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Disclosure to Participants
No conflicts of interest to disclose.

Outline
- Gut Microbiota and Microbiota Research
- Obesity, Diet, and Gut Microbiota
  - Mechanisms – Lipid Absorption
- Strategies to Promote Healthy Microbiota
  - Prebiotics – Supplementation Study
  - Probiotics
  - FMT
  - Postbiotics

The Human Microbiome:
Excitement in the scientific community

Diet, Gut Bacteria, and Metabolic Disease: Strategies to Promote Healthy Microbial Communities
What is the gut microbiota?

- **Gut microbiota**
  - Collection of microbes or the number of microbial cells harboring the gastrointestinal tract
  - May include bacteria, yeast, archaea, and viruses
- **Gut microbiome**
  - Collective number of genes within the gut microbiota
- **Microbial dysbiosis**
  - Disruption of normal microbial communities or imbalance of good vs bad bacteria (i.e., pathobionts)

How does our microbial community compare to us?

### Major Phyla of Bacteria in the Gut:
- Bacteroidetes
- Firmicutes
- Actinobacteria
- Proteobacteria
- Verrucomicrobia

Why do we need gut microbes?

**A vital organ?**
- Pathogen protection
- Immune development
- Digestion and caloric salvage
- Gut development
- Energy balance
- Drug metabolism
- Regulatory functions

How do we determine the functional role of gut microbes?

Germ-free mice are used to study the effect of gut microbiota on the host.

How do we study gut bacteria: 16S rRNA Sequencing

Other Technologies

- **Metagenomics** - Determine entire genome of bacterial communities
- **Metabolomics** - Determine collection of metabolites from bacterial communities
- **Metatranscriptomics** - Determine the collection of gene transcripts upregulated in bacterial communities

Factors Contributing to the Rise in Obesity
Factors Affecting Gut Microbial Structure

- Diet
- Host Genetics and Metabolic State
- Environment

Animal-based diet alters microbial communities within 1 week

- Firmicutes
- Proteobacteria
- Bacteroidetes
- Verrucomicrobia
- Actinobacteria

Microbial dysbiosis occurs in a genetic model of obesity


Diet regardless of genotype alters gut microbial structure


Lean male donors
Obese male recipients
BMI ≥ 30

4 weeks
- Improved symptoms of Type II Diabetes and Metabolic syndrome

Fecal Microbiota Transplant (FMT) from lean donors resolves insulin resistance in obese subjects

- Vrieze et al., Gastro. (2012) 143:913-916
Summary
- Many factors affect microbial assemblage including:
  - Diet
  - Genetics (and perhaps Diet > Genetics?)
  - Metabolic State (i.e., Obesity)
- Microbial communities from lean healthy donors may protect against symptoms associated with type 2 diabetes
- Microbial communities can transfer an obese phenotype if given to a lean donor

How does this occur?
What are the mechanisms?

GF mice are resistant to diet-induced obesity

Fat Digestion and Absorption is Regulated on Multiple Levels
Adequate lipid absorption relies on endocrine signaling, emulsification from bile, and absorptive capacity of small intestinal enterocytes
Overall Goal:
To determine if 1) gut microbes regulate lipid absorption in the small intestine of mice and 2) the mechanisms underlying this interaction.

Hypothesis:
GF mice have impaired lipid absorption on a high fat diet due to reduced hormonal signaling from the gut.

GF mice have altered intestinal expression of lipid transporters

Experimental Design
GF mice do not gain weight on high milkfat (MF) diet

Unpublished data, Chang Lab

GF mice have impaired pancreatic secretion of lipase and amylase

Unpublished data, Chang Lab

GF mice have larger gallbladders and elevated stool TG vs Conv mice

Unpublished data, Chang Lab

Conventionalizing GF mice with MF microbiota restores CCK expression

Unpublished data, Chang Lab

Corn Oil (CO) Challenge Experimental Design

Pancreas and intestinal Luminal contents collected

Conventionalization of GF mice with MF microbes restores TG and Chol absorption to a greater extent than LF microbes

Pancreas and intestinal Luminal contents collected

**Notes:**
- GF mice do not gain weight on a high milkfat (MF) diet.
- Conv GF mice have larger gallbladders and elevated stool TG compared to Conv mice.
- Conventionalizing GF mice with MF microbes restores CCK expression.
- CO Challenge Experimental Design:
  - Fast
  - CO or H2O Gavage
  - Harvest
  - Pancreas and intestinal Luminal contents collected
- Conventionalization of GF mice with MF microbes restores TG and Chol absorption to a greater extent than LF microbes.
HF diet-induced dysbiosis increases lipid absorption through impacting digestion and lipid uptake.

**How do prebiotics promote metabolic benefits?**
- Increase expression of antimicrobial peptides against deleterious bacteria
- Short chain fatty acid (SCFA) production and SCFA-mediated stimulation of intestinal gluconeogenesis and increased epithelial integrity
- Increase satiety and insulin sensitivity via release of gut peptide hormones including polypeptide YY (PYY) and glucagon-like peptide (GLP)-1, respectively
- Decrease relative abundance of pathogenic bacteria and increase abundance of commensal bacteria

**Targeting the Gut Microbiota**
- Prebiotics
- Probiotics
- Synbiotics
- Fecal Microbiota Transplant (FMT)
- Postbiotics

**Prebiotics**
- **Definition:** Prebiotics are foods that promote growth of beneficial bacteria
  - Resistant to gastric acidity
  - Non-digestible, fermented by bacteria
  - Promote healthy microbial communities (Viladomiua et al. 2013)
- **Non-digestible carbohydrates**
  - Inulin-type fructans
  - Oligofructose
  - Galactans
- **Examples**
  - Prebiotin

**Study Objective**
Determine if a dietary prebiotic (e.g., inulin) can remodel the gut microbiota (e.g., composition and function) in a well-defined human sample population (e.g., metabolic syndrome), while demonstrating possible effects on host metabolism (e.g., improved glucose tolerance).

**Hypothesis**
Daily supplementation with 8 g of a FOS-rich prebiotic for 3 weeks will improve glucose tolerance in pre-diabetic borderline obese males.

**Study Design**
- **Population:**
  - n = 8 males
  - Overweight/obese (BMI 25-35)
  - Prediabetic (FBG > 100)
- **Intervention:**
  - 3 week single arm intervention
  - Study product: Prebiotin FOS-enriched 100% inulin
- **Comparisons:**
  - Baseline vs End of Study
- **Experimental Outcomes:**
  - Host
    - Fasting blood glucose, insulin, GTT including glucose and insulin measurements
    - Dietary intake (3 day diet record)
    - Lipid Panel
  - Microbe
    - 16S rRNA sequencing
    - Biolog
    - Targeted metabolites: SCFAs, H2S, bile acids
Stool Collection
- 2 collection kits provided at baseline and end of study
- 2 samples to be collected within a 3 day period
- Time and date noted
- Provided 3 day bowel habits diary prior to and at end of 3 week supplementation

Demographics/Baseline Measures
- 4 non-Hispanic white, 3 African American, 1 Asian/Pacific Islander
- Mean Age: 44.1 +/- 2.9
- BMI: 30.2 +/- 1.2
- Body Fat % (DEXA): 33.4 +/- 1.4%
- FBG 109.3 +/- 2.7 mg/dL at screening

Prebiotic Supplementation
- A commercially-available oligofructose-rich fiber supplement
- Used in at least two NIH-funded studies.
- 8 g FOS-inulin per day for 3 weeks.
  - Maximum tolerable amount recommended by the company and found to be an effective dose in promoting bifidogenic activity in previous studies (Glen Gibson 2007).
- All Fiber product purchased for the study came from the same lot number.

Responders displayed improved glucose and insulin tolerance

Microbial communities differs b/t responders and non-responders
- P=0.003

Modest phylum level shifts are in responders but not non-responders
Conclusions

- 50% of subjects displayed improved glucose tolerance and/or insulin sensitivity ("Responders")
- Responders appeared to have alterations in microbial structure whereas Non-Responders did not
- Inulin significantly increased butyrate and acetate levels from baseline to endpoint but no differences in levels between responders and non-responders
- Inulin may promote mild improvement in glucose tolerance in some subjects after only 3 weeks of supplementation

Overall Conclusions

- Intimate link between gut microbes and host physiology including energy extraction and metabolism
  - Several mechanisms are being investigated to understand this relationship
- Many therapeutic options to restructure gut microbiota are available and on the rise
  - Much research is needed to solidify the best approach in using these therapies