# Obesity Modulates Gut Microbiome in Triple Negative Breast Cancer


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## Introduction

**The large intestine harbors a dynamic composition of microorganisms, which constitutes the gut microbiome. The diversity of the microbiome affects the host immune system and physiology, and alterations in the diversity lead to a state of dysbiosis. This dysbiosis plays an important role in the development of breast cancer and obesity due to its effects on inflammation and increased fat content of the host.**

**Triple negative breast cancer (TNBC) is an aggressive type of breast cancer that primarily affects premenopausal women and is characterized by low or no progesterone, estrogen, or HER2 receptors. Obesity leads to an increased incidence and worse prognosis of TNBC due to factors such as inflammation, reactive oxygen species, leptin, and hyperinsulinemia.**

**Because the gut microbiome plays a role in the development of obesity and breast cancer, the maintenance of a "healthy" gut microbiome could be a therapeutic target in the treatment of TNBC. The relationship between the gut microbiome, obesity, and TNBC has not been defined. This study aims to further the knowledge of how obesity associated with TNBC affects of the gut microbiome in order to improve therapeutic outcomes.**

## Methods

- **20 FVB female mice were fed control vs "Western diet" [diet-induced obesity, Adjusted calories diet: 42% from fat, High sucrose (34% by weight)] for sixteen weeks.**
- **Then, 1 million C0321 Mouse TNBC cells were injected into mammary fat pad (with Matrigel) of syngeneic FVB mice, and the tumor was allowed to grow for 3 weeks. Groups: (lean=5, lean-tumor=5, obese=5, obese-tumor=5)**
- **Tumors were collected for downstream processing and gut samples were collated for microbiome analysis (16s rRNA sequencing and metagenomics), which was done at Microbiome Insights in Canada.**

## Results

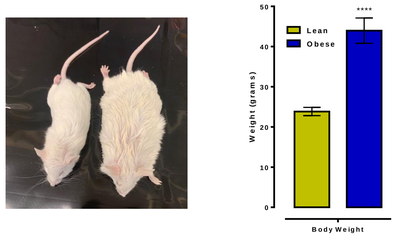


**Figure 1:** Lean and Obese mince weight after 16 weeks of control vs Western diet respectively.

## 16s rRNA Sequencing: Ordination Plot

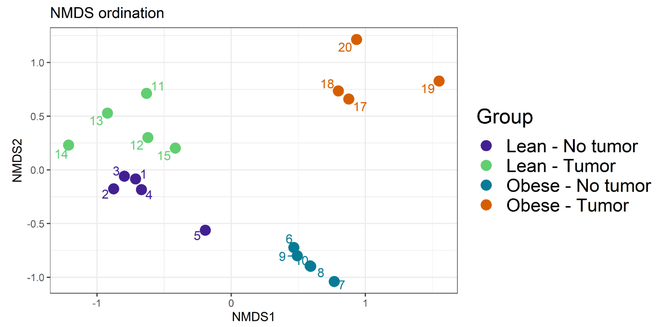


**Figure 2:** Ordination plot displaying similarity of community composition between samples

## 16s rRNA Sequencing: Alpha Diversity

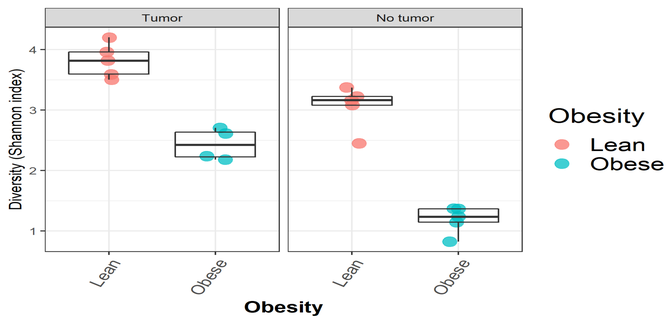


**Figure 3:** Alpha diversity was calculated using Shannon's index, a measure of diversity that takes abundance and evenness of species into account. Results show that obesity decreases the diversity of the gut microbiome in both tumor+ and tumor- mice. Lean mice have a higher gut microbial diversity compared to obese mice.

## Metagenomics Analysis: Taxonomic Profiles

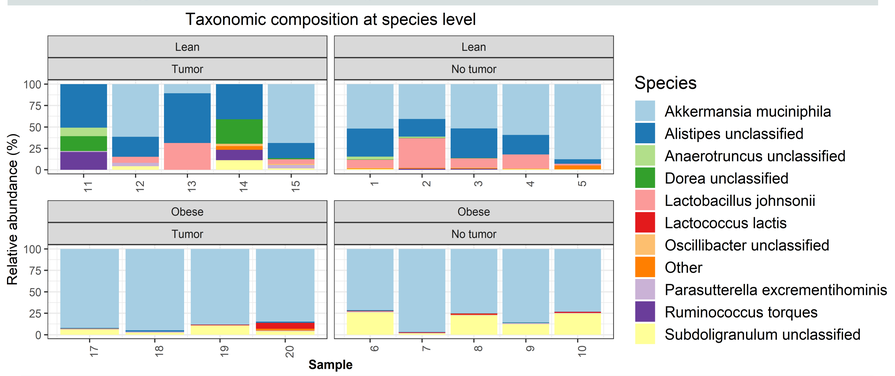


**Figure 4:** Metagenomic analysis revealed taxonomic profiles in order to highlight the genus and species changes among lean, obese, tumor+, and tumor- mice. Obesity was the only significant factor for alteration of taxonomic composition at the genus level. The most dominant species in obese mice was *Akkermansia muciniphila.*

## Metagenomics Analysis: Functional Profiles

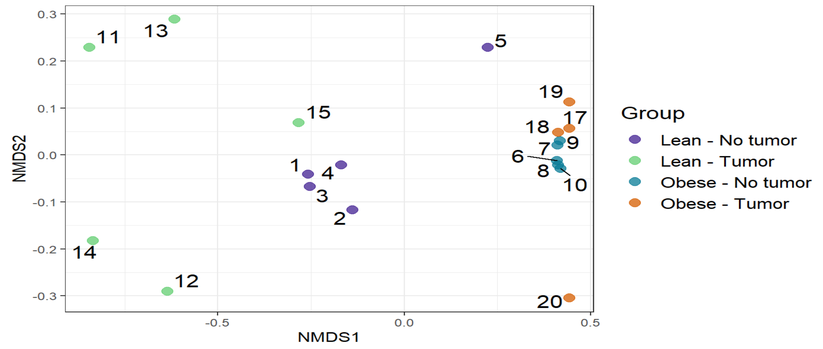


**Figure 5:** Analysis of similarities of functional profiles. Similarities were calculated using Bray-Curtis dissimilarities, which considers both presence/absence and abundance of pathways. It was then plotted on a Non-Metric Multidimensional Scaling (NMDS) plot. Functional groups near each other indicates similarities between mice.

| | Degrees of freedom | Sum of squares | F model | R2 | Pr(>F) |
|---|---|---|---|---|---|
| **Obesity** | **1** | **0.872** | **32.560** | **0.576** | **0.0001** |
| **Tumor** | **1** | **0.127** | **4.725** | **0.084** | **0.0196** |
| **Obesity:Tumor interaction** | **1** | **0.113** | **4.219** | **0.075** | **0.0295** |
| **Residuals** | **15** | **0.402** | **NA** | **0.265** | **NA** |
| **Total** | **18** | **1.513** | **NA** | **1.000** | **NA** |

**Table 1**: Permutational multivariate analysis using distance matrices (PERMANOVA) determined significant differences between treatments. Obesity, tumor, and obesity:tumor interaction were significant in explaining variance of profiles. Obesity was the strongest factor, accounting for 57% of the variation.

## Conclusions

- **Obese mice had a decreased diversity of the gut microbiome compared to lean mice.**
- **Although only obesity significantly explained the variation of taxonomic profiles, obesity, tumor, and obesity:tumor interaction significantly explained the variation of functional profiles.**
- **Obesity was the strongest factor for a change in the gut microbiome, but the analysis of functional profiles show that obesity and TNBC are correlated. Further studies are warranted to further understand this correlation.**
- **This study improved the knowledge of the interrelationship between obesity, TNBC, and the gut microbiome. Further understanding of this relationship can improve therapeutic options and outcomes for patients with TNBC in order to decrease morbidity and mortality.**