteroidetes ratio imbalance has also been observed in obese humans, being reversible after dietary interventions (*Ley, Turnbaugh & Klein, 2006*). While obesity is a multicomponent disease and dogs were classified as obese according to the BCS score, additional analysis, including measurements of fat percentage and metabolic markers would improve the power of these correlations.

Changes in the ratio Firmicutes/Bacteroidetes seem to contribute to the development and preservation of obesity in dogs (*Park et al., 2015*). In agreement with humans and other

animals, the increase in Firmicutes and decrease in Bacteroidetes generates an increase in the extraction of energy from the diet, mainly complex polysaccharides (*Ley et al., 2006*; *Palmas et al., 2021*). This has been suggested to lead to the induction of specific metabolic pathways involved in short-chain fatty acid production and finally causing an increase in adipose tissue in the individual (*Martínez-Cuesta et al., 2021*). Recently it has been shown by using metabolomics that weight loss in obese dogs induces several changes in fecal metabolites (*Bermudez Sanchez et al., 2021*). The actual contribution of alterations in the Firmicutes/Bacteroidetes and increased energy extraction to obesity has been challenged by several studies (*Duncan et al., 2008*; *Schwiertz et al., 2010*; *Xiao & Kang, 2020*). In addition, no studies have demonstrated that these alterations indeed contribute to obesity in dogs.

Of 119 genera found in the microbiota of these animals in this study, the *Bacteroides* genus was the most abundant in normal weight dogs. Comparatively, it showed a decrease in obese dogs (Fig. 3). These microorganisms carry important immunological and metabolic functions. They are related to healthy microbiomes in dogs and humans, participate in the production of IL-6 and IL-10, stimulating the expression of MHC class II (*Tsuda et al., 2007*). They are also major bacteria promoting the production of IgA in the large intestine (*Schofield & Palm, 2018*; *Yang et al., 2020*). *Bacteroides* species have been associated with the prevention of insulin resistance and correct energy metabolism (*Rios-Covian et al., 2017*; *Gurung et al., 2020*). They are believed to have a great therapeutic value in metabolic diseases such as diabetes and obesity (*Yang et al., 2016*). The role of Bacteroidetes in the gut microbiota of dogs has not been well studied, especially if they play similar roles as in the human gut.

The most abundant genera in obese dogs were *Peptoclostridium* and *Blautia* (Fig. 3). They belong to the *Clostridium* class and phylum Firmicutes. The increase in species of these genera has been related to certain disease states in dogs and humans, including obesity, metabolic syndrome, acute diarrhea, and IBD (*Leung et al., 2013*; *Woting et al., 2014*; *Guard et al., 2015*). For example, the *Blautia* genus has been related to visceral fat accumulation in adult humans between 20 and 76 years of age, independent of external factors such as diet (*Ozato et al., 2019*). Changes in certain KEGG categories here were associated with increases in *Blautia* and *Allobaculum*. Certain studies have shown this last genus to increase in high-fat diets in mice and dogs (*Kilburn et al., 2020*; *Zheng et al., 2021*).

Metabolic analyses have supported the hypothesis that microbial gut ecology creates functional changes that help perpetuate obesity (*Backhed & Crawford, 2010*). The microbiome of obese mice is enriched in genes that decode for the catabolism of complex polysaccharides, promoting higher absorption of polysaccharides from the diet and subsequent metabolism of monosaccharides (*Turnbaugh et al., 2008*). This precedes de novo lipogenesis (DNL), a hepatic pathway responsible for converting excess carbohydrates into fatty acids that are subsequently esterified to store triacylglycerols (TGs), providing energy for the energy pathway of β-oxidation of fatty acids (*Ameer et al., 2014*). It is believed that the increased absorption of polysaccharides from the diet occurs due to an increase in microbial glycosyl hydrolases present in multiple intestinal bacteria, including those belonging to *Bacteroidetes* and *Firmicutes*, increasing the transactivation of lipogenic enzymes and increasing the deposit of fat in peripheral tissues (*Backhed et al., 2004*).

In this study, we predicted the enrichment of KEGG orthologs K03406 and K07483 in the obese group. Previously, other authors have identified these genes in dogs with diarrhea compared with a healthy group (*Guard et al., 2015*). These genes, which code for methyl-accepting chemotaxis protein and transposases, are related to the formation of biofilms, biosynthesis of flagella, production of exopolysaccharides and toxins, among others. These changes are likely a reflection of the enrichment in pro-inflammatory, flagellated bacteria in the gut of obese animals, contributing to their obese phenotype (*Salah Ud-Din & Roujeinikova, 2017*).

CONCLUSIONS

Obesity is a multifactorial disease highly prevalent in dogs. In this study, we compared the gut microbiome of normal weight and obese dogs. Their microbiome compositions were observed to be different. At the phylum level, obese animals showed an increase in Firmicutes (*Blautia*, *Peptoclostridium*) and a decrese in Bacteroidetes (*Bacteroides* spp). An increase in pathways related to motility and chemotaxis was observed in obese animals, which could contribute to their phenotype. It is essential to understand the contribution of specific microbiome taxa and their metabolic activities to obesity in dogs and how this information could be used in combination with diet to manage this disease.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Pamela Thomson conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Rodrigo Santibáñez performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Camila Rodríguez-Salas performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

- Carla Flores-Yañez conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Daniel Garrido analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Clinica Veterinaria Los Avellanos approved the study (HCVLA-008).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The data is available at ENA: PRJEB38793.

Data Availability

The following information was supplied regarding data availability:

The relative abundances at the genus or ASV level is available in the Supplementary File.

Supplemental Information

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REFERENCES

Afgan E, Baker D, Batut B, Van Den Beek M, Bouvier D, Ech M, Chilton J, Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research* **46**:W537–W544 DOI 10.1093/nar/gky379.

Alessandri G, Milani C, Mancabelli L, Longhi G, Anzalone R, Lugli GA, Duranti S, Turroni F, Ossiprandi MC, Van Sinderen D, Ventura M. 2020. Deciphering the bifidobacterial populations within the canine and feline gut microbiota. *Applied and Environmental Microbiology* **86**:e02875–19.

Alessandri G, Milani C, Mancabelli L, Mangifesta M, Lugli GA, Viappiani A, Duranti S, Turroni F, Ossiprandi MC, Van Sinderen D, Ventura M. 2019. Metagenomic dissection of the canine gut microbiota: insights into taxonomic, metabolic and nutritional features. *Environmental Microbiology* **21**:1331–1343 DOI 10.1111/1462-2920.14540.

Ameer F, Scandiuzzi L, Hasnain S, Kalbacher H, Zaidi N. 2014. De novo lipogenesis in health and disease. *Metabolism* **63**:895–902 DOI 10.1016/j.metabol.2014.04.003.

Apper E, Privet L, Taminiau B, Le Bourgot C, Svilar L, Martin JC, Diez M. 2020. Relationships between gut microbiota, metabolome, body weight, and glucose homeostasis of obese dogs fed with diets differing in prebiotic and protein content. *Microorganisms* **8**:513 DOI 10.3390/microorganisms8040513.

Backhed F, Crawford PA. 2010. Coordinated regulation of the metabolome and lipidome at the host-microbial interface. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1801**:240–245 DOI 10.1016/j.bbalip.2009.09.009.

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Gordon JI. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America* **101**:15718–15723 DOI 10.1073/pnas.0407076101.

Barko PC, McMichael MA, Swanson KS, Williams DA. 2017. The gastrointestinal microbiome: a review. *Journal of Veterinary Internal Medicine* **32**:9–25.

Bermingham EN, Maclean P, Thomas DG, Cave NJ, Young W. 2017. Key bacterial families (Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae) are related to the digestion of protein and energy in dogs. *PeerJ* **5**:e3019 DOI 10.7717/peerj.3019.

Bermudez Sanchez S, Pilla R, Sarawichitr B, Gramenzi A, Marsilio F, Steiner JM, Lidbury JA, Woods GRT, German AJ, Suchodolski JS. 2020. Fecal microbiota in client-owned obese dogs changes after weight loss with a high-fiber-high-protein diet. *PeerJ* **8**:e9706 DOI 10.7717/peerj.9706.

Bermudez Sanchez S, Pilla R, Sarawichitr B, Gramenzi A, Marsilio F, Steiner JM, Lidbury JA, Woods GRT, Suchodolski JS, German AJ. 2021. Untargeted fecal metabolome analysis in obese dogs after weight loss achieved by feeding a high-fiber-high-protein diet. *Metabolomics* **17**:66 DOI 10.1007/s11306-021-01815-1.

Bjørnvada CR, Gloorab S, Johansenab SS, Sandøebc P, Lundc TB. 2019. Neutering increases the risk of obesity in male dogs but not in bitches—a cross-sectional study of dog- and owner-related risk factors for obesity in Danish companion dogs. *Preventive Veterinary Medicine* **170**:104730 DOI 10.1016/j.prevetmed.2019.104730.

Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* **11**:2639–2643 DOI 10.1038/ismej.2017.119.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**:581–583 DOI 10.1038/nmeth.3869.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335–336 DOI 10.1038/nmeth.f.303.

Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R, Patel A, Day MJ. 2017. Obesity and associated comorbidities in people and companion animals: a one health perspective. *Journal of Comparative Pathology* **156**:296–309 DOI 10.1016/j.jcpa.2017.03.006.

Chun JL, Bang HT, Ji SY, Jeong JY, Kim M, Kim B, Lee SD, Lee YK, Reddy KE, Kim KH. 2019. A simple method to evaluate body condition score to maintain the

optimal body weight in dogs. *Journal of Animal Science and Technology* **61**:366–370 DOI 10.5187/jast.2019.61.6.366.

Chun Ju L, Ji Sang Y, Lee Sung D, Lee Yoo K, Kim B, Kim Ki H. 2020. Difference of gut microbiota composition based on the body condition scores in dogs. *Journal of Animal Science and Technology* **62**:239–246 DOI 10.5187/jast.2020.62.2.239.

Clark M, Hoenig M. 2016. Metabolic effects of obesity and its interaction with endocrine diseases. *Veterinary Clinics of North America: Small Animal Practice* **46**:797–815 DOI 10.1016/j.cvsm.2016.04.004.

Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki-Maulden G, Hayward MR, Forslund SK, Schmidt TSB, Descombes P, Jackson JR, Li Q, Bork P. 2018. Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* **6**:72 DOI 10.1186/s40168-018-0450-3.

Craven MD, Washabau RJ. 2019. Comparative pathophysiology and management of protein-losing enteropathy. *Journal of Veterinary Internal Medicine* **33**:383–402 DOI 10.1111/jvim.15406.

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2020. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* **38**:685–688 DOI 10.1038/s41587-020-0548-6.

Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. 2008. Human colonic microbiota associated with diet, obesity and weight loss. *International Journal of Obesity* **32**:1720–1724 DOI 10.1038/ijo.2008.155.

Forster GM, Stockman J, Noyes N, Heuberger AL, Broeckling CD, Bantle CM, Ryan EP. 2018. Comparative study of serum biochemistry, metabolome and microbiome parameters of clinically healthy, normal weight, overweight, and obese companion dogs. *Topics in Companion Animal Medicine* **33**:126–135 DOI 10.1053/j.tcam.2018.08.003.

Garcia-Mazcorro JF, Minamoto Y, Kawas JR, Suchodolski JS, De Vos WM. 2020. Akkermansia and microbial degradation of mucus in cats and dogs: implications to the growing worldwide epidemic of pet obesity. *Veterinary Sciences* **7**:44 DOI 10.3390/vetsci7020044.

Gavazza A, Rossi G, Lubas G, Cerquetella M, Minamoto Y, Suchodolski JS. 2018. Faecal microbiota in dogs with multicentric lymphoma. *Veterinary and Comparative Oncology* **16**:E169–E175 DOI 10.1111/vco.12367.

German AJ, Holden SL, Bissot T, Morris PJ, Biourge V. 2009. Use of starting condition score to estimate changes in body weight and composition during weight loss in obese dogs. *Research in Veterinary Science* **87**:249–254 DOI 10.1016/j.rvsc.2009.02.007.

Guard BC, Barr JW, Reddivari L, Klemashevich C, Jayaraman A, Steiner JM, Vanamala J, Suchodolski JS. 2015. Characterization of microbial dysbiosis and metabolomic changes in dogs with acute diarrhea. *PLOS ONE* **10**:e0127259 DOI 10.1371/journal.pone.0127259.

Guard BC, Mila H, Steiner JM, Mariani C, Suchodolski JS, Chastant-Maillard S. 2017. Characterization of the fecal microbiome during neonatal and early pediatric development in puppies. *PLOS ONE* **12**:e0175718 DOI 10.1371/journal.pone.0175718.

Gupta VK, Paul S, Dutta C. 2017. Geography, ethnicity or subsistence specific variations in human microbiome composition and diversity. *Frontiers in Microbiology* **8**:1162 DOI 10.3389/fmicb.2017.01162.

Guptill L, Glickman L, Glickman N. 2003. Time trends and risk factors for diabetes mellitus in dogs: analysis of veterinary medical data base records (1970-1999). *Veterinary Journal* **165**:240–247 DOI 10.1016/S1090-0233(02)00242-3.

Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, Shulzhenko N. 2020. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* **51**:102590 DOI 10.1016/j.ebiom.2019.11.051.

Hand D, Wallis C, Colyer A, Penn CW. 2013. Pyrosequencing the canine faecal microbiota: breadth and depth of biodiversity. *PLOS ONE* **8**:e53115 DOI 10.1371/journal.pone.0053115.

Handl S, German AJ, Holden SL, Dowd SE, Steiner JM, Heilmann RM, Grant RW, Swanson KS, Suchodolski JS. 2013. Faecal microbiota in lean and obese dogs. *FEMS Microbiology Ecology* **84**:332–343 DOI 10.1111/1574-6941.12067.

Haro C, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, Quintana-Navarro GM, Landa BB, Navas-Cortés JA, Tena-Sempere M, Clemente JC, López-Miranda J, Pérez-Jiménez F, Camargo A. 2016. Intestinal microbiota is influenced by gender and body mass index. *PLOS ONE* **11**(**5**):e0154090 DOI 10.1371/journal.pone.0154090.

Heeley AM, O'Neill DG, Davison LJ, Church DB, Corless EK, Brodbelt DC. 2020. Diabetes mellitus in dogs attending UK primary-care practices: frequency, risk factors and survival. *Canine Medicine and Genetics* **7**:6 DOI 10.1186/s40575-020-00087-7.

Heintz-Buschart A, Wilmes P. 2018. Human gut microbiome: function matters. *Trends in Microbiology* **26**:563–574 DOI 10.1016/j.tim.2017.11.002.

Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, Tameda M, Shiraki K, Ito M, Takei Y, Takase K. 2015. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterology* **15**:100 DOI 10.1186/s12876-015-0330-2.

Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG. 2016. Role of gut microbiota in the aetiology of obesity: proposed mechanisms and review of the literature. *Journal of Obesity* **2016**:7353642 DOI 10.1155/2016/7353642.

Khera AV, Chaffin M, Wade KH, Zahid S, Brancale J, Xia R, Distefano M, Senol-Cosar O, Haas ME, Bick A, Aragam KG, Lander ES, Smith GD, Mason-Suares H, Fornage M, Lebo M, Timpson NJ, Kaplan LM, Kathiresan S. 2019. Polygenic prediction of weight and obesity trajectories from birth to adulthood. *Cell* **177**:587–596.e9 DOI 10.1016/j.cell.2019.03.028.

Kieler IN, Shamzir Kamal S, Vitger AD, Nielsen DS, Lauridsen C, Bjornvad CR. 2017. Gut microbiota composition may relate to weight loss rate in obese pet dogs. *Veterinary Medicine and Science* **3**:252–262 DOI 10.1002/vms3.80.

Kilburn LR, Koester LR, Schmitz-Esser S, Serão NV, Rossoni Serão MC. 2020. High-fat diets led to OTU-level shifts in fecal samples of healthy adult dogs. *Frontiers in Microbiology* **11**:564160 DOI 10.3389/fmicb.2020.564160.

Kim J, An JU, Kim W, Lee S, Cho S. 2017. Differences in the gut microbiota of dogs (Canis lupus familiaris) fed a natural diet or a commercial feed revealed by the Illumina MiSeq platform. *Gut Pathogens* **9**:68 DOI 10.1186/s13099-017-0218-5.

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies.. *Nucleic Acids Research* **41**:1–11 DOI 10.1093/nar/gks808.

Leung J, Burke B, Ford D, Garvin G, Korn C, Sulis C, Bhadelia N. 2013. Possible association between obesity and clostridium difficile infection. *Emerging Infectious Diseases* **19**:1791–1798 DOI 10.3201/eid1911.130618.

Ley RE, Mardis ER, Magrini V, Mahowald MA, Turnbaugh PJ, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**:1027–1031 DOI 10.1038/nature05414.

Ley RE, Turnbaugh PJ, Klein S. 2006. Human gut microbes associated with obesity. *Nature* **444**:1022–1023 DOI 10.1038/4441022a.

Li Q, Lauber CL, Czarnecki-Maulden G, Pan Y, Hannah SS. 2017. Effects of the dietary protein and carbohydrate ratio on gut microbiomes in dogs of different body conditions. *mBio* **8**:1703–1716.

Lin H, Peddada SD. 2020. Analysis of microbial compositions: a review of normalization and differential abundance analysis. *npj Biofilms and Microbiomes* **6**:60 DOI 10.1038/s41522-020-00160-w.

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. 2011. UniFrac: an effective distance metric for microbial community comparison. *ISME Journal* **5**:169–172 DOI 10.1038/ismej.2010.133.

Mann HB, Whitney DR. 1947. On a test of whether one of two random variables is stochastically larger than the other. *Annals of Mathematical Statistics* **18**:50–60 DOI 10.1214/aoms/1177730491.

Mao JZ, Xia J, Chen JY. 2013. Prevalence and risk factors for canine obesity surveyed in veterinary practices in Beijing, China. *Preventive Veterinary Medicine* **112**:438–442 DOI 10.1016/j.prevetmed.2013.08.012.

Marchesi JR, Ravel J. 2015. The vocabulary of microbiome research: a proposal. *Microbiome* **3**:31 DOI 10.1186/s40168-015-0094-5.

Marshall WG, Bockstahler BA, Hulse DA, Carmichael S. 2009. A review of osteoarthritis and obesity: current understanding of the relationship and benefit of obesity treatment and prevention in the dog. *Veterinary and Comparative Orthopaedics and Traumatology* **22**:339–345 DOI 10.3415/VCOT-08-08-0069.

Martínez-Cuesta MC, Campo Rdel, Garriga-García M, Peláez C, Requena T. 2021. Taxonomic characterization and short-chain fatty acids production of the obese microbiota. *Frontiers in Cellular and Infection Microbiology* **11**:516 DOI 10.3389/fcimb.2021.598093.

Middelbos IS, Vester Boler BM, Qu A, White BA, Swanson KS, Fahey GCJ. 2010. Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. *PLOS ONE* **5**:e9768 DOI 10.1371/journal.pone.0009768.

Moinard A, Payen C, Ouguerram K, André A, Hernandez J, Drut A, Biourge VC, Suchodolski JS, Flanagan J, Nguyen P, Leray V. 2020. Effects of high-fat diet at two energetic levels on fecal microbiota, colonic barrier, and metabolic parameters in dogs. *Frontiers in Veterinary Science* **7**:699 DOI 10.3389/fvets.2020.566282.

Montoya-Alonso JA, Bautista-Castaño I, Peña C, Suárez L, Juste MC, Tvarijonaviciute A. 2017. Prevalence of canine obesity, obesity-related metabolic dysfunction, and relationship with owner obesity in an obesogenic region of Spain. *Frontiers in Veterinary Science* **4**:59 DOI 10.3389/fvets.2017.00059.

Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, Katsuragi Y, Kakuta M, Imoto S, Ihara KSN. 2019. Blautia genus associated with visceral fat accumulation in adults 20–76 years of age. *npj Biofilms Microbiomes* **5**:28 DOI 10.1038/s41522-019-0101-x.

Palmas V, Pisanu S, Madau V, Casula E, Deledda A, Cusano R, Uva P, Vascellari S, Loviselli A, Manzin A, Velluzzi F. 2021. Gut microbiota markers associated with obesity and overweight in Italian adults. *Scientific Reports* **11**:5532 DOI 10.1038/s41598-021-84928-w.

Park H-J, Lee S-E, Kim H-B, Isaacson RE, Seo K-W, Song K-H. 2015. Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. *Journal of Veterinary Internal Medicine* **29**:43–50 DOI 10.1111/jvim.12455.

Pilla R, Suchodolski JS. 2020. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. *Frontiers in Veterinary Science* **6**:498 DOI 10.3389/fvets.2019.00498.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**:D590–D596 DOI 10.1093/nar/gks1219.

Rios-Covian D, Salazar N, Gueimonde M, Gdelos Reyes-Gavilan C. 2017. Shaping the metabolism of intestinal bacteroides population through diet to improve human health. *Frontiers in Microbiology* **8**:376 DOI 10.3389/fmicb.2017.00376.

Salah Ud-Din AIM, Roujeinikova A. 2017. Methyl-accepting chemotaxis proteins: a core sensing element in prokaryotes and archaea. *Cellular and Molecular Life Sciences : CMLS* **74**:3293–3303 DOI 10.1007/s00018-017-2514-0.

Salas-Mani A, Jeusette I, Castillo I, Manuelian CL, Lionnet C, Iraculis N, Sanchez N, Fernández S, Vilaseca L, Torre C. 2018. Fecal microbiota composition changes

after a bw loss diet in beagle dogs. *Journal of Animal Science* **96**:3102–3111 DOI 10.1093/jas/sky193.

Schofield WB, Palm NW. 2018. Gut Microbiota: IgA protects the pioneers. *Current Biology* **28**:R1117–R1119 DOI 10.1016/j.cub.2018.08.019.

Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. 2010. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* **18**:190–195 DOI 10.1038/oby.2009.167.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biology* **12**:R60 DOI 10.1186/gb-2011-12-6-r60.

Siddiqui R, Akbar N, Khan NA. 2021. Gut microbiome and human health under the space environment. *Journal of Applied Microbiology* **130**:14–24 DOI 10.1111/jam.14789.

Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, Caporaso JG, Knights D, Clemente JC. 2013. Cohabiting family members share microbiota with one another and with their dogs. *eLife* **2**:e00458 DOI 10.7554/eLife.00458.

Stubbs RJ, Tolkamp BJ. 2006. Control of energy balance in relation to energy intake and energy expenditure in animals and man: an ecological perspective. *British Journal of Nutrition* **95**:657–676 DOI 10.1079/BJN20041361.

Suchodolski JS. 2011. Companion animals symposium: microbes and gastrointestinal health of dogs and cats. *Journal of Animal Science* **89**:1520–1530 DOI 10.2527/jas.2010-3377.

Suchodolski JS. 2016. Diagnosis and interpretation of intestinal dysbiosis in dogs and cats. *The Veterinary Journal* **215**:30–37 DOI 10.1016/j.tvjl.2016.04.011.

Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, Kachroo P, Ivanov I, Minamoto Y, Dillman EM, Steiner JM, Cook AK, Toresson L. 2012. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. *PLOS ONE* **7**:e51907 DOI 10.1371/journal.pone.0051907.

Tsuda M, Hosono A, Yanagibashi T, Hachimura S, Hirayama K, Itoh K, Kaminogawa S. 2007. Prior stimulation of antigen-presenting cells with Lactobacillus regulates excessive antigen-specific cytokine responses in vitro when compared with Bacteroides. *Cytotechnology* **55**:89–101 DOI 10.1007/s10616-007-9104-1.

Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host & Microbe* **3**:213–223 DOI 10.1016/j.chom.2008.02.015.

Vemuri R, Shankar EM, Chieppa M, Eri R, Kavanagh K. 2020. Beyond just bacteria: functional biomes in the gut ecosystem including virome, mycobiome, archaeome and helminths. *Microorganisms* **8**:483 DOI 10.3390/microorganisms8040483.

Vital M, Gao J, Rizzo M, Harrison T, Tiedje JM. 2015. Diet is a major factor governing the fecal butyrate-producing community structure across Mammalia, Aves and Reptilia. *ISME Journal* **9**:832–843 DOI 10.1038/ismej.2014.179.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**:16 DOI 10.1128/AEM.00062-07.

Wernimont SM, Radosevich J, Jackson MI, Ephraim E, Badri DV, MacLeay JM, Jewell DE, Suchodolski JS. 2020. The effects of nutrition on the gastrointestinal microbiome of cats and dogs: impact on health and disease. *Frontiers in Microbiology* **11**:1266 DOI 10.3389/fmicb.2020.01266.

Woting A, Pfeiffer N, Loh G, Klaus S, Blaut M. 2014. Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. *mBio* **5(5)**:e01530–14 DOI 10.1128/mBio.01530-14.

Xiao H, Kang S. 2020. The role of the gut microbiome in energy balance with a focus on the gut-adipose tissue axis. *Frontiers in Genetics* **11**:297 DOI 10.3389/fgene.2020.00297.

Xu J, Verbrugghe A, Lourenço M, Cools A, Liu DJX, Van de Wiele T, Marzorati M, Eeckhaut V, Van Immerseel F, Vanhaecke L, Campos M, Hesta M. 2017. The response of canine faecal microbiota to increased dietary protein is influenced by body condition. *BMC Veterinary Research* **13**:374 DOI 10.1186/s12917-017-1276-0.

Yang J-Y, Lee Y-S, Kim Y, Lee S-H, Ryu S, Fukuda S, Kweon M. 2016. Gut commensal Bacteroides acidifaciens prevents obesity and improves insulin sensitivity in mice. *Mucosal Immunology* **10**:104–116.

Yang C, Mogno I, Contijoch EJ, Borgerding JN, Aggarwala V, Li Z, Siu S, Grasset EK, Helmus DS, Dubinsky MC, Mehandru S, Cerutti A, Faith JJ. 2020. Fecal IgA levels are determined by strain-level differences in bacteroides ovatus and are modifiable by gut microbiota manipulation. *Cell Host and Microbe* **27**:467–475.e6 DOI 10.1016/j.chom.2020.01.016.

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and all-species Living Tree Project (LTP) taxonomic frameworks. *Nucleic Acids Research* **42**:D643–D648 DOI 10.1093/nar/gkt1209.

Zheng Z, Lyu W, Ren Y, Li X, Zhao S, Yang H, Xiao Y. 2021. Allobaculum involves in the modulation of intestinal ANGPTLT4 expression in mice treated by high-fat diet. *Frontiers in Nutrition* **8**:242.