

# Obese dog fecal microbiota shifts towards lean dog fecal microbiota after weight loss intervention

Sandra Bermudez-Sanchez<sup>1,2</sup>, Rachel Pilla<sup>1</sup>, Alessandro Gramenzi<sup>2</sup>, Fulvio Marsilio<sup>2</sup>, Joerg M. Steiner<sup>1</sup>, Jonathan A. Lidbury<sup>1</sup>, Alex J. German<sup>3</sup>, Jan S. Suchodolski<sup>1</sup>

1. Gastrointestinal Laboratory, department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX
2. Facoltà di Medicina Veterinaria, Università degli studi di Teramo, Teramo, Italy
3. Small Animal Teaching Hospital, School of Veterinary Science, University of Liverpool, Leahurst Neston, United Kingdom

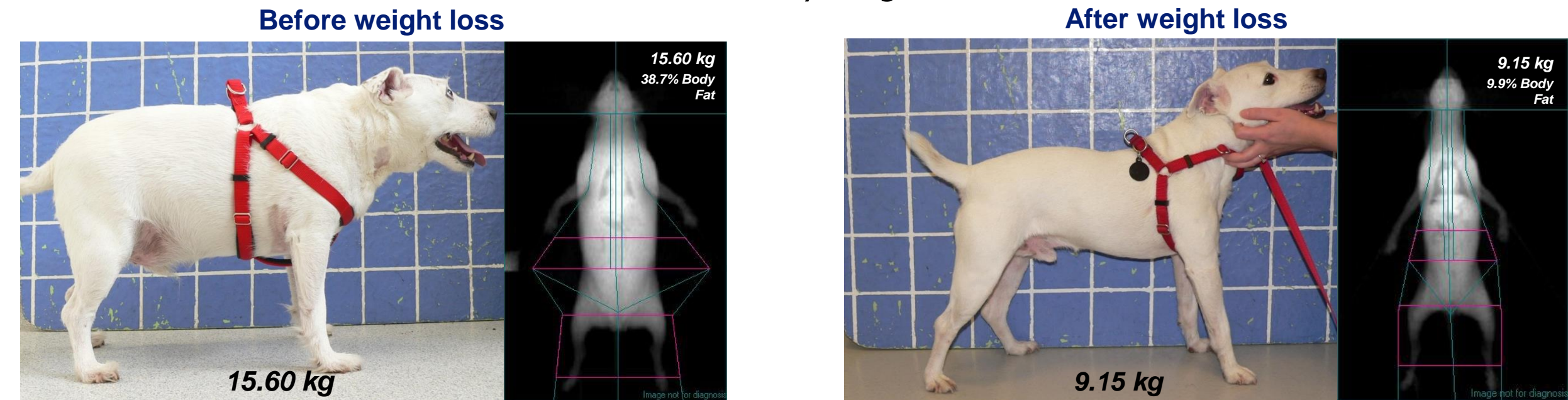
## Introduction

Obesity has become epidemic in the past decades not only in humans. The incidence of obesity in companion animals has increased dramatically, and it has become a serious concern in veterinary medicine. A causal relationship between intestinal microbiota dysbiosis and body weight control has been demonstrated in rodent models. In humans, several studies have investigated fecal microbiota differences between obese and lean individuals and its possible effect on weight loss. In dogs, however, most of the differences found did not reach significance. Further studies are required to increase our understanding of the role of gut microbiota in obesity in companion animals.

## Objectives

Evaluate the fecal microbiota composition in client-owned obese dogs before and after losing weight, in comparison with control lean dogs, using 16S rRNA sequencing:

1. To identify the differences between obese and lean dogs
2. To evaluate how those differences are affected by weight loss



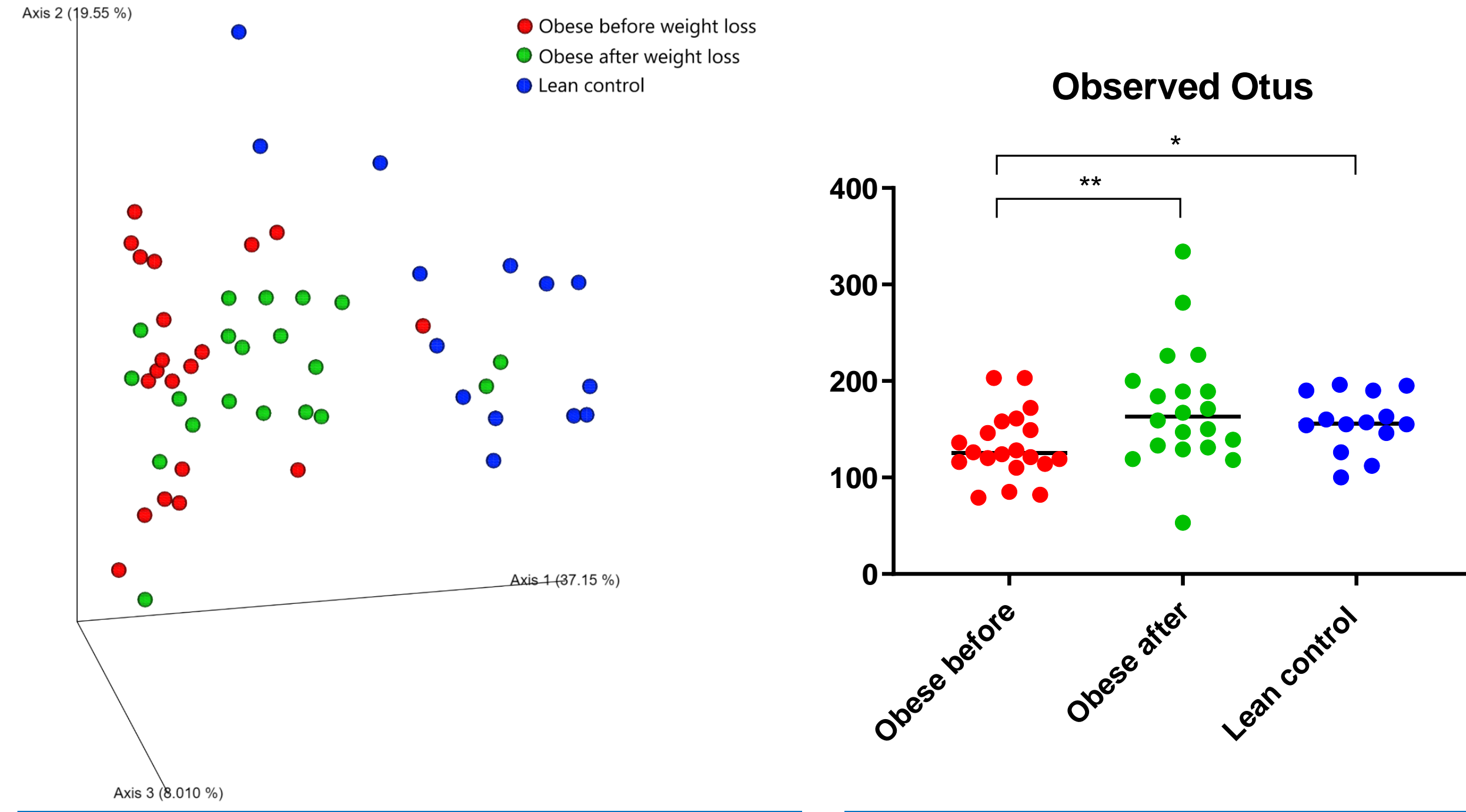
**Figure 1.** Sample image of obese dog included in the study. Left image shows visual and DEXA scan at enrollment. Right image shows visual and DEXA scan after weight loss. DEXA measurement showed 15.60 kg of weight and 38.7% starting body fat vs. 9.15 kg of final weight and 9.9% body fat after weight loss.

OBESSE DOGS (n=20)	Days on weight loss diet	Age in months	Gender	Neutered status	BCS before weight loss	BCS after weight loss
MEAN (min-max)	330.9 (112-768)	69.4 (16-135)	10F/10 M	18N/2I	8 (6-9)	5.2 (4-7)

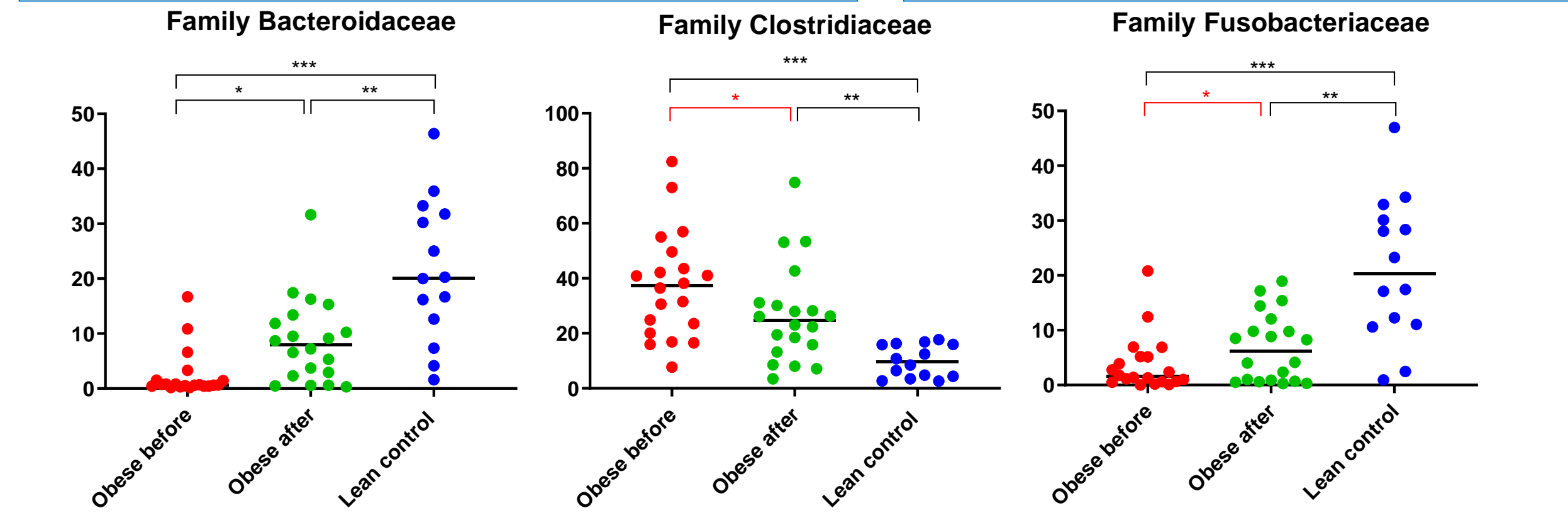
LEAN DOGS (n=14)	Age in months	Gender	Neutered status	BCS
MEAN (min-max)	48.9 (12-120)	8F/6M	12N/2I	4.1 (3-5)

**Table 1.** Demographics of obese and lean dogs enrolled in the study.

## Results



**Figure 2.** 3D Principal coordinate analysis (PCoA) plot based on weighted UniFrac distances of 16S rRNA gene. Visible clustering was confirmed by ANOSIM, showing that the fecal microbiota of obese dogs significantly changed after weight loss ( $p=0.016$ ,  $r=0.358$ ), but was still significantly different from the fecal microbiota of lean control dogs ( $p=0.001$ ,  $r=0.358$ ).



**Figure 3.** Observed OTUs, an indicator of richness, was lower in obese dogs when compared to lean dogs ( $p=0.050$ ). After weight loss, the richness of the fecal microbiota was significantly increased ( $p=0.007$ ), and no longer different from lean control dogs.

## Discussion

Fecal microbiota of obese dogs showed significant differences when compared to fecal microbiota of lean dogs and after weight loss:

Lower bacteria diversity (richness) was observed in obese dogs, which has been associated to obesity and to other metabolic and gastrointestinal diseases before.

The observed increase of Bacteroidetes, Fusobacteria, and decrease of Firmicutes after weight loss was coherent with bacterial abundance in samples from lean dogs.

- Similar to humans, obese dogs had a higher ratio of Firmicutes:Bacteroidetes that decreased after weight loss
- Within the phylum Firmicutes, we observed a decrease of the genus Clostridium after weight loss, as previously reported in research beagle dogs
- Higher Fusobacteria abundance has been previously associated with healthy dogs, when compared to different canine diseases

## Materials and Methods

This retrospective study was conducted at the Small Animal Teaching Hospital of the University of Liverpool:

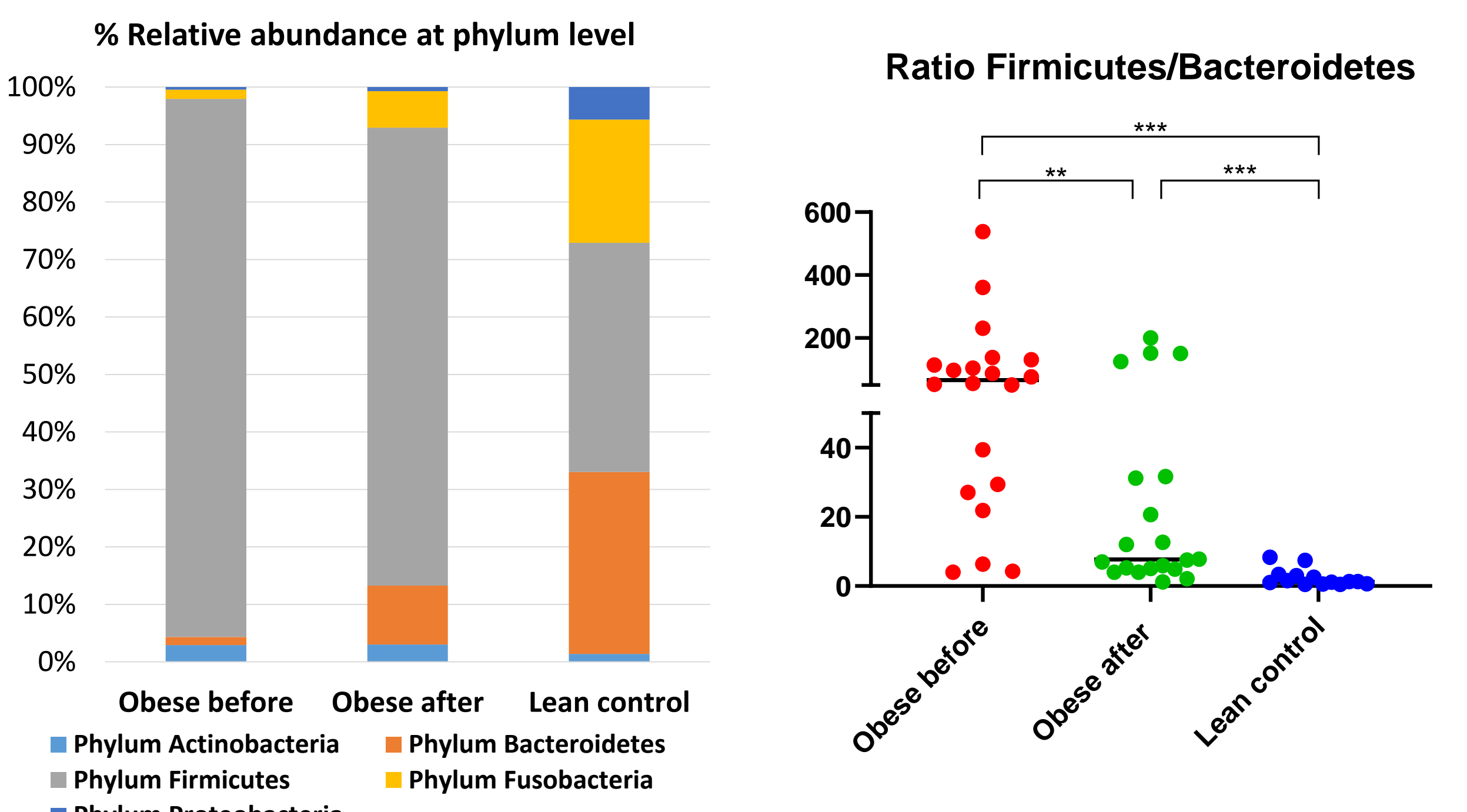
- Animal recruitment:
- ✓ Obese dogs (6>BCS), but otherwise healthy (n=20)
- Weight loss diet intervention (Royal Canin Satiety Dry)

### Microbiota analysis:

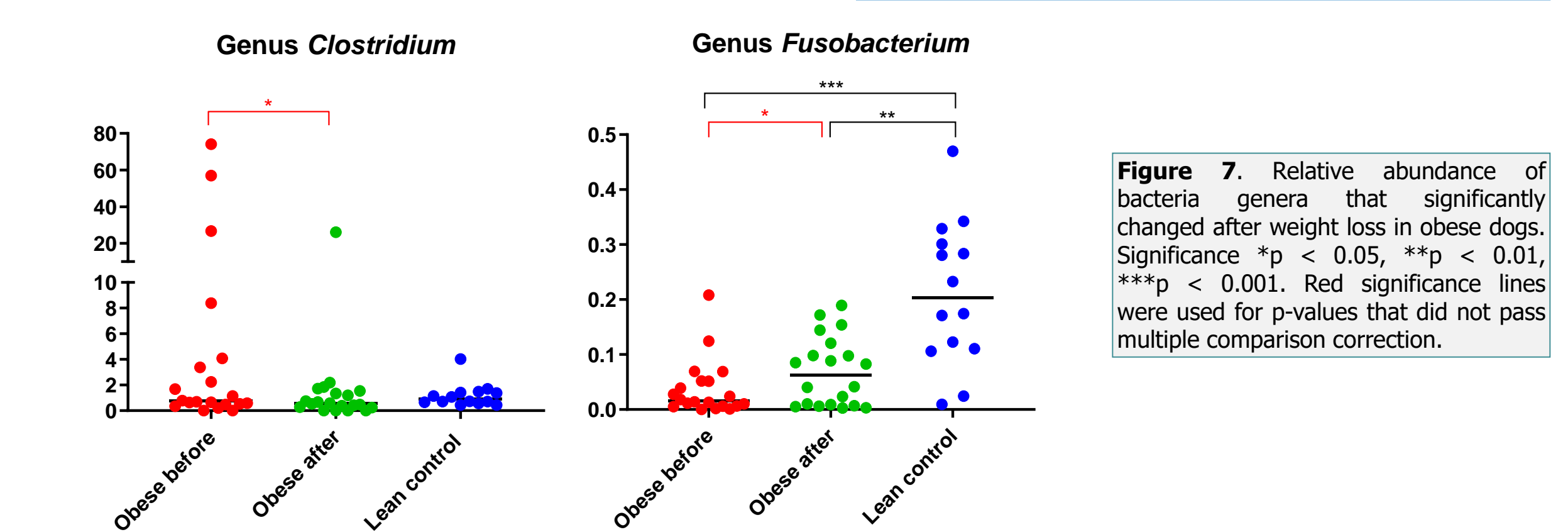
- Fecal samples collection before and after weight loss
- Lean dogs were enrolled as controls, and sampled once (n=14)
- DNA extraction with Mo Bio PowerSoil® DNA isolation kit
- 16S rRNA gene was amplified and sequenced by Illumina MiSeq and QIIME 2 pipeline was used for analysis of sequences

### Statistical analysis:

- ✓ Normality was tested using Shapiro-Wilk test for all variables
- ✓ Analysis of similarities (ANOSIM) was used to compare microbial communities between groups
- ✓ Alpha diversity indexes (Shannon, Chao1 and Observed Otus) and % bacterial relative abundance were compared between groups using Mann-Whitney or Wilcoxon test
- ✓ P-values were adjusted using the Benjamini-Hochberg Step-up method with a false discovery rate (FDR) of 0.05
- ✓ For all statistical analyses significance set at P value < 0.05



**Figure 4.** Relative abundance of the most abundant phyla found in fecal samples of obese dogs before weight loss, after weight loss and lean control dogs. Significant differences were found in all phyla when compared obese dogs at baseline and lean control dogs ( $p<0.05$ ). Increase of Bacteroidetes ( $p=0.002$ ) and Fusobacteria ( $p=0.040$ ), and decrease of Firmicutes ( $p=0.001$ ) abundances were shown after weight loss.



**Figure 5.** Firmicutes/Bacteroidetes ratio values for each sample. Firmicutes/Bacteroidetes ratio was significantly higher in obese dogs when compared to lean dogs ( $p<0.001$ ). After weight loss, Firmicutes/Bacteroidetes ratio significantly decreased ( $p=0.004$ ), but remained significantly higher than lean control dogs ( $p<0.001$ ).

## Conclusion

- The fecal microbiota of obese dogs showed similarities to the fecal microbiota of obese humans, characterized for a higher Firmicutes:Bacteroidetes ratio and a lower bacterial diversity richness.
- Weight loss significantly changed the fecal microbiota composition and the relative abundance of specific bacteria populations following a trend toward the fecal microbial of lean dogs.
- However, fecal microbiota of obese dogs after weight loss was still significantly different from the fecal microbiota of lean dogs.

## References

1. German AJ, et al. Dietary energy restriction and successful weight loss in obese client-owned dogs. Journal of Veterinary Internal Medicine. 2007; 21, 1174–1180.
2. Ley RE, et al. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022-3.
3. Cotillard A, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013; 500, 585–588.
4. Handl S, et al. Faecal microbiota in lean and obese dogs. FEMS Microbiol Ecol. 2013; 84(2):332-43.
5. Park HJ, et al. Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. J Vet Intern Med. 2015; 29(1):43-50.
6. Kiehl JN, et al. Gut microbiota composition may relate to weight loss rate in obese pet dogs. Vet Med Sci. 2017; 3(4):252-62.
7. Salas-Mani A, et al. Fecal microbiota composition changes after a BW loss diet in Beagle dogs. J Anim Sci. 2018; 96(8):3102-11.

## Conflict of Interest

There are no conflicts of interest to disclose.

Sandra Bermudez Sanchez  
Gastrointestinal Laboratory  
[ssanchez@cvm.tamu.edu](mailto:ssanchez@cvm.tamu.edu)  
[sbermudezsanchez@unite.it](mailto:sbermudezsanchez@unite.it)