THC Supplementation Results in Weight Loss and Sex-Dependent Gut Microbiota Changes

Avi Kaye[1] DO'26, Matthew Rusling[1] DO, Ken Mackie[2] MD, LiLian Yuan[1] PhD

[1]College of Osteopathic Medicine, Des Moines University, Des Moines, IA; [2]Gill Center & Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN

DES MOINES UNIVERSITY
MEDICINE & HEALTH SCIENCES

Introduction

- There is increasing evidence of the reciprocal relationship between the gut microbiome and host physiological conditions.[1]
- Previous research demonstrated consistent and reproducible alterations in gut microbiota with obesity and high-fat diets,[2,3] prompting investigations into the role of the microbiome in countering the global obesity epidemic.
- Observations from epidemiological reviews[4] illustrate that cannabis users have lower body mass indexes than non-users despite its reputation for increasing appetite, provoking analysis of delta(9)-tetrahydrocannabinol's (**THC**) potential weight regulation effects via the endocannabinoid system.[5,6]
- **Purpose:** We aimed to examine the possible relationship between gut microbiome changes and THC-induced weight loss.

Methods

- Obese mouse models were treated with oral THC supplementation without changing their diet and compared with controls (**VEH**).
- In addition to measuring weight, fecal samples were obtained at various timepoints, sequenced for bacteria 16s rRNA content and analyzed using QIIME2.[7]
- Alpha diversity was determined using pairwise Shannon t-tests and beta diversity weighted Unifrac utilizing PERMANOVA in QIIME2.
- Microbiome changes with THC and weight change were assessed by employing linear mixed effects (**LME**) in R.[8]
- A LME statistical model was generated in R to predict weight change from baseline based on bacterial relative abundance. The model was tested by estimating weight change from baseline based solely on bacterial relative abundance in a different mouse cohort that underwent the same experimental procedure and comparing to actual weight change using linear regression.

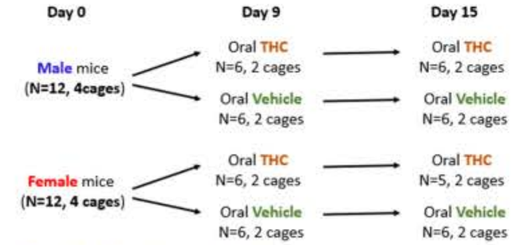

Figure 1. Experimental design

Acknowledgments

A big thank you to my faculty mentor, Dr. Yuan, and fellow lab member Dr. Rusling for their vision, guidance, support in this project. The Yuan lab also greatly appreciates our partnership with the Gill Center & Department of Psychological and Brain Sciences at Indiana University who performed the experiments for this study.

Results

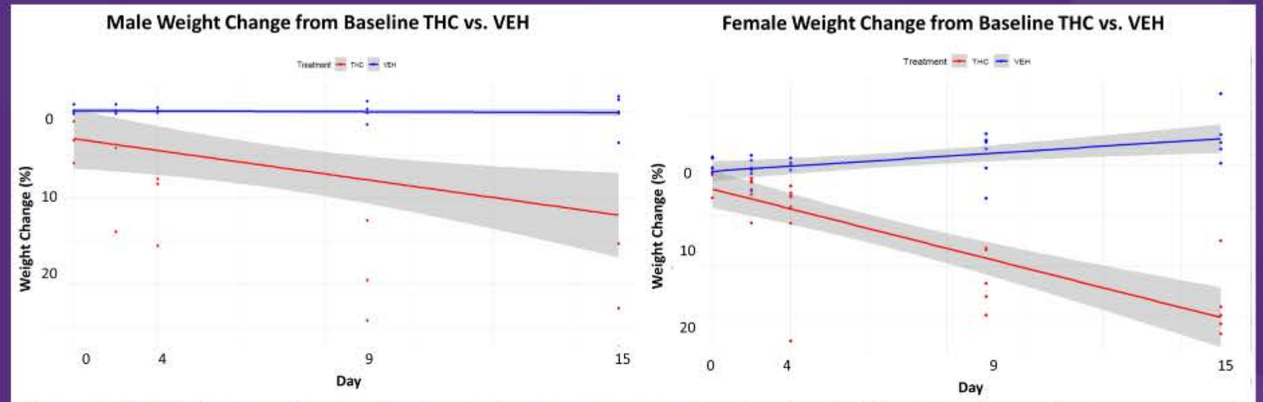

Figure 2. THC induces weight loss in obese mice in both sexes. In male mice, the THC-treated group lost an average of 17.8% of their body weight over the 15-day experimental duration compared with an average loss of 0.23% in controls (p=0.00002). In female mice, the THC-treated group lost an average of 13.8% of their body weight compared with an average gain of 2.9% of body weight in controls (p=0.00006). [*95% CI illustrated by grey shading*]

Alpha and beta diversity: Male and female mice have different baseline alpha diversity (Shannon, p=9.7e-5) and beta diversity (Weighted Unifrac p=0.001) at baseline. In male mice, THC had significantly greater alpha diversity than VEH on day 9 and 15 and different beta diversity on day 9 (p=0.011).

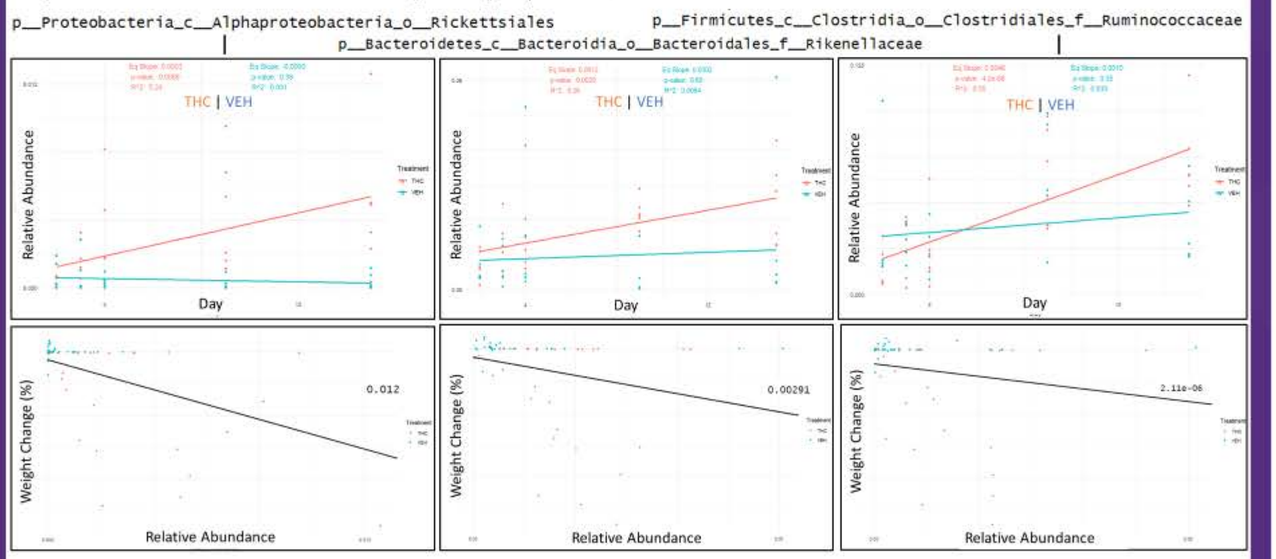

Figure 3. LME models of bacterial taxonomic features in male mice. For each graph pair representing a bacterial taxonomic feature, the top illustrates change in relative abundance from day=1 to day=15 separated by treatment and the bottom displays weight change from baseline plotted against bacterial relative abundance. 8 bacterial taxonomic features had both significantly greater change in relative abundance with THC versus VEH and were correlated with weight change. Three of the bacterial features presented above were selected for an LME model to predict weight change.

```
Formula: weight_change ~ p__Firmicutes_c__Clostridia_o__Clostridiales_f__Ruminococcaceae +
    p__Bacteroidetes_c__Bacteroidia_o__Bacteroidales_f__Rikenellaceae +
    p__Proteobacteria_c__Alphaproteobacteria_o__Rickettsiales +      (1 | ratid)
   Data: data_no_baseline

REML criterion at convergence: 285.4

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.8802 -0.2915  0.0456  0.3381  2.0065

Random effects:
 Groups   Name        Variance Std.Dev.
 ratid    (Intercept) 23.30    4.827
 Residual             11.04    3.325
Number of obs: 56, groups:  ratid, 12

Fixed effects:
                                                                  Estimate Std. Error t value
(Intercept)                                                        -0.1054     1.8566  -0.064
p__Firmicutes_c__Clostridia_o__Clostridiales_f__Ruminococcaceae    -9.6522    20.0972  -0.479
p__Bacteroidetes_c__Bacteroidia_o__Bacteroidales_f__Rikenellaceae -77.2393    48.8229  -1.582
p__Proteobacteria_c__Alphaproteobacteria_o__Rickettsiales        -1007.5951   256.1473  -3.934

Correlation of Fixed Effects:
            (Intr) p__F__ p__B__
p__F___C___ -0.259
p__B___B___ -0.266 -0.205
p__P___A___  0.023 -0.498 -0.119

          R2m       R2c
0.2365065 0.7545481
```

Figure 4. R statistics of 3-bacterial feature LME model predicting weight change in male mice. By assessing the fixed effects of just three bacterial features' relative abundance, we can explain a larger percentage of variance than final treatment alone (R2m of 23.6% versus 20.5% respectively). Including the random effects of individual mice, the model can explain 75.5% of the variance in weight change. *R2M=Marginal R Squared. R2C=Conditional R-Squared*

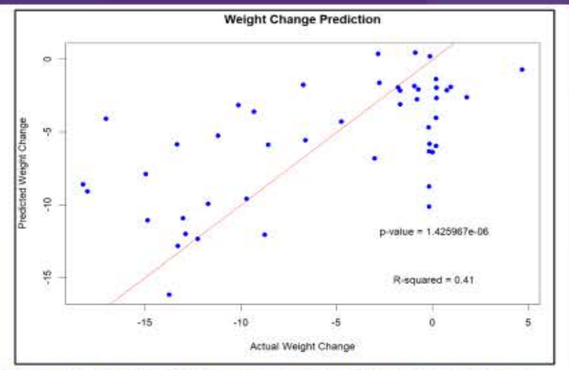

Figure 5. Testing 3-feature model on a new cohort. The 3-feature model explained 39% of the variance in weight change in the new cohort. Predicted vs. actual weight change was statistically significant (p=1.43e-6)

Discussion

- THC induces stark weight loss in obese mice.
- In male mice, 1/3 of variation in weight change induced by THC could be explained by the relative abundance of just 3 bacterial taxonomic features impacted by THC.
- We corroborated efficacy of the model by accurately predicting weight change in a new male mice cohort.
- Two of the features identified in the model have known associations with weight loss:
  - Ruminococcacae is associated with improved insulin sensitivity and enhanced mitochondrial function due to butyrate production. It is also decreased in high-fat diets and obesity.
  - Rikenellaceae is associated with reduced visceral adipose tissue and increased lipid metabolism.
- Rickettsiales' connection to weight loss is less clear, but it was consistently higher in THC samples and strongly correlated with weight loss.
- Female mice proved harder to model with bacterial relative abundance.
  - The 3-feature model did not account for weight change variation, but remarkably predicted weight change accurately (R=0.66, R^2=0.44, p=7e-9).
- **Conclusions:** Results indicate sex-specific gut microbiome changes play a role in THC-induced weight loss. Furthermore, we proved the concept of predicting weight change with bacterial abundance models.

References

1. Gilbert, J., Blaser, M., Caporaso, J., Janson, J., Lynch, S., Knight, R. Current understanding of the human microbiome. 2018. *Nature Medicine*; 24(4): 392-400. doi: 10.1038/nm.4517
2. Davis, C. The gut microbiome and its role in obesity. 2016. *Nutrition Today*; 51(4): 167-174. doi: 10.1097/NT.0000000000000167
3. Bisanz, J., Upadhyay, V., Turbaugh, J., Ly, K., Turnbaugh, P. Meta-analysis reveals reproducible gut microbiome alterations in response to a high-fat diet. *Cell Host & Microbe*; 26(2): 265-272. doi: 10.1016/j.chom.2019.06.013
4. Sansone, R., Sansone, L. Marijuana and body weight. 2014. *Innovations in Clinical Neuroscience*; 11(7-8):50-54. PMID: 25337447
5. Vemuri, V., Janero., D., Makriyannis, A. Pharmacotherapeutic targeting of the endocannabinoid signaling system: Drugs for obesity and the metabolic syndome. 2008. *Physiologic Behavior*; 93(0): 671-686. PMID: 18155257
6. Mir, H., Giorgini, G., Di Marzo, V. The emerging role of the endocannabinoidome-gut microbiome axis in eating disorders. 2023. *Psychoneuoendocrinology*; 154: 106295. doi: 10.1016/j.psyneuen.2023.106295
7. Boylen, E., Rideout, J., Dillon, M. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. 2019. *Nature Biotechnology*; 37: 852–857. doi: 10.1038/s41587-019-0209-9
8. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.