

TOTUM-63 DECREASES INSULIN RESISTANCE AND ADIPOSIITY, AND ALTERS GUT MICROBIOTA IN HIGH-FAT FED MICE

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BACKGROUND

- Diabetic population will rise by almost 55% to 640 million by 2040 (International Diabetes Federation, 2015).
- Prediabetes is a condition associated with obesity and insulin resistance, defined by a high risk of developing type 2 diabetes (T2D).
- 70% of prediabetics will eventually develop T2D (Tuso *et al.*, 2014).
- Gut microbiota is involved in the development of obesity and obesity-associated complications such as insulin resistance (Cani *et al.*, 2007).

OBJECTIVES

- We have previously shown that TOTUM-63 (T63) improves body composition and insulin sensitivity in high fat-fed mice.
- The aim of this work was to study the effects of TOTUM-63 on gut microbiota in high-fat fed mice.

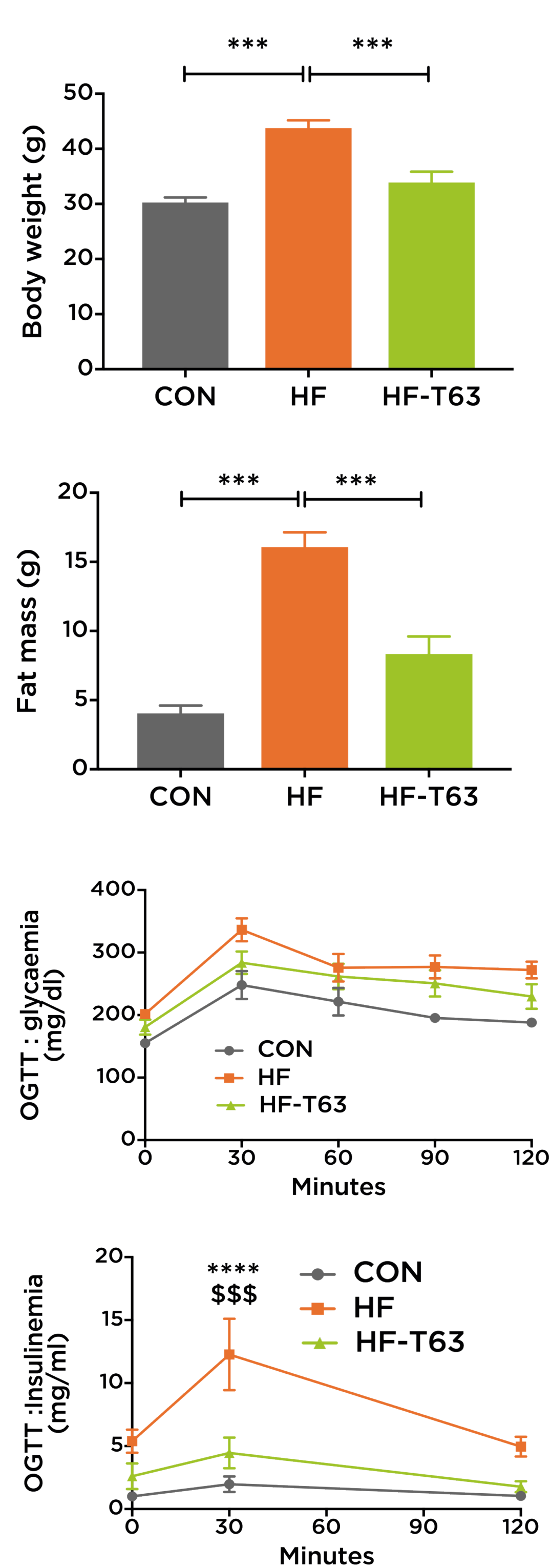
STUDY DESIGN

- Five week-old mice were fed a standard diet (CON), a high-fat diet (HF) or a HF diet + T63 (HF-T63, 2.7% diet) for 16 weeks, ad libitum.
- Whole body composition was determined by Echo MRI™ analyses (Houston, USA), at the end of the experiment.
- Oral Glucose Tolerance Test (OGTT) was performed at the end of the experiment. Blood glucose was monitored before (0), 30, 60, 90 and 120 mn after an oral gavage of 2.3g of glucose/kg of lean mass.
- Plasma insulin levels were determined by ELISA (Alpco Diagnostics) before (0), 30 and 120 mn after the oral gavage.
- Caecal content was harvested immediately after sacrifice of the animal and frozen into liquid nitrogen. Composition of caecal microbiota was assessed by 16S rDNA taxonomical metasequencing approach (Ondov *et al.*, 2011, Schloss *et al.* 2009).

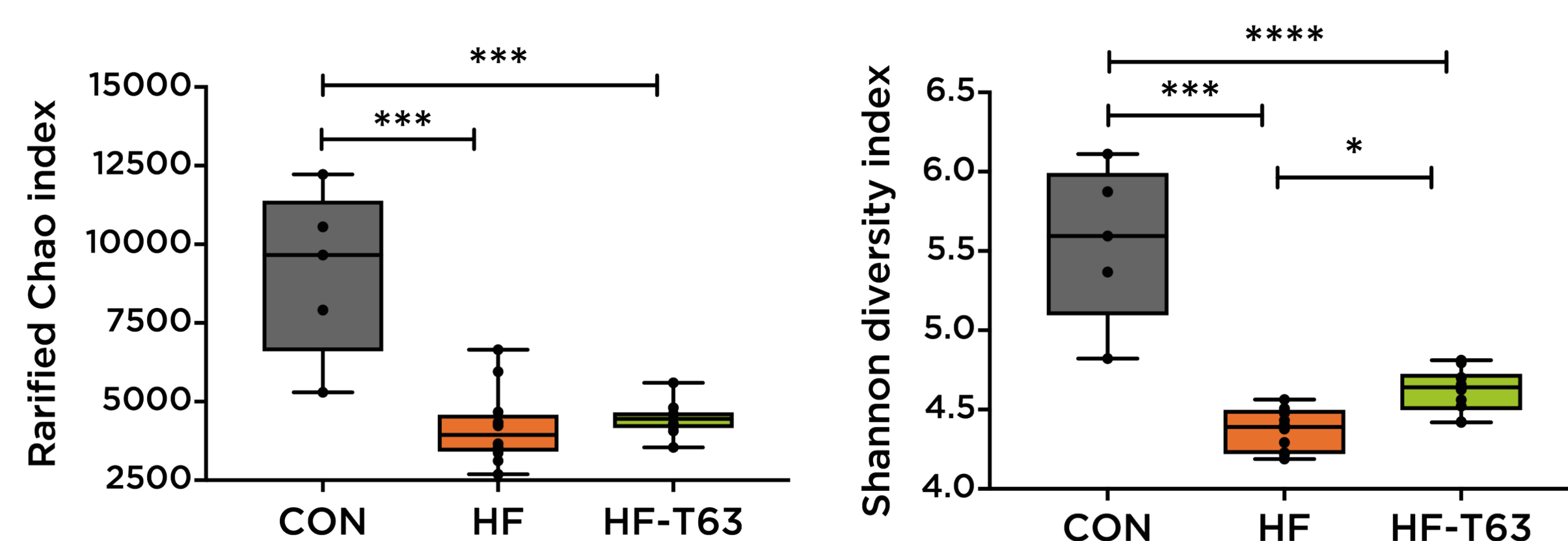
- Rarefied Chao1 richness index was calculated following the formula defined in Chao A. (1984).
- Shannon diversity index was calculated following the formula defined in Shannon, C. E. (1948).
- Taxa present in average in all samples at a threshold $\geq 0.5\%$ or present in at least 10% of samples at a threshold $\geq 0.5\%$ are individually represented. In other cases, taxa are grouped and labelled «other» in barplots.
- Each taxon (expressed in relative abundance at the phylum, family and genus levels) and rarefied Chao/ Shannon indices were analyzed using a one-way (treatment) analysis of variance statistical model (ANOVA) with the MIXED SAS® procedure. For the analysis of the relative abundances of taxa, a Benjamini & Hochberg procedure was applied to control the False Discovery Rate (FDR) due to multiple hypothesis tests on all taxa. For the analysis of Shannon and rarefied Chao1 indexes, a Dunnett correction was applied for the pairwise comparisons.

RESULTS

BODY COMPOSITION AND GLYCAEMIC CONTROL

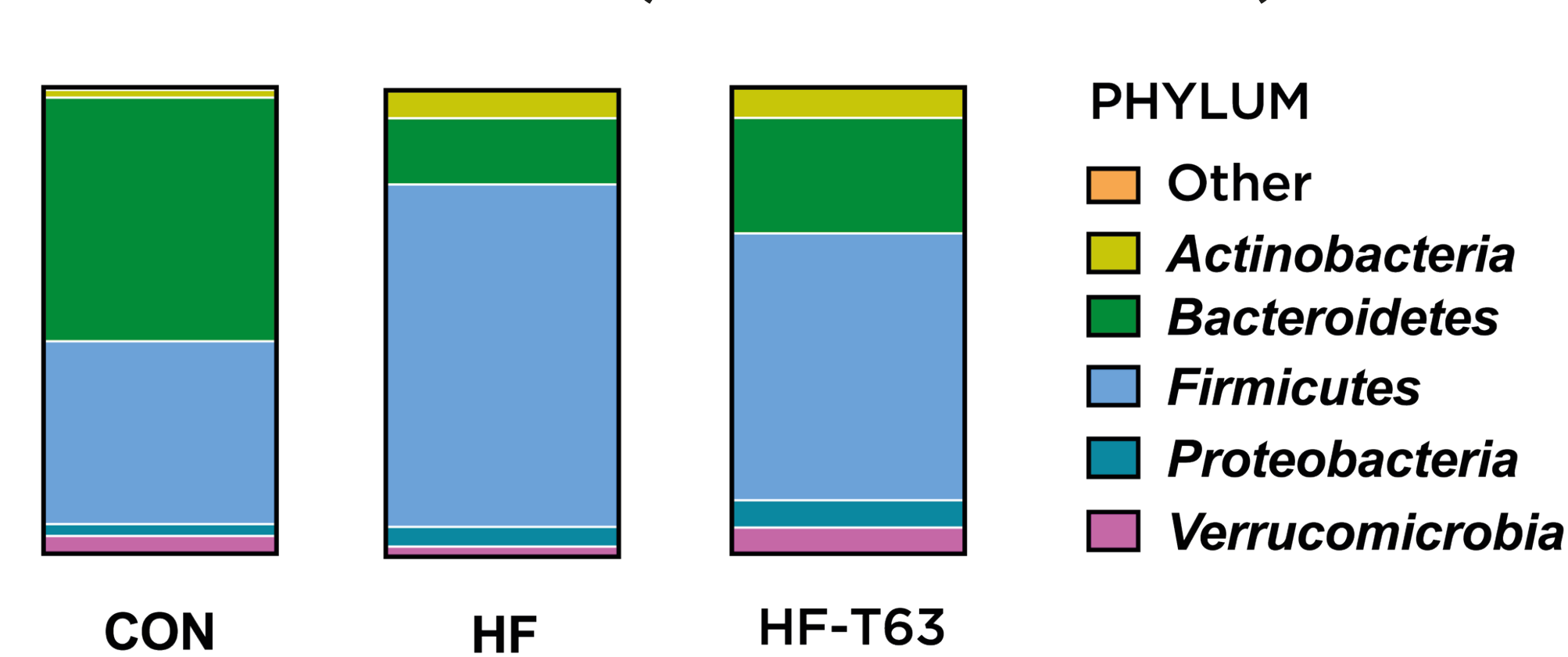


RICHNESS AND DIVERSITY INDEXES



- Richness (rarefied Chao1) and diversity (Shannon) indexes were reduced by HF diet ($p < 0.0001$).
- Diversity (Shannon) index was higher in HF-T63 group, vs HF ($p < 0.05$).

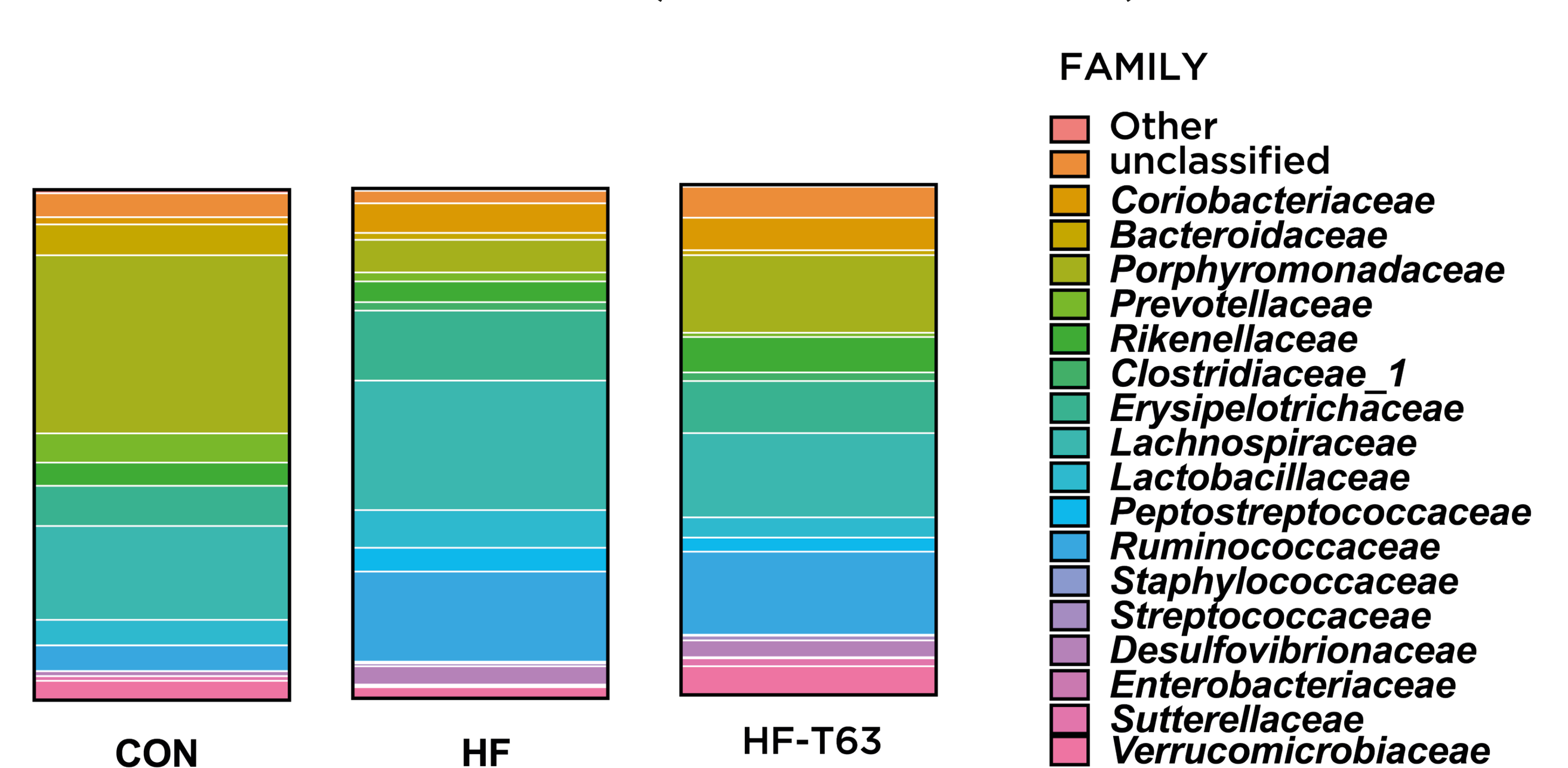
TAXONOMIC COMPOSITION OF THE INTESTINAL FLORA: PHYLUM LEVEL (RELATIVE ABUNDANCE)



| Phylum | Relative abundance (mean and SEM in %) and adjusted p-values | | |
|----------------|--|--|---|
| | HF vs CON | HF-T63 vs HF | HF-T63 vs CON |
| Bacteroidetes | 14.19% (2.69%) < 52.23% (4.16%) | 24.76% (2.69%) > 14.19% (2.45%) adj. p= 0.0272 | 24.76% (2.69%) < 52.23% (3.80%) adj. p<0.0001 |
| Firmicutes | 73.41% (2.95%) > 39.20% (4.57%) | 57.22% (2.70%) < 73.41% (2.46%) adj. p=0.0012 | 57.22% (2.70%) > 39.20% (3.81%) adj. p=0.0021 |
| Proteobacteria | NS | NS | 5.86% (0.55%) > 2.55% (0.77%) adj. p=0.0033 |

- Statistically significant differences and adjusted p-values at phylum level are presented in the table above.
- There were no statistically significant differences for *Actinobacteria*, *Candidatus Saccharibacteria*, *Tenericutes* and *Verrucomicrobia* (adjusted $p > 0.05$).
- We noted an inversion of ratio *Bacteroidetes/Firmicutes* for HF at the Phylum level, compared to CON.
- HF-T63 seems to display an intermediate profile (in-between HF and CON).

TAXONOMIC COMPOSITION OF THE INTESTINAL FLORA: FAMILY LEVEL (RELATIVE ABUNDANCE)



| Phylum | Family | Relative abundance (mean and SEM in %) and adjusted p-values | | |
|----------------|---------------------------|--|---|--|
| | | HF vs CON | HF-T63 vs HF | HF-T63 vs CON |
| Bacteroidetes | <i>Bacteroidaceae</i> | 1.36% (0.46%) < 6.06% (0.71%) | NS | 0.97% (0.42%) < 6.06% (0.59%) adj. p<0.0001 |
| | <i>Porphyromonadaceae</i> | 6.40% (1.57%) < 34.89% (2.43%) | 15.21% (1.62%) > 6.40% (1.48%) adj. p=0.0056 | 15.21% (1.62%) > 6.40% (1.48%) < 34.89% (2.29%) adj. p<0.0001 |
| | <i>Prevotellaceae</i> | 1.76% (0.81%) < 5.72% (1.26%) | NS | 0.83% (0.71%) < 5.72% (1.01%) adj. p=0.0031 |
| Firmicutes | <i>Lachnospiraceae</i> | NS | 16.50% (1.99%) < 25.40% (1.82%) adj. p=0.0167 | NS |
| | <i>Ruminococcaceae</i> | 17.67% (1.40%) > 5.02% (2.17%) adj. p=0.0011 | NS | 16.28% (1.57%) > 5.02% (2.22%) adj. p=0.0027 |
| | <i>Streptococcaceae</i> | 0.57% (0.06%) > 0.04% (0.09%) adj. p=0.0010 | NS | 0.92% (0.16%) > 0.04% (0.23%) adj. p=0.0021 |
| Proteobacteria | <i>Sutterellaceae</i> | 0.33% (0.09%) < 0.91% (0.14%) adj. p=0.0117 | 1.55% (0.17%) > 0.33% (0.16%) adj. p=0.0004 | NS |

- Statistically significant differences and adjusted p-values at family level are presented in the table above.
- The inversion of *Bacteroidetes/Firmicutes* ratio, at the phylum level (for HF vs CON), was mainly explained by a lower level of *Porphyromonadaceae* (a *Bacteroidetes* Family) to the benefit of *Ruminococcaceae* (a *Firmicutes* Family).
- Differences were observed at the family level on *Porphyromonadaceae* (a *Bacteroidetes* family) and on *Lachnospiraceae* (at *Firmicutes* family) between HF and HF-T63.
- At the genus level, T63 increased *Barnesiella* (*Porphyromonadaceae*) and *Parasutterella* (*Sutterellaceae*), while *Dorea* (*Lachnospiraceae*) was decreased.

CONCLUSION

- T63 reduced total adiposity and improved insulin sensitivity in HF-fed mice.
- Analysis of caecal microbiota revealed that T63 may have improved the diversity of the intestinal flora, and partially limited some of the HF-induced alteration of gut microbiota.
- Interestingly, key families previously linked with obesity, metabolic impairments and T2D, were affected by T63.
- These results suggest that gut microbiota participates to the mechanisms involved in the beneficial effects of T63 in this mouse model of prediabetes.

REFERENCES

IDF, IDF Diabetes Atlas, 7th ed., 2015; Tuso *et al.*, The Permanente Journal 2014 ; Ondov, B. D. *et al.* BMC Bioinformatics, 2011; Schloss *et al.*, Appl Environ Microbiol. 2009 ; Cani *et al.*, Diabetes, 2007; Chao A, Scan J of stat, 1984; Shannon A, The Bell Syst Tech J, 1948.