The Gut Microbiota During a Behavioral Weight Loss Intervention: The DRIFT2 Randomized Clinical Trial

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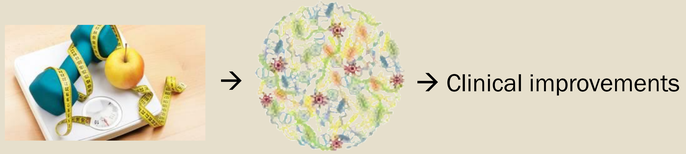
Summary

Altered gut microbiota has been linked to obesity and may influence weight loss. **DRIFT2** an ongoing, 1-year weight loss trial comparing daily caloric restriction (**DCR**) to intermittent fasting (**IMF**) in adults with overweight or obesity. This study is ancillary to DRIFT2 and examines the gut microbiota and selected obesity-related parameters at baseline and after the first three months of the interventions. During this time, participants experienced significant improvements in clinical health measures, along with altered composition and diversity of fecal microbiota. We observed significant associations between gut microbiota features and clinical measures, including weight and waist circumference, as well as change in these clinical measures over time. Analysis by intervention group found between-group differences in the response of *Akkermansia* to the interventions. Our results provide insight into the impact of baseline gut microbiota on weight loss responsiveness, as well as the early effects of DCR and IMF on gut microbiota.

Conceptual Model



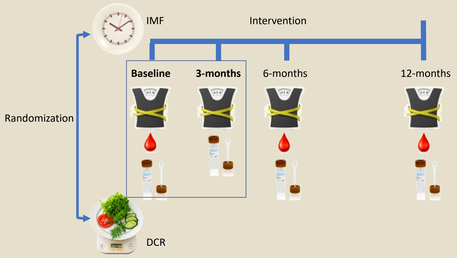
The gut microbiota may mediate the cardiometabolic benefits of a lifestyle weight loss intervention

- The gut microbiota may play a causal role in obesity - and a key role in weight loss - through effects on energy balance, nutrient absorption, inflammation, appetite regulation and host signaling[4]
- The gut microbiota may also play a key mediating role in the clinical improvements associated with changes in diet and exercise.[6]

Methods

Study Design

This study uses data collected at baseline and 3-months from a one-year comprehensive, group-based behavioral weight loss intervention of DCR versus IMF ("DRIFT2"; R01 DK111622), which began in May 2018.



Study Population

- Healthy sedentary adults with overweight / obesity, BMI 27-45kg/m^2
- Mean BMI: 33.1 (SD: 4.4), age: 40.7 (SD: 9.8) y; 76.3 % female

Study sample: N=111 fecal samples / N=59 people (25 DCR; 34 IMF)

Data collection: Demographics; Fecal and blood samples; Height & weight; Waist circumference

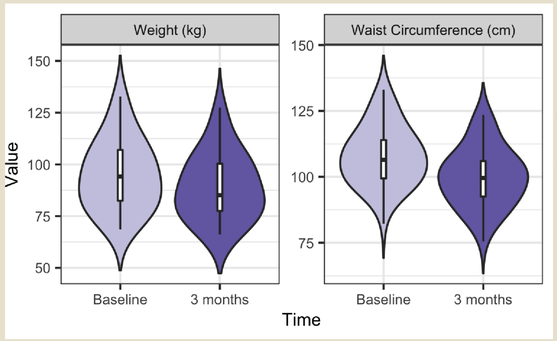
Processing of samples: V3V4 region of 16S rRNA gene sequencing; Denoising and sequences assigned using Dada2 and Silva 12.8; Rarefied OTU table at 3407 sequences / sample

Statistical Methods

- Examined changes in gut microbiota over time and by intervention group using:
  - Mixed effects linear regressions for changes in alpha diversity
  - Longitudinal permutational ANOVA (permanovaFL)[7] of weighted and unweighted UniFrac distance metrics and their change (PLDist) for changes in composition[8]
  - ANCOM for taxonomic changes[9]
- Examined clinical outcomes: change in weight and waist circumference: MIRKAT[10] with weighted and unweighted UniFrac distance metrics and their change (PLDist)[8]

Results

Participants lost weight and waist circumference at 3 months

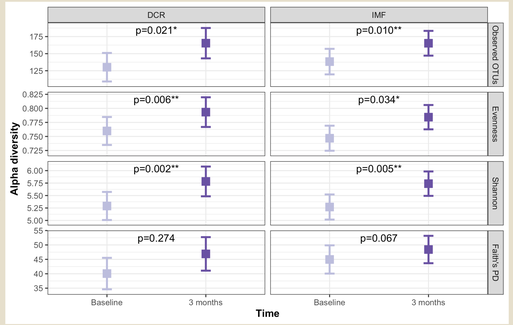


60% of participants had lost a clinically significant amount of weight (≥5%) at 3 months.

Figure 1. From baseline to 3 months of the intervention, participants lost significant weight and waist circumference as shown in these violin plots.

Alpha diversity increased significantly at 3 months

Figure 2. Alpha diversity metrics (Observed OTUs, Evenness, Shannon diversity index, and Faith's phylogenetic diversity (PD)) at baseline and 3-months for DCR and IMF. All measures except Faith's PD increased significantly in both groups, with no significant differences between groups (p>0.847).



Gut microbiota taxonomy shifted significantly at 3 months

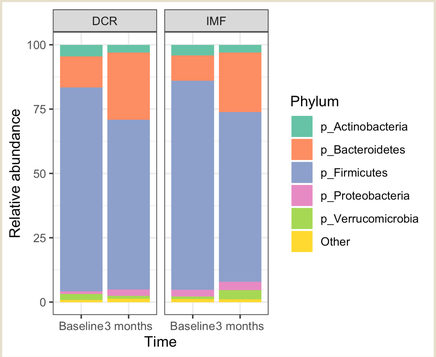


Figure 3. Phylum-level gut microbiota taxa relative abundance at baseline and 3-months for DCR and IMF.

- Microbiota composition (beta diversity) changed significantly (R^2=7.1%; p=0.001) over the first 3 months of the intervention, with no significant differences between groups (p>0.325).
- Numerous genera changed significantly over this time period.
- Only the genus *Akkermansia* showed significant between-group differences in change over time with an increase in IMF and no change in DCR. (FDR<0.05).

Results

Change in gut microbiota taxonomy predicts change in waist circumference

Figure 4. P-values for tests of association between the baseline gut microbiota composition (top) and change from baseline to 3-months (bottom) and change in weight (left) and change in waist circumference (right). Significance ($p \leq 0.05$) shown with red dotted line.

- Plots show p-values for 1) Weighted UniFrac, which takes into account types of microbes and their relative abundance, and 2) Unweighted UniFrac, which takes into account presence / absence of microbes.
- Baseline / Change in gut microbiota composition not significantly associated with change in weight.
- Quantitative differences (weighted UniFrac) in baseline and change in gut microbiota composition associated with change in waist circumference.

Discussion

Gut microbiota composition and alpha diversity and show substantial change over the course of the intervention

- No strong evidence of between group differences in metrics of overall change (α/β diversity), but one specific taxon, *Akkermansia*, showed between-group differences.

The gut microbiota at baseline and its change from baseline to 3 months are significantly predictive of change in waist circumference at 3-months

- Numerous taxa (baseline and change in abundance; not shown here) predictive of change in weight and waist circumference.
- *Potential for clinical applications?*
  - Can we predict who will be most responsive before trial begins?
  - Can we target gut microbiota of people unlikely to be responsive in order to improve responsiveness?
    - Prebiotics, probiotics, targeted dietary therapy

Current / future work

- Additional outcome measures: BMI, blood pressure; Body composition (DXA), lipids, inflammation
- Influence of genetics, epigenetics, metabolomics

References

1. de Cabo, R. & Mattson, M. P. Effects of Intermittent Fasting on Health, Aging, and Disease. *New England Journal of Medicine* 381, 2541–2551 (2019).
2. Francesco, A. D., et. al. de. A time to fast. *Science* 362, 770–775 (2018).
3. Catenacci, V. A. et al. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction. *Obesity* 24, 1874–1883 (2016).
4. Meijnikman, A. S., et. al.. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev* 39, 133–153 (2018).
5. Fabbiano, S., Suárez-Zamorano, N. & Trajkovski, M. Host–Microbiota Mutualism in Metabolic Diseases. *Front. Endocrinol.* 8, (2017).
6. Li, G. et al. Intermittent Fasting Promotes White Adipose Browning and Decreases Obesity by Shaping the Gut Microbiota. *Cell Metabolism* 26, 672-685.e4 (2017).
7. Hu, Y.-J. & Satten, G. A. Testing hypotheses about the microbiome using the linear decomposition model (LDM). *bioRxiv* 229831 (2020) doi:10.1101/229831.
8. Plantinga, A. M., et. al.. pldist: ecological dissimilarities for paired and longitudinal microbiome association analysis. *Bioinformatics* 35, 3567–3575 (2019).
9. Mandal, S. et al. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease* 26, (2015).
10. Zhao, N. et al. Testing in Microbiome-Profiling Studies with MiRKAT. *The American Journal of Human Genetics* 96, 797–807 (2015).
11. Fang, S. et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nature Medicine* 21, 159–165 (2015).
12. González Hernández, et. al.. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients* 11, (2019).

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