THE GUT MICROBIOTA AND WEIGHT LOSS

RESULTS FROM A WEIGHT LOSS INTERVENTION OF DAILY CALORIC RESTRICTION VERSUS INTERMITTENT FASTING

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Introduction

BA, Mathematics

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LEAD CENTER

Lifecourse Epidemiology of Adiposity and Diabetes

uchealth

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Outline

- Background on the human microbiome and its role in obesity
- Weight loss intervention
  - ***DRIFT2:*** *Daily caloric Restriction (**DCR**) versus Intermittent Fasting (**IMF**) trial*
- Changes in gut microbiota (**GM**) during the first three months of the intervention
- Gut microbiota and weight loss outcomes
- Current / future work

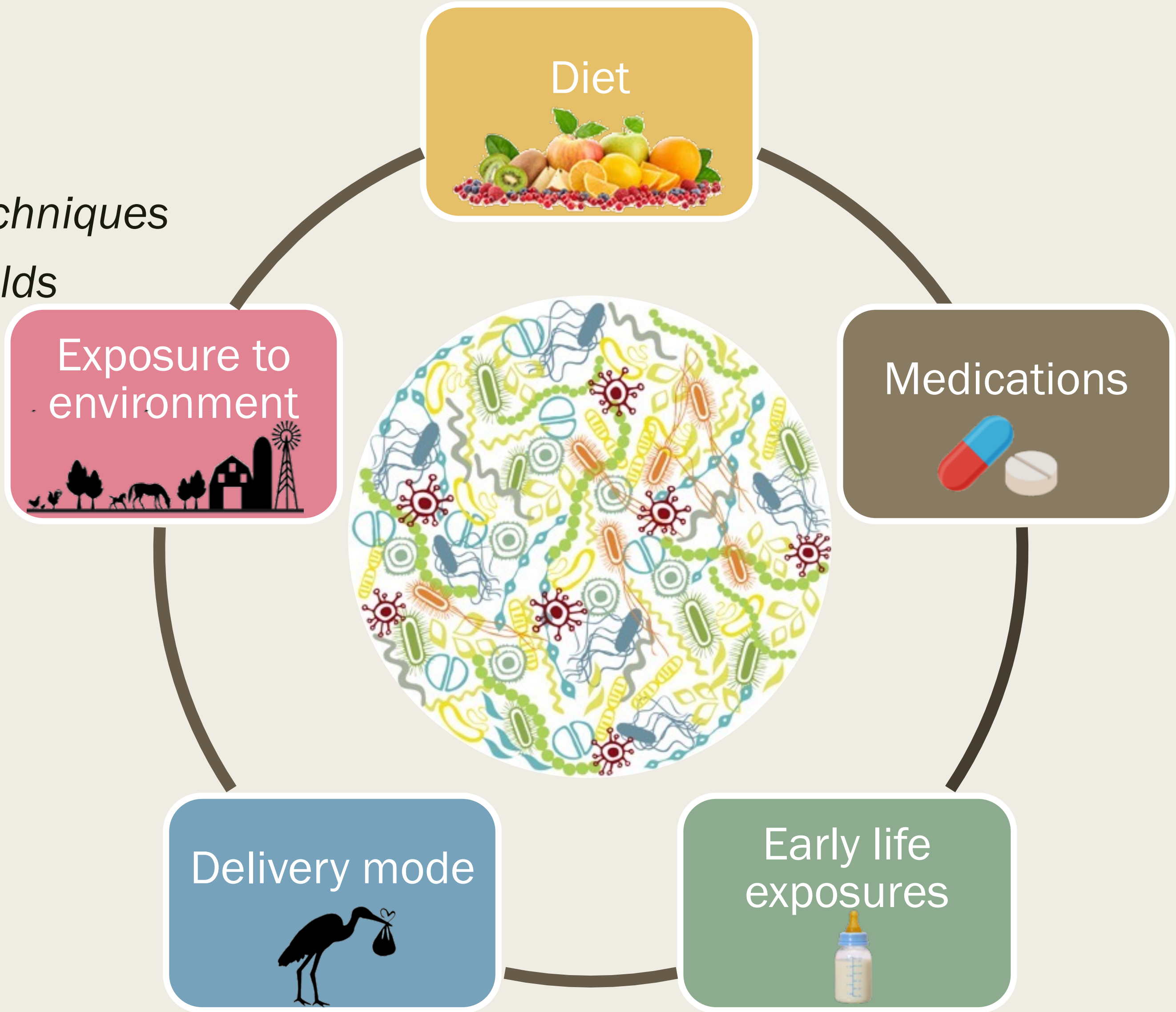
vs.

What is the human microbiome?

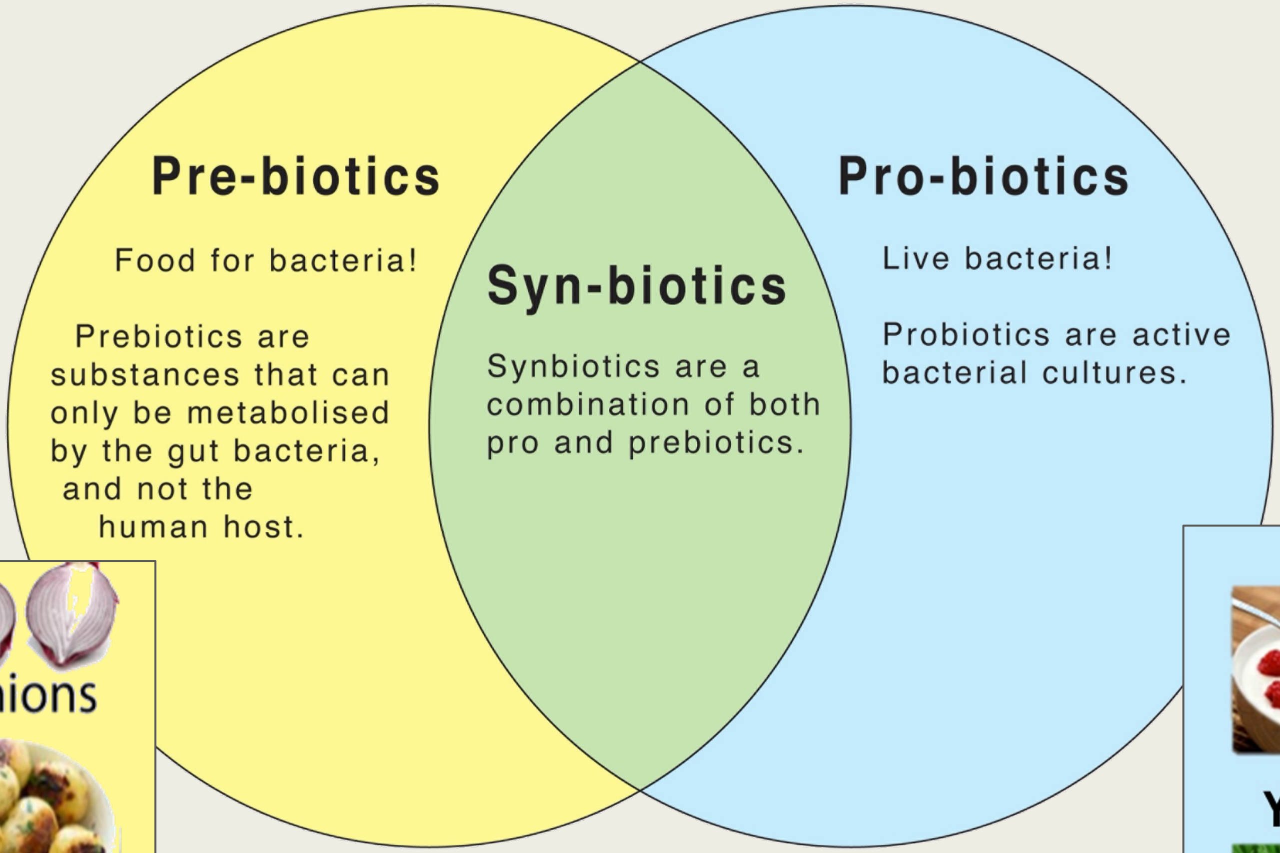
- Definitions
  - *Probiotics: Live beneficial or “healthy” bacteria/microorganisms*
  - *Microbiota: Community of commensal, symbiotic and pathogenic microorganisms that live in & on the body*
  - *Microbiome: the combination of these microbes, their genomes, and their interactions with the environment*
    - Bacteria, archaea, fungi and viruses
- Different regions of the body all have characteristic microbiota

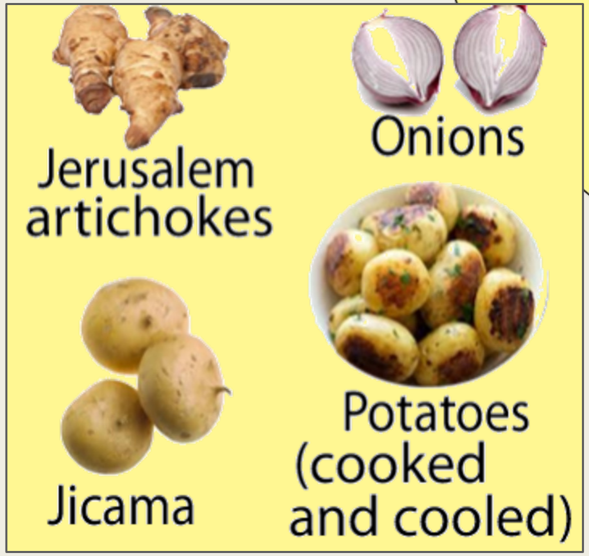
Human microbiome

- Hot topic in research
  - *Advances in DNA sequencing techniques*
  - *Research connects disparate fields*
  - *Human microbiome is changing*
  - ★ *Potential for disease prevention and/or treatment*



Disease Treatment / Prevention





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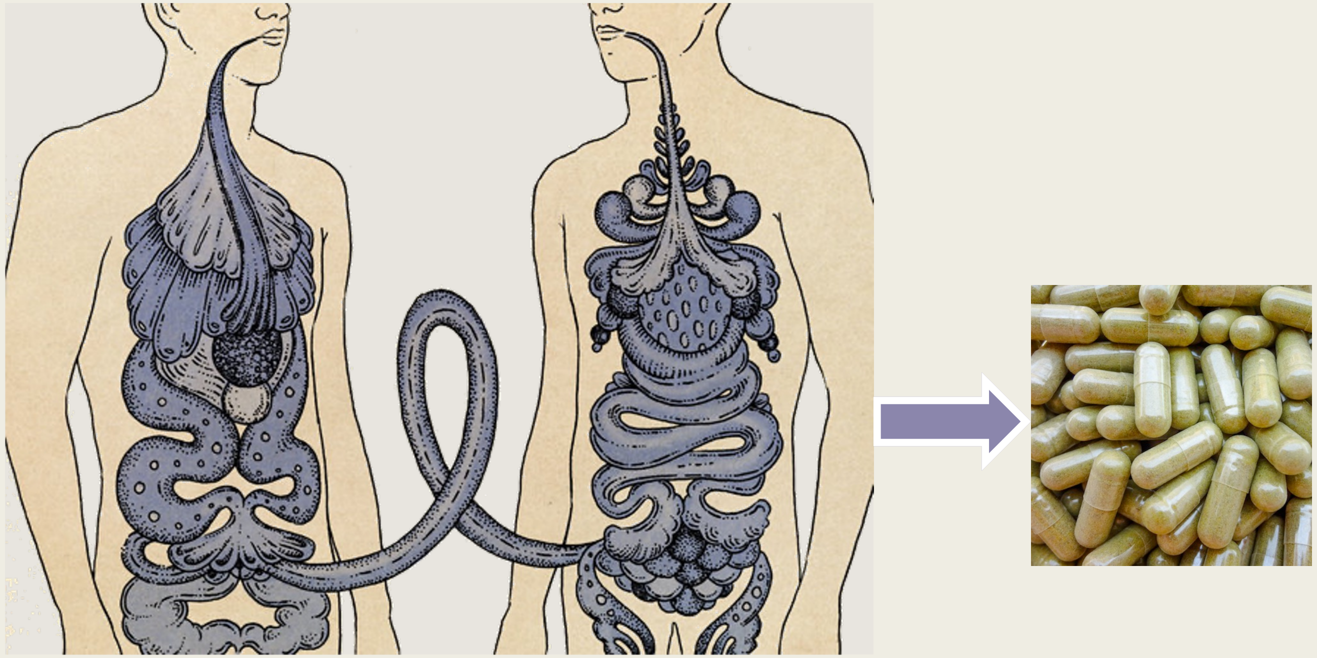
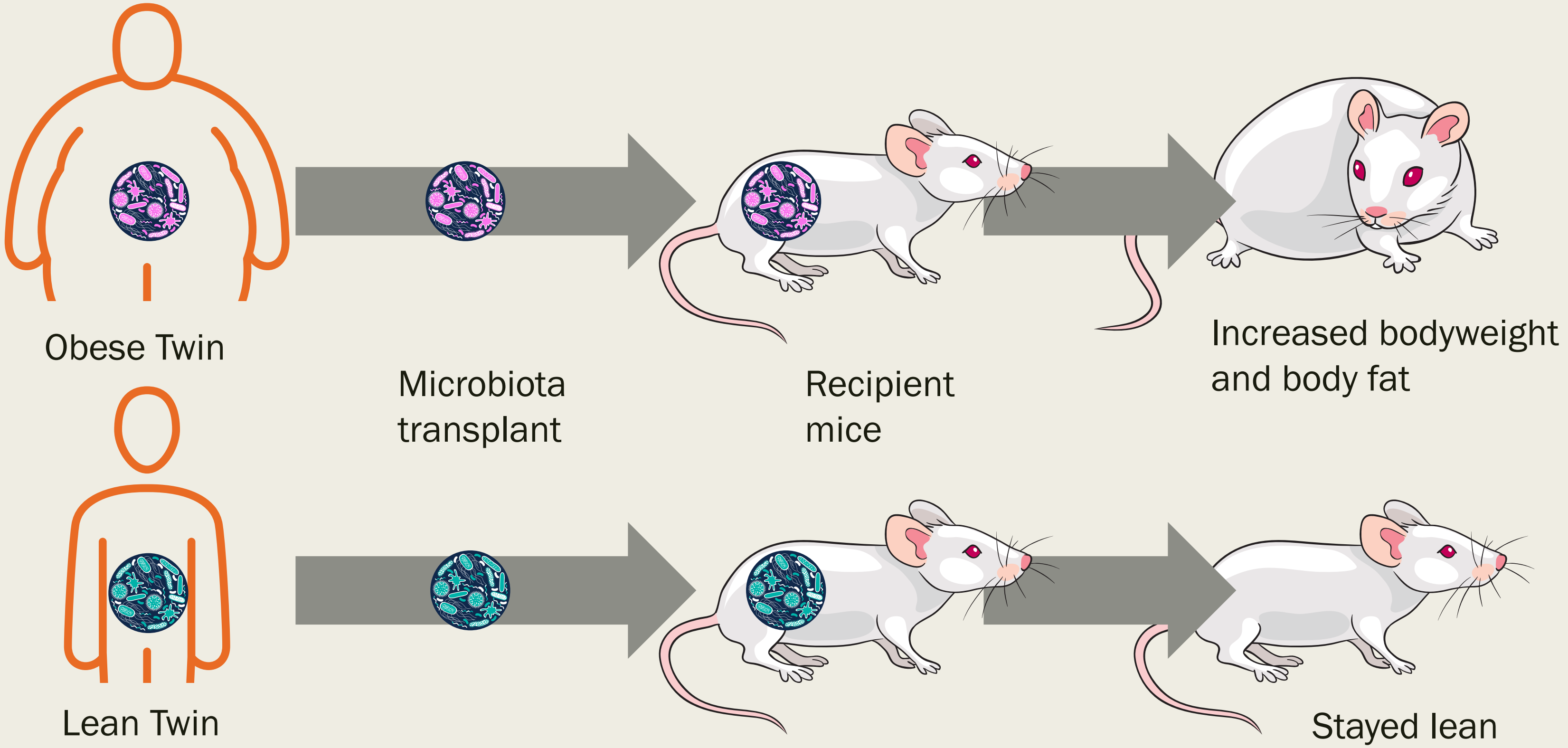
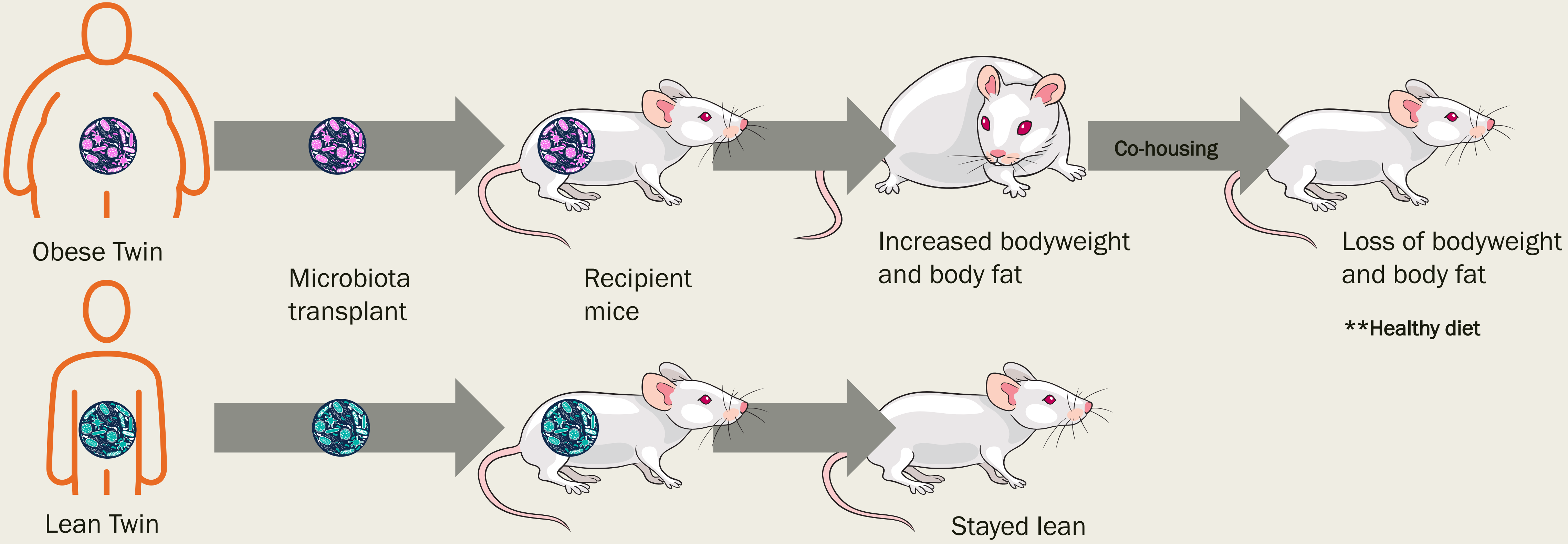


Gut microbiome & obesity



Source: Turnbaugh, 2009, *Nature* 457.7228: 480; Ridaura, 2013, *Science* 341.6150; Davie, 2014, Feel Better Already! Microbiome Health.

Gut microbiome & obesity



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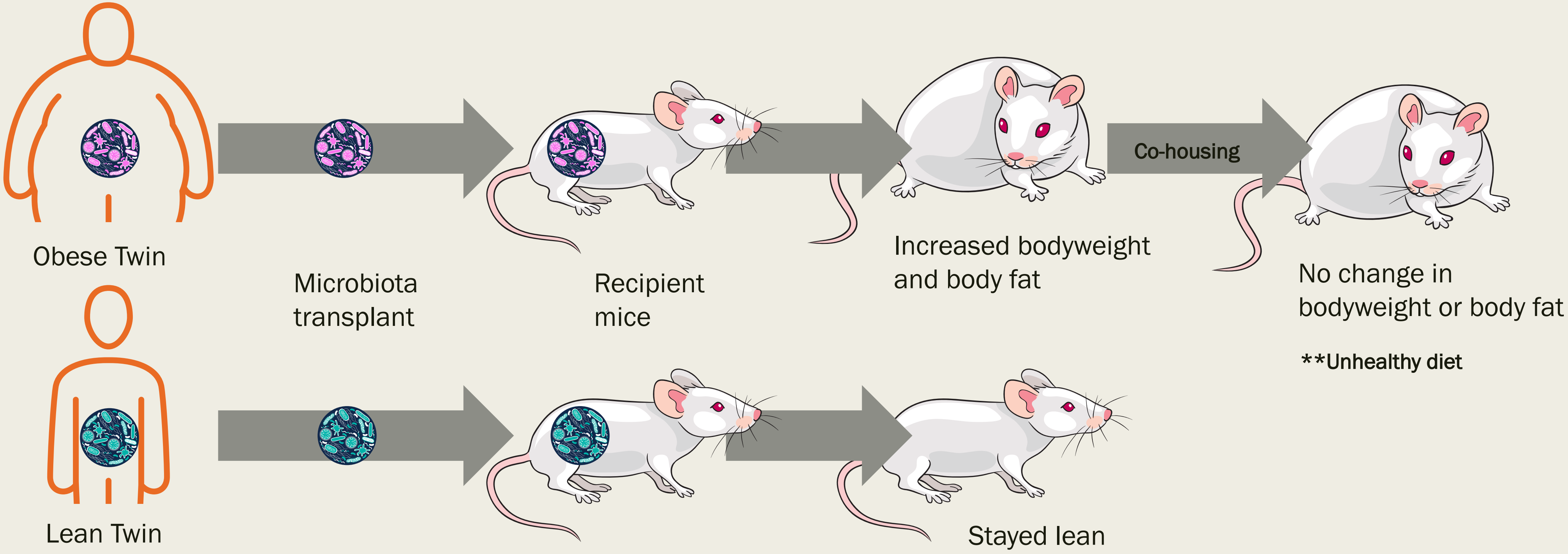
Gut microbiome & obesity



Diet interacts with gut microbiota in relation to obesity

→ Two individuals may have a similar diet, but one may be more/less prone to obesity due to differences in gut microbiota

Source: Turnbaugh, 2009, *Nature* 457.7228: 480; Ridaura, 2013, *Science* 341.6150; Davie, 2014, Feel Better Already! Microbiome Health.

Gut microbiome & obesity

Mechanisms

What mechanisms link microbes to obesity?

- Influence energy extraction / nutrient absorption (Jumpertz, 2011)
- Effects on inflammatory pathways through interactions with immune system or effects on gut permeability which drives systemic inflammation (Janssen , 2015; Gauffin, 2012)
- Metabolites that affect metabolic system – short chain fatty acids/bile acids (Janssen , 2015)

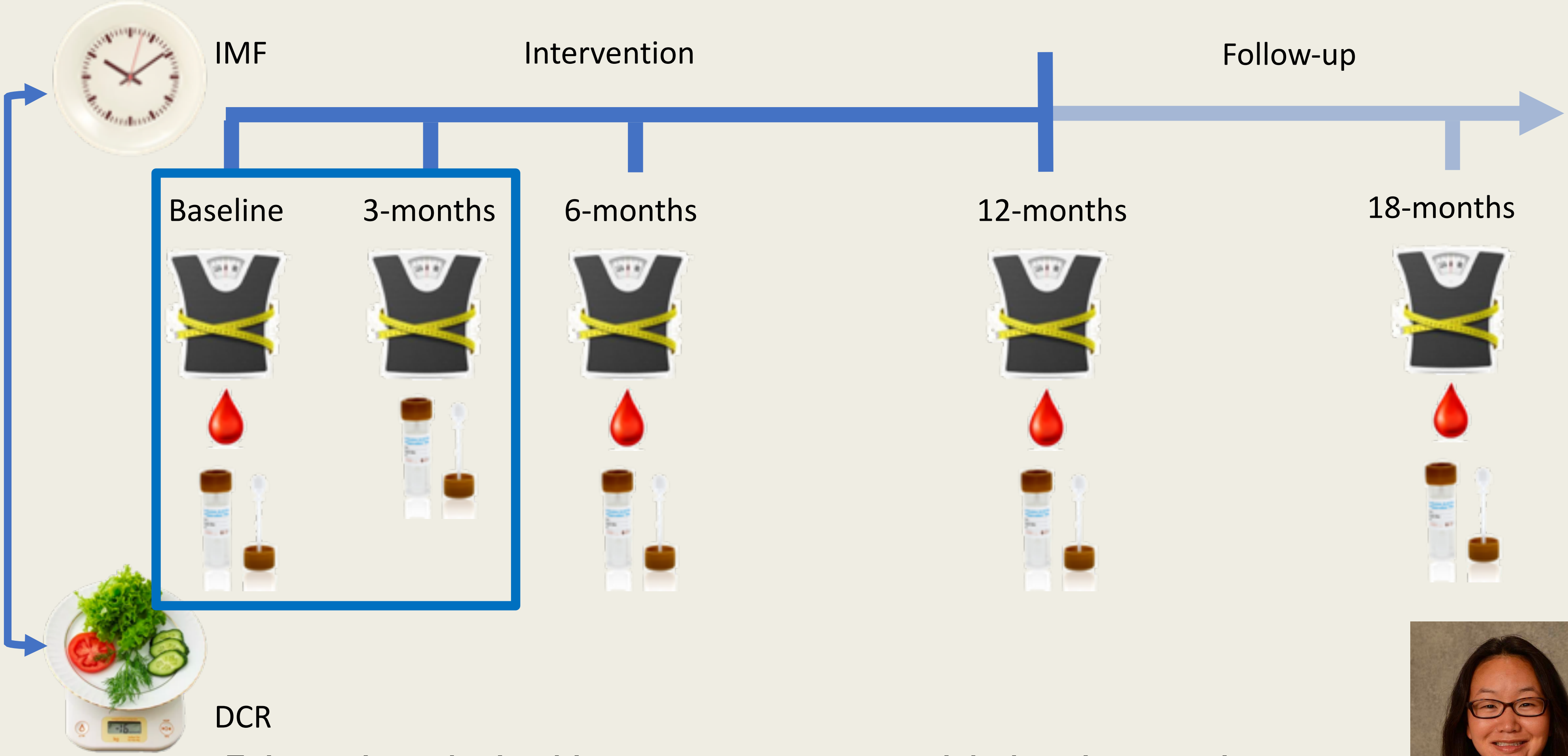
How do you lose weight?

Daily caloric Restriction vs Intermittent Fasting Trial

DRIFT2

Randomization

5 recruitment cohorts over 6 years



<u>Epigenetic and microbiome responses to a weight loss intervention</u>

AHA innovative project: First two cohorts @ baseline and 3 months; N~70

DCR and IMF

DCR

- Daily reduction of caloric intake by ~30%

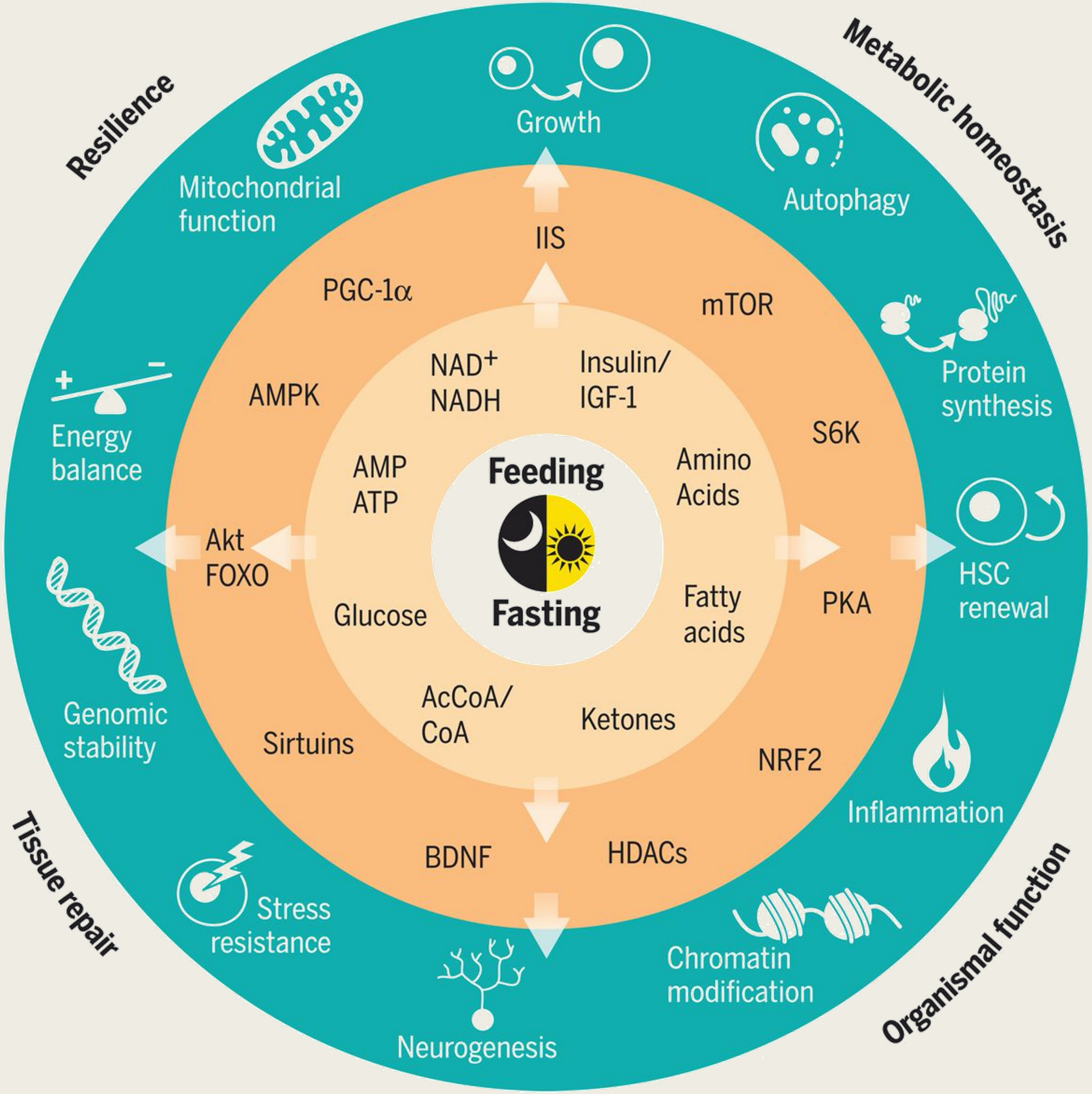
IMF

- Fasting on 3 non-consecutive days/week, reduction of caloric intake to ~25% of weight maintenance requirements
  → *weekly deficit of ~30%*
- Every other day fasting; Time restricted feeding, etc.

Drift2 Study Goals

DRIFT2 is a comprehensive group-based behavioral intervention

- *Powered to establish non-inferiority of IMF compared to DCR*
- *Clinician bias against skipping meals during weight loss*
  - Shift clinician perspective in order to offer a broader range of options for people who want to lose weight



Response to intermittent fasting involves:

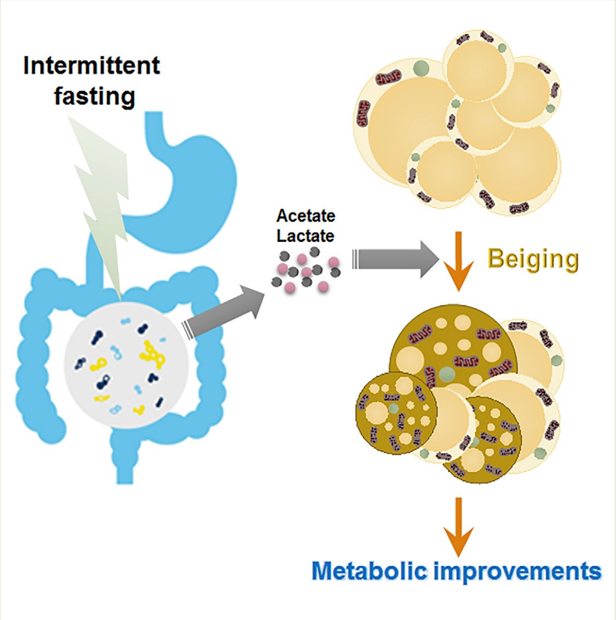
- Effects similar to those of regular aerobic exercise
- Impacts glucose and lipid metabolism, inflammation
- Enhances stress resistance
- Possible benefits in terms of satiety & appetite regulation

➢ ***Benefits of IMF are hard to separate from benefits of caloric restriction generally or weight loss***

De Cabo, N Engl J Med 2019;381:2541-51.
Di Francesco et al. Science 2018;362:770-775

IMF & Gut microbiota

- Effects of fasting that may impact the gut microbiota
  - *Changes in acetic acid (↑), secondary bile acids (↑), gut pH (↑)*
  - *Fasting animals produce less mucus on gut lining, return to feeding increases mucus lining*
  - *Reduction in size of intestines → housing crisis*
  - ➢ *Alter diversity*
  - ➢ *Growth of different types of microorganisms*
- Fasting may lead to metabolic improvements through changes in adipose tissue composition
  - *These changes may be mediated by the gut microbiota*

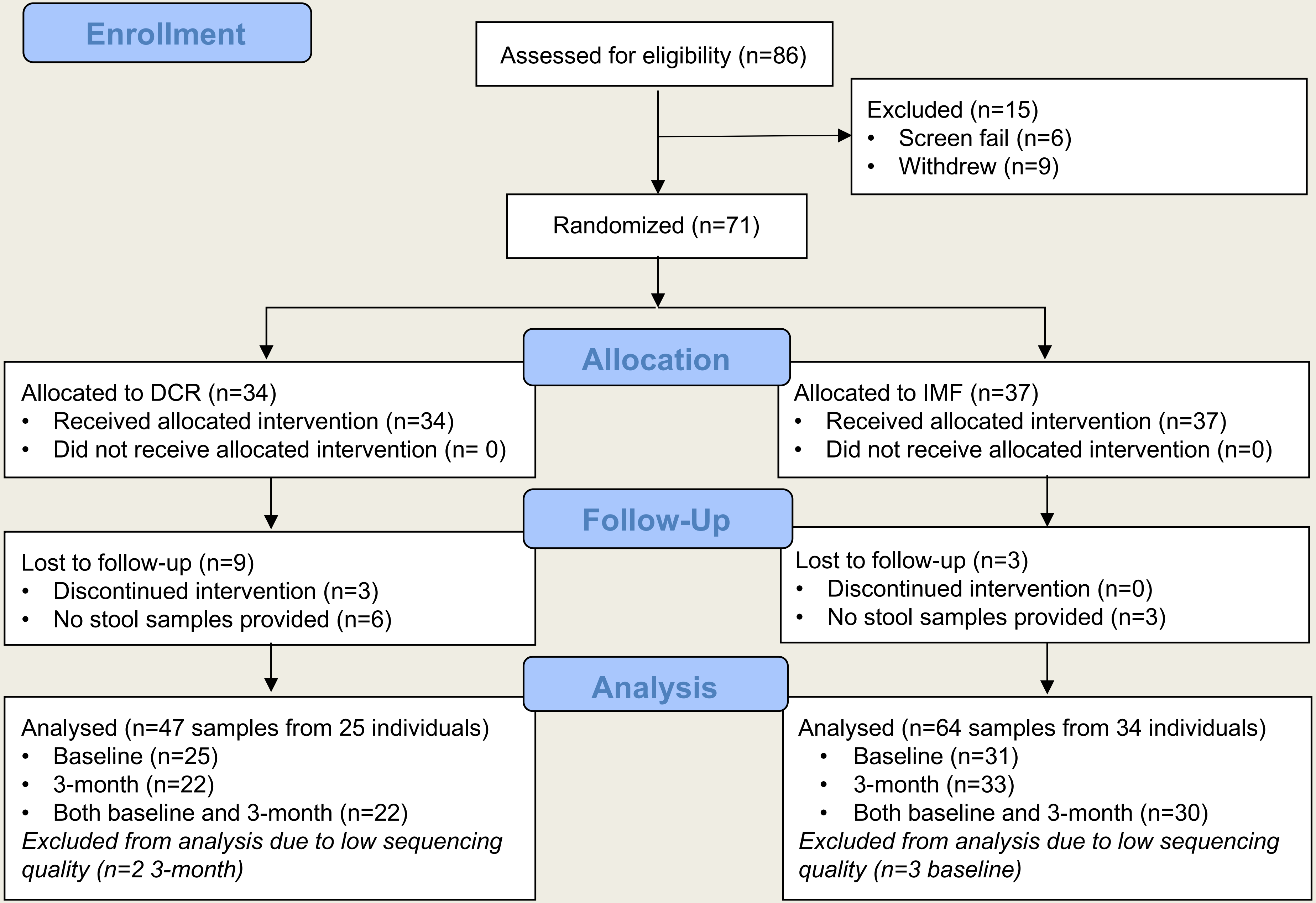


Li et al., 2017, Cell Metabolism 26, 672–685

Kohl, 2014; Patterson, 2017; Thompson, 2006; Banas, 1988; Sonnenburg, 2005; Martens, 2008; Hooper, 2001; Marcobal, 2013; Ward, 1987; Palframan, 2002; Karasov, 2004

Research aims

- Understand changes in gut microbiota during the first three months of the intervention
- Examine associations between gut microbiota and clinical measures
  - *Weight, waist circumference (baseline and 3 months)*
  - *MetS score – metabolic syndrome score (baseline)*
    - Triglycerides, glucose, HDL cholesterol, waist circumference, blood pressure
- Preliminarily examine differences in these relationships by DCR vs IMF



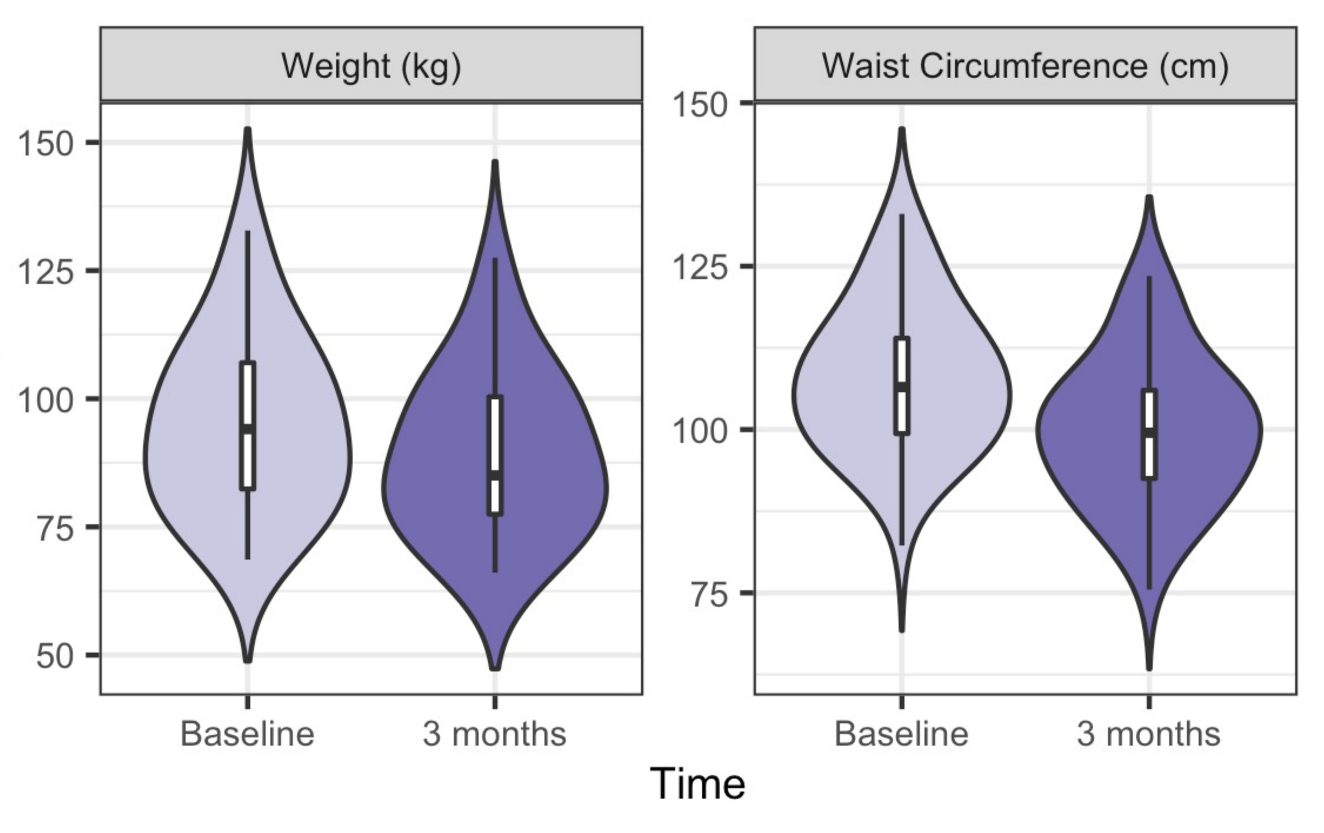
Enrollment
Assessed for eligibility (n=86)
Excluded (n=15)
• Screen fail (n=6)
• Withdrew (n=9)
Randomized (n=71)
Allocation
Allocated to DCR (n=34)
• Received allocated intervention (n=34)
• Did not receive allocated intervention (n= 0)
Allocated to IMF (n=37)
• Received allocated intervention (n=37)
• Did not receive allocated intervention (n=0)
Follow-Up
Lost to follow-up (n=9)
• Discontinued intervention (n=3)
• No stool samples provided (n=6)
Lost to follow-up (n=3)
• Discontinued intervention (n=0)
• No stool samples provided (n=3)
Analysis
Analysed (n=47 samples from 25 individuals)
• Baseline (n=25)
• 3-month (n=22)
• Both baseline and 3-month (n=22)
Excluded from analysis due to low sequencing quality (n=2 3-month)
Analysed (n=64 samples from 34 individuals)
• Baseline (n=31)
• 3-month (n=33)
• Both baseline and 3-month (n=30)
Excluded from analysis due to low sequencing quality (n=3 baseline)

Cohort

	Overall	DCR	IMF	p-value
N	59	25	34	
Age (mean (SD))	40.7 (9.8)	42.0 (10.4)	39.8 (9.3)	0.384
Female sex (%)	45 (76.3)	18 (72.0)	27 (79.4)	0.725
Race (%)				>0.99
White	53 (89.8)	22 (88.0)	31 (91.2)	
Black or African American	4 (6.8)	2 (8.0)	2 (5.9)	
Asian	2 (3.4)	1 (4.0)	1 (2.9)	
Hispanic ethnicity (%)	10 (16.9)	6 (24.0)	4 (11.8)	0.297
Stool collection				
Stool at baseline (%)	56 (94.9)	25 (100.0)	31 (91.2)	0.355
Stool at 3 months (%)	55 (93.2)	22 (88.0)	33 (97.1)	0.399
Stool at both times (%)	52 (88.1)	22 (88.0)	30 (88.2)	>0.99

Follow-up measures at 3 months

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

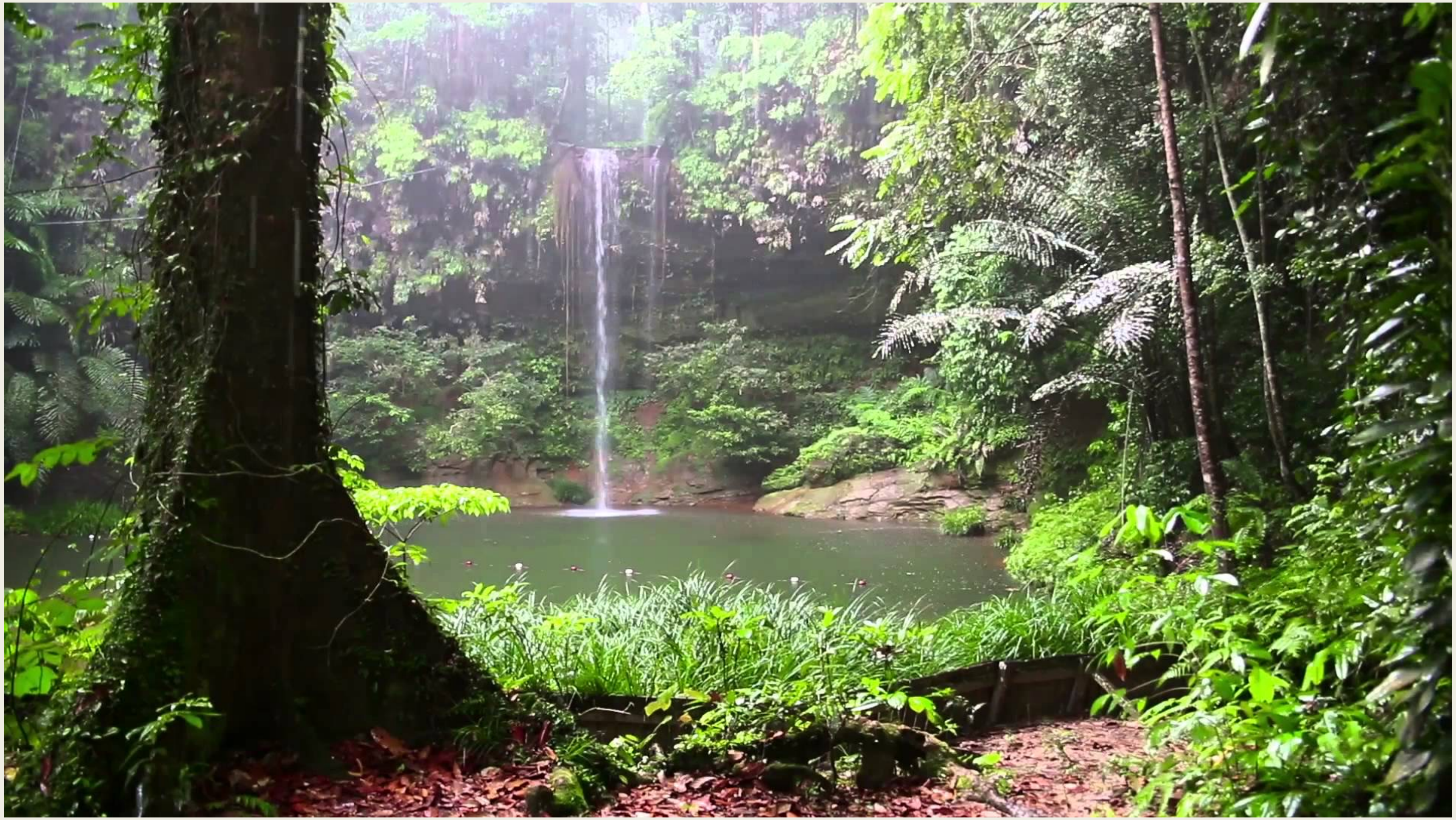


Stanislawski et al. Nutrients 13.9, 2021.

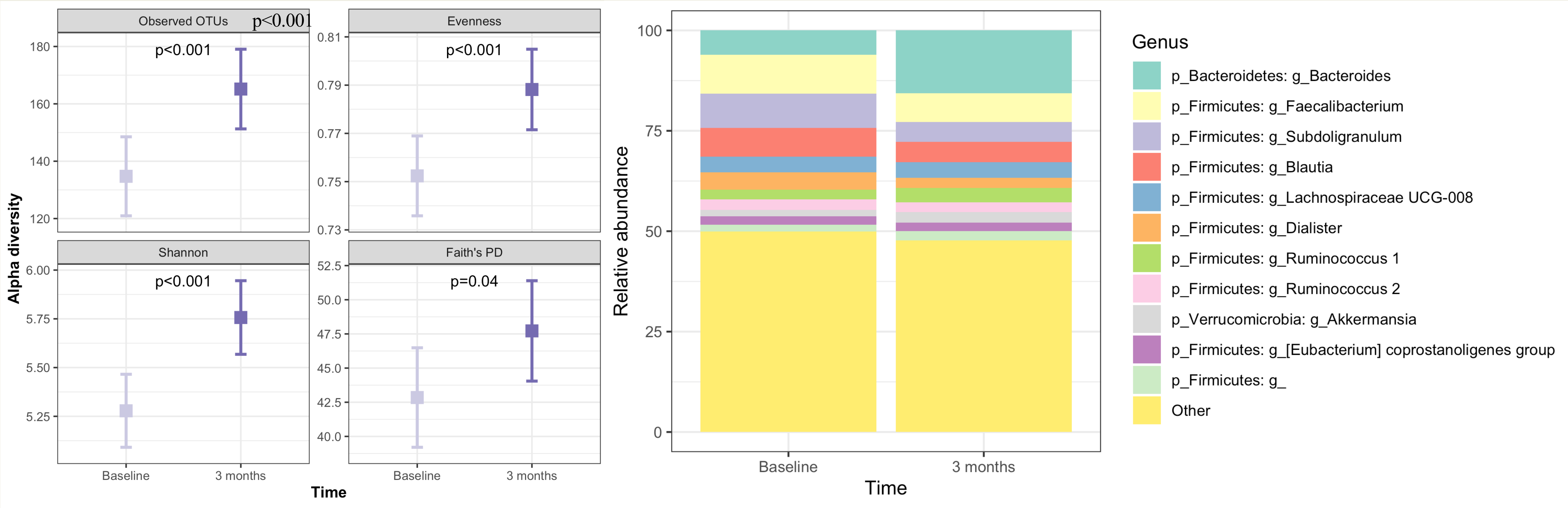
Methods

- GM sample processing
  - *16S rRNA gene sequencing V3V4 region*
  - *DADA2 run (default parameters) for denoising & finding sequence abundances*
  - *SEPP insertion tree using Silva 12.8*
- Alpha diversity: Linear mixed models
- Overall composition: Permutational ANOVA (longitudinal - PermanovaFL, adonis); MiRKAT
- Taxa
  - *Change in taxa during intervention: analysis of composition of microbiomes (ANCOM)*
  - *Taxa predictive of change in clinical outcomes: variable selection using random forests (VSURF)*
- Covariates: age, sex, time, intervention group, evaluated time * intervention

Alpha diversity



Change in gut microbiota



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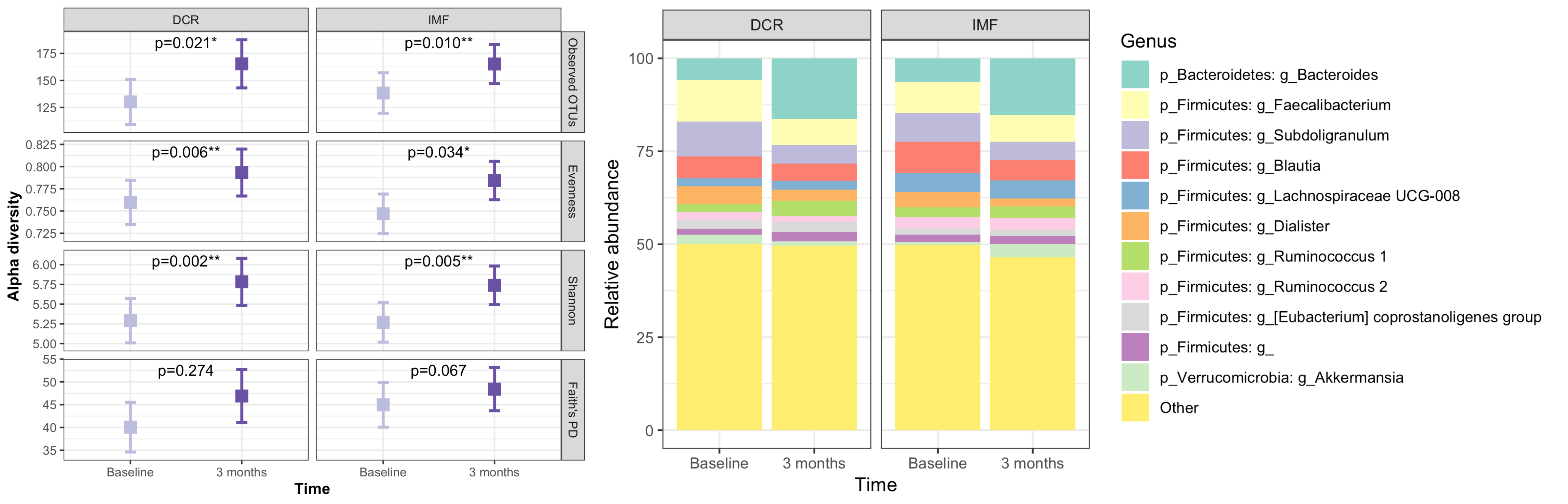
Similar overall changes in DCR vs IMF



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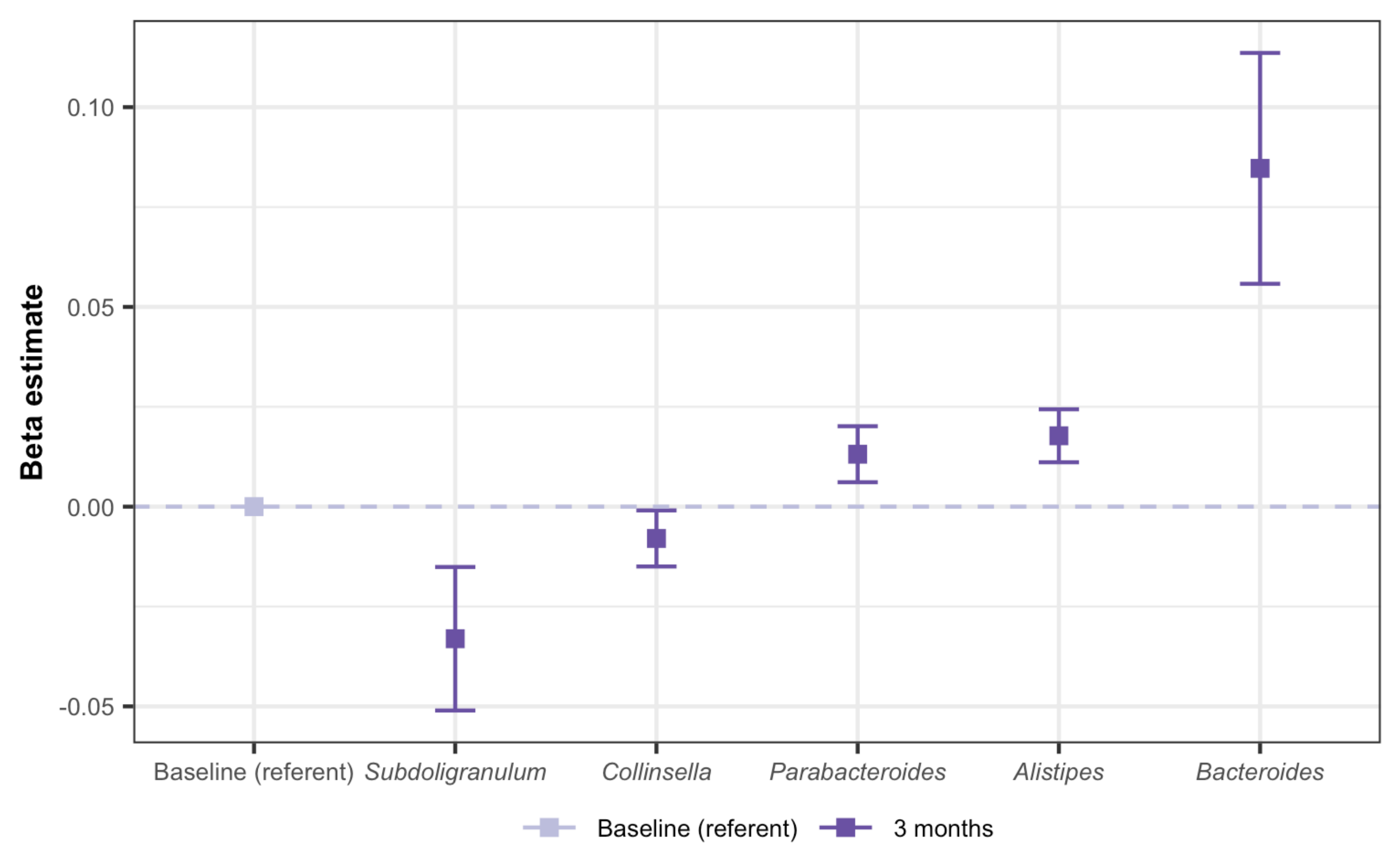
Change in gut microbiota taxa



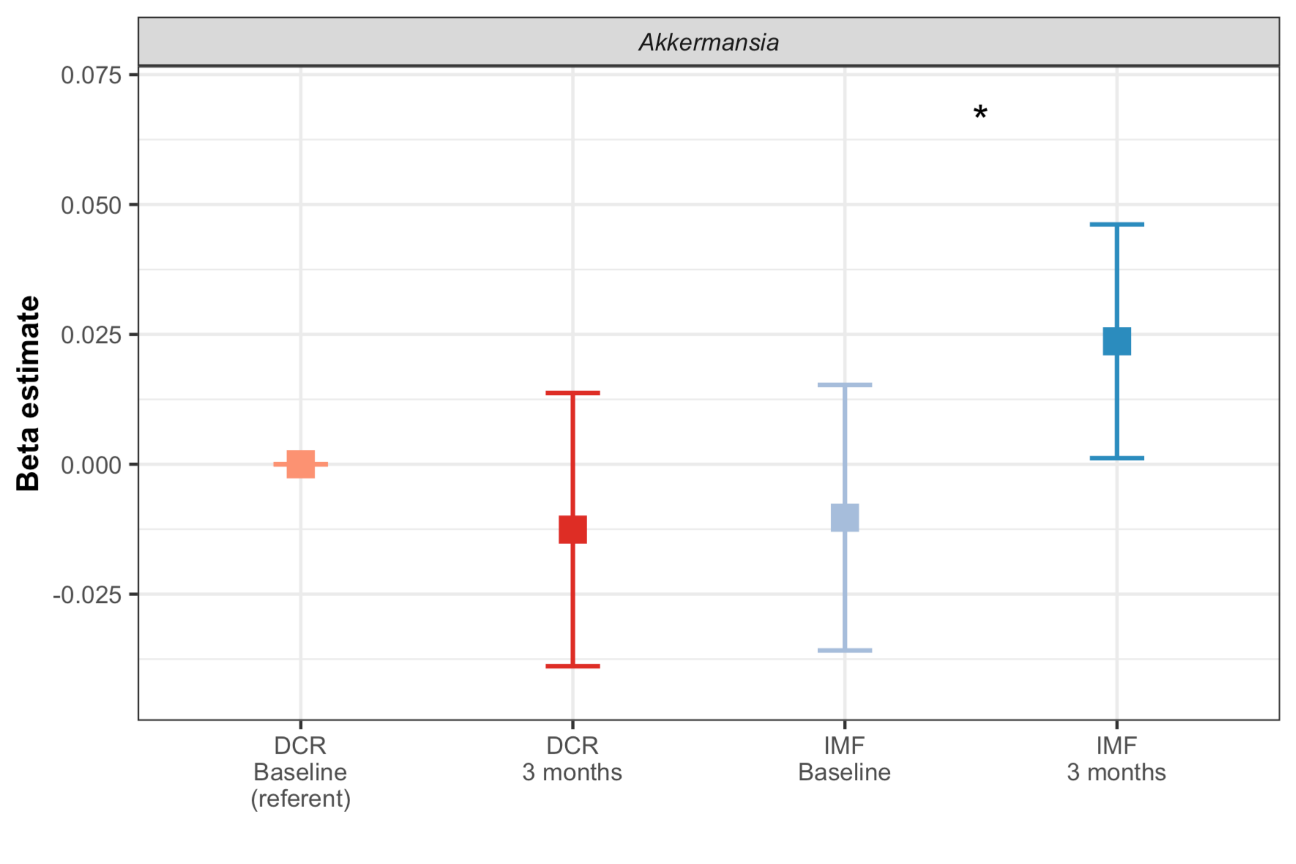
Stanislawski et al. Nutrients 13.9, 2021.

Consistent findings with prior weight loss literature

- Increased abundance of *Bacteroides* in hypocaloric weight-loss diets and of *Alistipes* following surgical weight loss interventions
- Higher baseline *Alistipes* abundance correlated with greater success in long-term weight-loss maintenance following a diet/lifestyle intervention
- Reduction in *Collinsella* abundance observed during a hypocaloric weight loss program in type 2 diabetics with obesity and in a reduced carbohydrate intervention of overweight men

Nadal, I. *Int. J. Obes*. 2008; Santacruz, A. *Obesity* 2009; Simões, C.D. *Eur. J. Nutr*. 2014; Louis, S. *PLoS ONE* 2016; Frost, F. *PLoS ONE* 2019; Walker, A. *ISME J*. 2011; Seganfredo, F.B. *Obes. Rev*. 2017.

Akkermansia increases in IMF

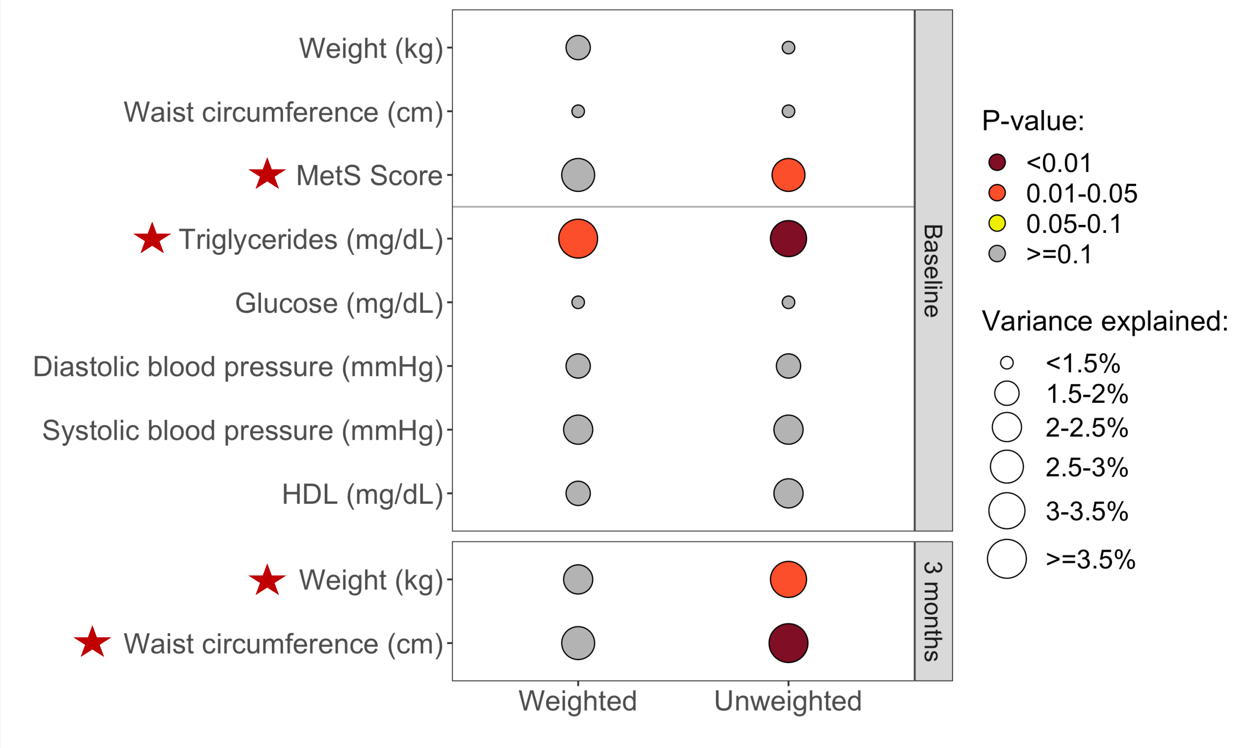


- *Akkermansia Muciniphila* is most common species in this genus
  - Mucin-degrading bacterium causally linked in animal models to lowering body fat mass, improving glucose homoeostasis, decreasing adipose tissue inflammation and increasing gut integrity, as well as cardiometabolic improvements during dietary energy restriction (Dao et al., 2016; Everard et al., 2013; Shin et al., 2014)
- *Akkermansia* important producer of acetate
  - Linked to microbiota-mediated cardiometabolic improvements during IMF in animal models (Li et al., 2017)

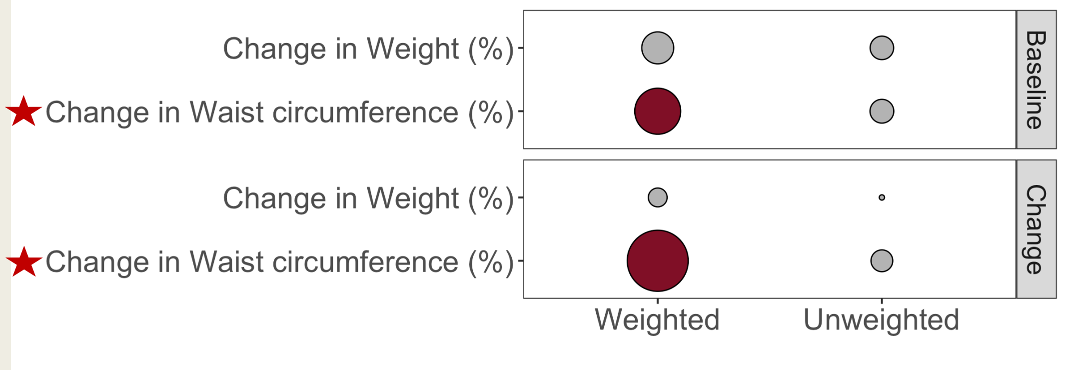
Stanislawski et al. Nutrients 13.9, 2021.

Association with clinical measures

Cross-sectional associations between gut microbiota composition and clinical measures

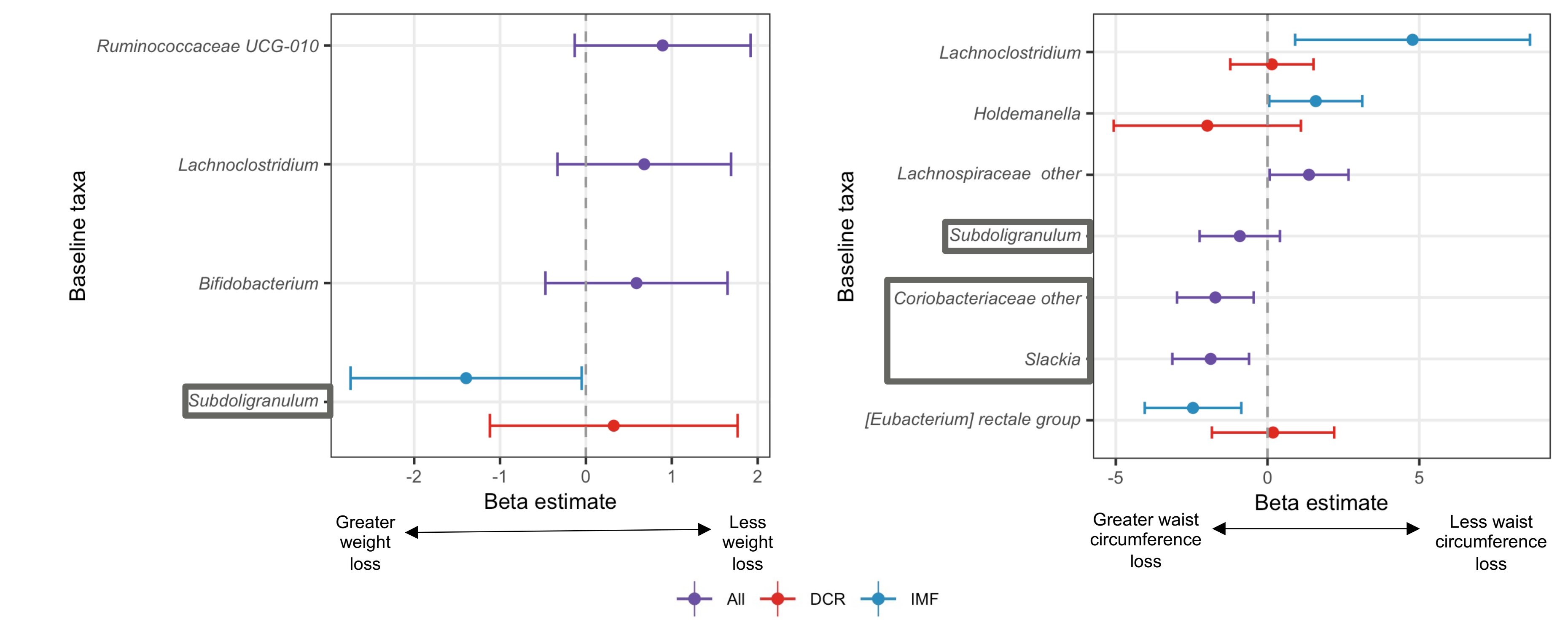


Associations with change in clinical measures



Stanislawski et al. Nutrients 13.9, 2021.

Taxa predictive of clinical outcomes



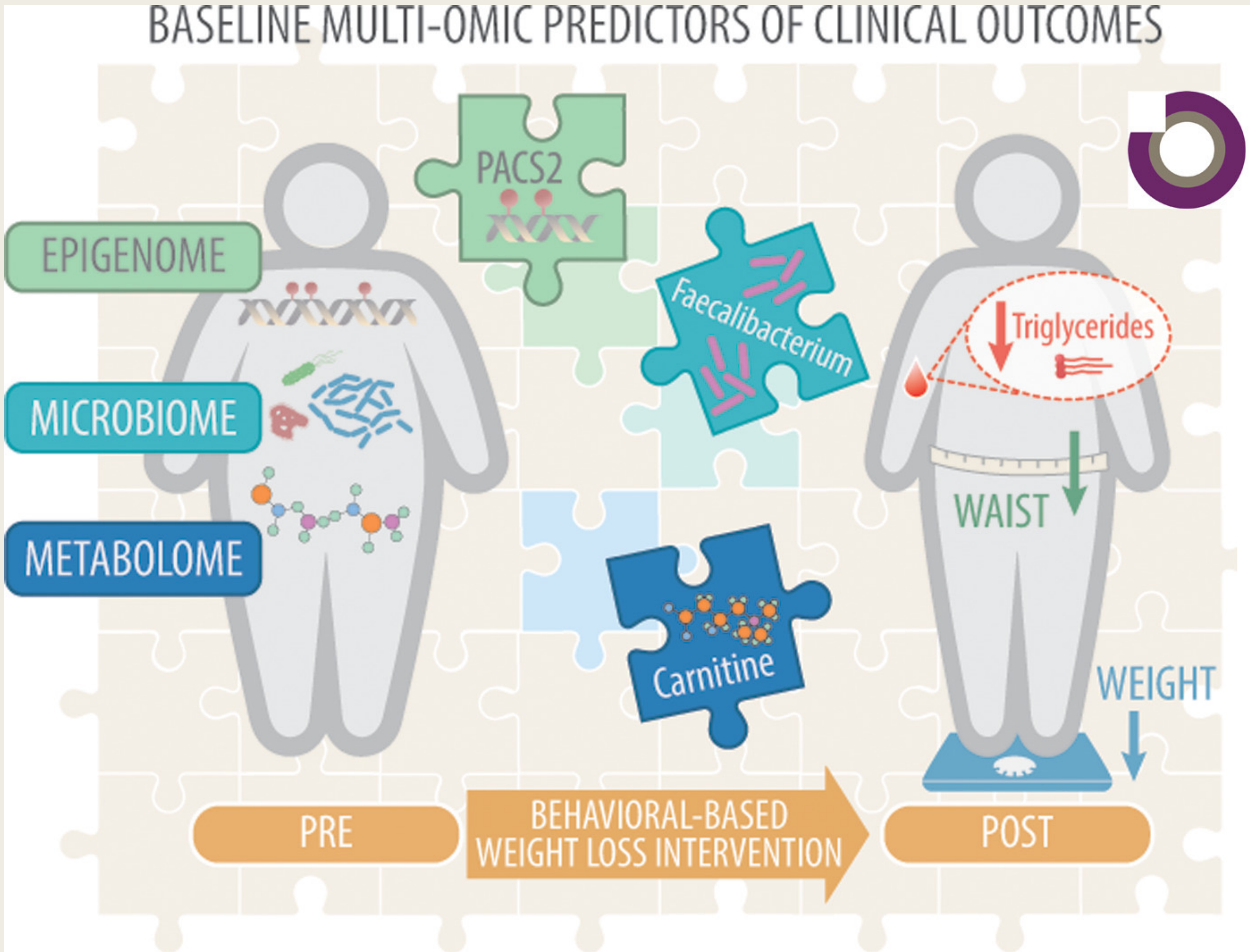
- *Subdoligranulum* has only one species, *S. variabile*, which was **predictive of greater improvements in insulin sensitivity during an FMT intervention** study from lean donors to men with metabolic syndrome
- Higher baseline levels of Coriobacteriaceae were also identified as contributing towards the beneficial effects of **Roux-en-Y gastric bypass among people with type 2 diabetes**
- *Slackia* may help to **increase the bioavailability of polyphenols**, with possible benefits for cardiometabolic health

Conclusions

- During the first three months of a lifestyle weight loss intervention involving an energy restricted diet and increased physical activity
  - *Gut microbiota of participants changed significantly*
  - *Baseline gut microbiota composition – and change in composition from baseline to 3 months - were predictive of change in waist circumference at three months*
  - *Numerous bacterial taxa (at baseline and their change) were associated with improvements in weight and waist circumference measures*
- Growing body of literature demonstrates that gut microbiota play an important role in body weight regulation and may contribute towards responsiveness during a weight loss intervention
- Critical area for further research because gut microbiota profiles are alterable through various means, such as probiotics/prebiotics, personalized dietary changes or targeting gut microbiota pathways and metabolites

Kunnackal John et al., 2018; Zeevi et al., 2015; Joyce and Gahan, 2016

Related Work



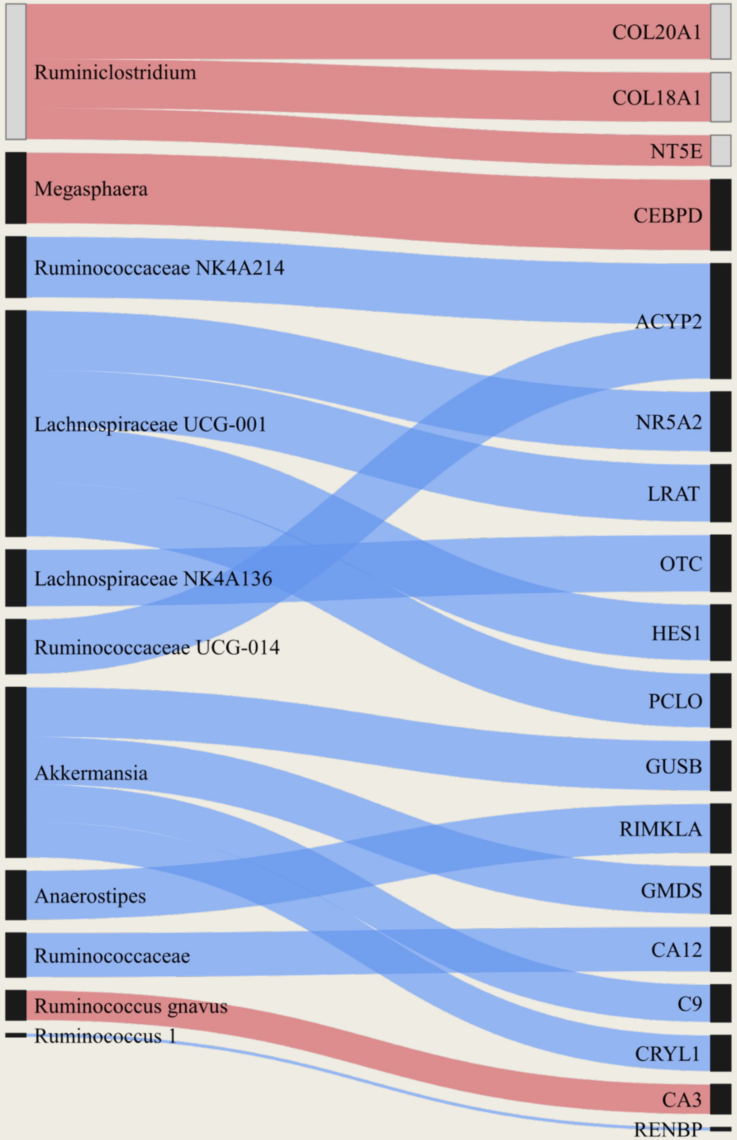
A network of multiomic relationships informed predictive models for change in 10 clinical measures. The models identified specific DNA methylation sites, gut microbes, and metabolites that were predictive of variability in weight loss, waist circumference, and circulating triglycerides and that are biologically relevant to obesity and metabolic pathways.

Siebert, Janet C., et al. "Multiomic Predictors of Short-Term Weight Loss and Clinical Outcomes During a Behavioral-Based Weight Loss Intervention." *Obesity 29.5* (2021): 859-869.

Epigenetic / Gut microbiota

- Interplay between epigenetics and gut microbiota virtually unknown in any disease context
  - *Evidence that epigenetics can influence the gut microbiota*
  - *Gut microbiota or related metabolites may elicit changes in DNA methylation*

Current Work



- Relationships among diet, gut microbiota taxa and DNA methylation
  - *Numerous associations between gut microbes and CPGs*
  - *No significant associations with dietary food groups or a targeted panel of CVD-associated metabolites*

Hill E, Konigsberg, I, et al. In Preparation for *Nutrients*.

Current/Future Work

- Microbiota for all participants & all timepoints
  - *Baseline, 3, 6, 12 months & 6 months post-intervention*
  - *16S rRNA + shotgun metagenomic sequencing of subset*
  - *What drives changes in microbiota? How do microbiota relate to outcomes?*
  - *Weight loss maintenance*
- Metabolomics
  - *Metabolites may mediate GM effects on weight loss*
- Genetics
  - *Does genetic propensity for obesity impact weight loss success?*
  - *Genetic relationships with GM/metabolites*




Aims

- Understand changes and patterns in longitudinal microbiome/metabolomic data: baseline, 3, 6, 12 & 18 months
  - *Assess effects of intervention, diet, physical activity*
  - *Evaluate longitudinal association with outcomes*
- Mediation analysis
  - *Microbiome as mediator of intervention effects*
  - *Microbial metabolites as mediators of intervention effects*

Methodological Issues

- Challenges of microbiome data
  - *High-dimensional*
  - *Compositional*
  - *Phylogenetic tree structure*
  - *Sparse*
  - *Often non-normally distributed*
  - *Often have large portion of zero values* → *skewed*
  - *Heteroscedastic and overdispersed*
  - *Microbes can play a lot of roles (exposure, mediator, outcome)*

Methodological Issues

- Challenges of microbiome data
  - *High-dimensional*
  - *Compositional*
  - *Phylogenetic trees structure*
  - *Sparse*
  - *Often non-normally distributed*
  - *Often have large portion of zero values → skewed*
  - *Heteroscedastic and overdispersed*
  - *Microbes can play a lot of roles (exposure, mediator, outcome)*
- Many tools and methods addressing these issues are not designed for longitudinal data

Methodological Approaches

- Understand changes and patterns in longitudinal microbiome data: baseline, 3, 6, 12 & 18 months
  - *Assess effects of intervention, diet, physical activity*
    - Microbial Trend Analysis (Wang, Huilin Li)
    - Identifies dominant taxa contributing to common trends; a microbial trend group differential test to confirm the statistical significance of group comparison and identify key taxa contributing to the group differential trend; a distance-based classification algorithm to assign a group label to a given subject
    - Integrates spline-based method for time-course data analysis with principal component analysis for dimension reduction. Matrix decomposition and lasso technique used to address high-dimensionality, and graph Laplacian penalty additionally used to incorporate phylogenetic tree structure.

Wang, et al. *BMC genomics* 22 (2021): 1-16.

Methodological Approaches

- Understand longitudinal association of microbiome/metabolites with outcomes
  - *Correlated sequence kernel association test (Zhan, Jun Chen)*
    - Detect longitudinal association of overall microbiome with outcomes using a linear mixed model approach with small sample correction (recalibrate the null distribution)
  - *Adaptive Microbiome Association Test (Banerjee, Xiang Zhan) – feature selection and association testing*
    - Distance correlation learning followed by data-adaptive association test under flexible generalized linear model framework

Zhan, et al. *Genetic epidemiology* 42.8 (2018): 772-782;
Banerjee, et al. *NAR Genomics and Bioinformatics* 4.1 (2022): lqab120.

Methodological Approaches

- Mediation analysis
  - *SparseMCMM – counterfactual approach (Wang, Huilin Li) uses linear log-contrast regression models and Dirichlet regression models to: identify key causal microbes using regularization; incorporate control variables; assess treatment-mediator interactions; evaluate the overall and taxon-level effects; account for the compositional nature of microbiota data*
    - Not currently designed for longitudinal data - authors plan to extend w/ microbial dynamic system modelling
  - *LDM-Med - Linear Decomposition Model mediation approach based on inverse regression (Yue, Hu)*
- Understand relationship between genetics / microbiome / metabolomics
  - *Dual dual kernel based association (Zhan, Wu)*

Wang, et al. *Bioinformatics* 36.2 (2020): 347-355.
Yue and Hu. *Bioinformatics* 38.12 (2022): 3173-3180.
Zhan, Xiang, et al. *Genetics* 206.4 (2017): 1779-1790.

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