What’s Your Gut Telling You?
The Role of the Gastrointestinal Microbiome in Diabetes

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Learning Objectives
1. Identify potential mechanisms by which the microbiome affects human health
2. Explain how the microbiome influences the development of diabetes mellitus
3. Describe the differences seen in gut microbiome composition between patients with diabetes and healthy subjects
4. Compare gut microbiome alpha diversity and bacterial taxa composition of Mexican Americans with and without diabetes
Role of the Microbiome in Human Health

I. Overview of the human microbiome
   a. Definitions
      i. Microbiota: microbes that collectively inhabit a given ecosystem
      ii. Microbiome: collection of all genomes of microbes in an ecosystem
      iii. Dysbiosis: disturbance or change in the composition or function of microbes
   b. Scope
      i. Body’s bacteria laid end-to-end would circle the Earth 2.5 times
      ii. Microbiome weighs up to 1 to 2 kg
      iii. Bacterial cells outnumber human cells by 10:1
      iv. 95% of bacteria located in gastrointestinal (GI) tract
   c. Studying the microbiome
      i. Transition from culture-based methods to culture-independent molecular assays
      ii. Methods are used to discern the structure (i.e., anatomy) and function (i.e., physiology) of the microbiota
   d. Tools for analyzing the microbiome (Figure 1)

Figure 1: Analyzing the Microbiome

i. 16S rRNA gene sequencing – measures bacterial community composition
   1. Most common approach
   2. 16S gene encodes for the 16S rRNA molecule that is unique to bacteria and archaea, thus distinguishes these cells from human cells
   3. 16S gene is amplified using polymerase chain reaction and sequenced using next-generation sequencing technology
   4. Machine learning is used to cluster similar sequences and reference databases (e.g., Greengenes) assist with assigning taxonomy
ii. Metatranscriptomics – measures gene expression through next-generation sequencing

iii. Metaproteomics – measures protein expression using mass spectrometry

iv. Metabolomics – measures metabolic productivity using mass spectrometry

e. Reporting on the microbiome
    i. Alpha diversity – measure of diversity of a microbial community
    ii. Shannon diversity – accounts for diversity and evenness of microbial community
        1. Higher values indicate greater evenness and diversity

f. Composition
    i. Varies substantially by body site
        1. Outer body sites predominated by Gram-positive aerobic organisms from the Actinobacteria and Firmicutes phyla
        2. Gut microbiome (represented by stool) predominated by anaerobic Gram-positive and Gram-negative bacteria
            a. Firmicutes (e.g., Lachnospiraceae, Ruminococcaceae)
            b. Bacteroidetes (e.g., Bacteroidaceae, Prevotellaceae)
            c. Actinobacteria (e.g., Bifidobacteriaceae)
    ii. Microbiota extensively conserved at high taxonomic levels; variation increases at progressively lower taxonomic levels
    iii. Large inter-individual variability in microbiota composition, but not ecosystem function

II. Global gut microbiota functions
    a. Mature and train the immune system
    b. Colonization resistance
    c. Mediate host-cell proliferation and vascularization
    d. Regulate intestinal endocrine functions, neurologic signaling, and bone density
    e. Provide a source of energy biogenesis
    f. Biosynthesize vitamins, neurotransmitters, and related compounds
    g. Metabolize bile salts
    h. Xenobiotic metabolism and elimination

III. Microbiome characteristics associated with health
    a. Impossible to define core set of microbial taxa
    b. Increased microbial diversity/richness
    c. Increased functional diversity (gene richness)
    d. Stability or resilience
        i. Ability of microbiome to rapidly return to baseline functional profile after internal and external insults

IV. Associations between gut dysbiosis and human disease
    a. Endogenous and exogenous factors influence gut microbiota
        i. Neonatal mode of delivery
        ii. Host genetic features
        iii. Host immune response
        iv. Diet
        v. Medications
        vi. Environmental exposures
vii. Age
viii. Physical activity
ix. Smoking and alcohol consumption
b. Disruption of microbial communities associated with a host of chronic and acute diseases

| Table 1: Influence of Gut Microbiome on Health⁸ |
|-----------------|-----------------|-----------------|
| Health          | Microbial products or activities | Disease          |
| Nutrient & energy supply | • SCFA production & vitamin synthesis  |
|                  | • Energy supply, gut hormones, & satiety | Obesity & metabolic syndrome |
|                  | • Lipopolysaccharides, inflammation | |
| Cancer prevention | • Butyrate production, phytochemical release | Cancer promotion |
|                  | • Toxin and carcinogen inflammation | |
|                  | • Mediates inflammation | |
| Pathogen inhibition | • SCFA production, intestinal pH, bacteriocins | Pathogen invasion |
|                  | • Competition for substrates and/or binding sites | |
|                  | • Toxin production, tissue invasion, inflammation | |
| GI immune function | • Balance of pro- and anti-inflammatory signals | IBD |
|                  | • Inflammation, immune disorders | |
| Gut motility     | • Metabolites (SCFAs, gases) from non-digestible carbohydrates | IBS (constipation, diarrhea, bloating) |
| Cardiovascular health | • Lipid & cholesterol metabolism | Cardiovascular disease |

SCFA=short-chain fatty acid; IBD=inflammatory bowel disease; IBS=irritable bowel syndrome

**Gut Dysbiosis and Type 2 Diabetes Mellitus (T2DM)**

I. Overview of T2DM
   a. Disease prevalence⁹,¹⁰
      i. As of 2015, 30.3 million Americans (9.4% of the population) with diabetes
         1. 23.1 million people diagnosed
         2. 7.2 million people undiagnosed
      ii. 29 million Americans (9% of the population) have T2DM
   b. Morbidity and mortality
      i. Seventh leading cause of death in the United States in 2015
      ii. Associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy
   c. Cost: 2017 total cost in the United States was $327 billion
II. Pathophysiology: Egregious Eleven

![Diagram of β-cell-Centric Construct: Egregious Eleven]

Figure 2: β-cell-Centric Construct: Egregious Eleven

a. Dysfunctional pathways
   i. Pancreatic β-cells: decreased insulin production
   ii. Muscle: disruptions in insulin signal transduction resulting in insulin resistance
   iii. Liver: decreased inhibition of hepatic glucose production (HGP) by hyperinsulinemia
   iv. Adipose: enlarged fat cells exhibit insulin resistance; fat “spill-over” can worsen insulin resistance in muscle and liver
   v. Decreased incretin effect
   vi. Glucagon-like peptide-1 (GLP-1) diminished in diabetes
   vii. GLP-1 aids in glucose disposal as well as inhibition of HGP
   viii. α-cell: overproduction of glucagon contributes to increased basal hepatic glucose production
   ix. Kidney: increased sodium-glucose cotransporter-2 (SGLT2) threshold
   x. Brain: delayed satiety in response to increases in insulin
   xi. Stomach/small intestine: increased glucose absorption
   xii. Immune dysregulation/inflammation: macrophage and interleukin-1 (IL-1) recruitment to pancreas results in β-cell apoptosis
   xiii. Colon/microbiome: influences host metabolism in three main ways that can affect multiple other facets of Egregious Eleven

III. Microbiome influences on host metabolism
   a. Increased production of lipopolysaccharides (LPS)
      i. LPSs shed from Gram-negative bacterial cell walls (i.e., *E. coli*)
1. Bind to toll-like receptor-4 (TLR4)/CD14 complex
2. TLR4 activates innate immune system, resulting in pro-inflammatory response
3. Decrease expression of tight junction proteins and increase mucosa integrity
   ii. Decreased integrity of intestinal mucosa increases release of LPS into bloodstream\(^\text{12}\)
   1. Higher plasma LPS levels in DM patients compared to healthy counterparts
b. Decreased short-chain fatty acids (SCFAs) production\(^\text{13}\)
   i. SCFAs (butyrate, acetate, propionate) produced by bacterial fermentation of dietary fiber and resistant starches
   1. Main energy source for gut epithelium (mainly butyrate)
   2. Bind G-protein coupled receptors (GPCRs) 41 and 43 in intestinal mucosa, immune cells, liver, and adipose tissues
      a. Intestinal mucosa: SCFAs bind to GPCRs on enterohepatic L-cells in colon \(\rightarrow\) increase GLP-1 secretion
      b. Immune cells: inhibit NF-κB activation; decrease TNF-α and IL-6 suppression and decreased inflammation
c. Bile acids\(^\text{14}\)
   i. Gut bacteria convert primary bile acids to secondary bile acids via bile salt hydrolases
   ii. Secondary bile acids act as signaling molecules to induce GLP-1 secretion from small intestine L-cells
d. Trimethylamine-N-oxide\(^\text{15}\)
   i. Breakdown product of phosphatidylcholine
   ii. Promotes atherosclerosis through upregulation of macrophage scavenger receptors

IV. Gut microbes implicated in specific mechanisms of dysbiosis\(^\text{12-14,16}\)
a. Firmicutes:Bacteroidetes ratio
   i. Increased ratio implicated in metabolic dysregulation (e.g. increased BMI and obesity)\(^\text{4}\)

**Table 2: Bacteria Production or Conversion of Metabolites**

<table>
<thead>
<tr>
<th>LPS production</th>
<th>SCFA production</th>
<th>Bile salt hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• E. coli</td>
<td>• Roseburia sp.</td>
<td>• Lactobacillus</td>
</tr>
<tr>
<td>• Salmonella</td>
<td>• Faecalibacterium prausnitzii</td>
<td>• Bifidobacterium</td>
</tr>
<tr>
<td>• Shigella</td>
<td>• Eubacterium hallii</td>
<td>• Firmicutes</td>
</tr>
<tr>
<td>• Pseudomonas</td>
<td>• Eubacterium rectale</td>
<td>• Enterococcus</td>
</tr>
<tr>
<td>• Neisseria</td>
<td></td>
<td>• Clostridium</td>
</tr>
<tr>
<td>• H. influenza</td>
<td></td>
<td>• Bacteroides</td>
</tr>
<tr>
<td>• Bordetella pertussis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Vibrio cholerae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Gut Microbiome in Patients with Diabetes

I. Microbiome studies
   a. Historically, studies have yielded diverse results\textsuperscript{17-19}
   b. Findings from previous comparative studies

Table 3: Bacterial Taxa Differences in Previous Studies\textsuperscript{20-24}

<table>
<thead>
<tr>
<th>Bacterial Taxa</th>
<th>Previous Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium</td>
<td>• Significantly enriched in non-diabetic subjects</td>
</tr>
<tr>
<td>Prevotella</td>
<td>• RA higher in diabetic subjects compared to control\textsuperscript{21}</td>
</tr>
<tr>
<td></td>
<td>• Higher RA in healthy subjects (58.8% versus 10.7%; p&lt;0.05)\textsuperscript{20}</td>
</tr>
<tr>
<td></td>
<td>• Trend toward correlation with plasma glucose (PG) (R=0.32, p=0.15)\textsuperscript{22}</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>• Higher RA in diabetic subjects\textsuperscript{20}</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>• Higher RA in diabetic group (53% versus 11.8%; p &lt; 0.05)\textsuperscript{20}</td>
</tr>
<tr>
<td>Firmicutes:Bacteroidetes ratio</td>
<td>• Firmicutes higher in controls\textsuperscript{22}</td>
</tr>
<tr>
<td></td>
<td>• Firmicutes:Bacteroidetes ratio negatively correlated with plasma glucose values\textsuperscript{22}</td>
</tr>
<tr>
<td></td>
<td>◦ Conflicts with previous studies on obesity and BMI\textsuperscript{4}</td>
</tr>
<tr>
<td>Clostridia</td>
<td>• Low RA in T2DM gut microbiome (53% versus 34%; p=0.03)\textsuperscript{22}</td>
</tr>
<tr>
<td></td>
<td>• Particular species correlated negatively with blood glucose, hemoglobin A1c, insulin, C-peptide, and plasma triglycerides\textsuperscript{23}</td>
</tr>
<tr>
<td>Roseburia</td>
<td>• Negative correlation with plasma glucose, though not significant (R=-0.53, p=0.06)\textsuperscript{22}</td>
</tr>
<tr>
<td></td>
<td>• Significantly depleted in T2DM subjects\textsuperscript{22}</td>
</tr>
</tbody>
</table>

RA: relative abundance

c. Discordant findings due to:
   i. Diet
   ii. Age
   iii. Birth (Caesarian section versus vaginal delivery)
   iv. Host genotype
   v. Physical activity
   vi. Smoking
   vii. Alcohol consumption
   viii. Medications, especially metformin
   ix. Geographic location
The Gut Microbiome of Mexican Americans with and without T2DM

Research Rationale

I. Prevalence of T2DM by race and ethnicity
   a. National rate of diagnosed diabetes: 9.4%
   b. By race and ethnicity
      i. Non-Hispanic whites: 7.4%
      ii. Asian Americans: 8.0%
      iii. Hispanics: 12.1%
      iv. Non-Hispanic blacks: 12.7%
      v. American Indians/Alaskan Natives: 15.1%
   c. Breakdown among Hispanics
      i. Central and South Americans: 8.5%
      ii. Cubans: 9.0%
      iii. Mexican Americans: 13.8%
      iv. Puerto Ricans: 12.0%
   d. Local DM prevalence
      i. Texas: 10%
      ii. Bexar County 13%
         1. 64% of San Antonio is Hispanic\textsuperscript{25}
         2. 90% of San Antonio Hispanics are of Mexican origin
   e. No published research investigating the gut microbiome composition in DM in Hispanic or Mexican American subjects

Research Objective:
To compare the gut microbiome composition between Mexican Americans with and without T2DM

Methods

I. Study design: cross-sectional study of volunteers from San Antonio and surrounding areas from June 2017 to July 2018

II. Study population
   a. At least 18 years of age
   b. Self-identify as Mexican American
   c. Exclusion criteria
      i. Prior GI surgery that altered anatomy of stomach, esophagus, or intestine
      ii. Chronic daily use of medications for the purpose of altering GI secretory or motor function, e.g.:
         1. Prokinetic agents
         2. Laxatives
         3. Anti-diarrheals
      iii. Use of antibiotics, gastric acid suppressant medications, or probiotics within two months of stool sample collection
   d. Subjects divided into two groups during prescreening based on self-report
Figure 3: Study Groups

e. Data collection
   i. Performed at First Outpatient Research Unit at Medical Arts and Research Center
   ii. Demographic and health questionnaire
   iii. Food diary and stool sample collection kit provided for completion by subjects (Figure 4)

Figure 4: Overview of Study Design

III. Sample processing and sequencing
   a. 16S rRNA gene PCR amplification
   b. Primers targeting V4 region
   c. Sequences classified using Greengenes database
   d. Operational taxonomic unit (OTU) clustering with Mothur Software

IV. Microbiome Analysis
   a. Alpha diversity measured using Shannon Index
      i. Measures richness and evenness of an ecological community
      ii. Compared between groups using the Wilcoxon rank sum test
   b. Beta diversity
      i. Measured using Bray-Curtis indices
      ii. Visualized using Principle Coordinate Analysis (PCoA)
**Results**

I. Baseline characteristics (Table 4)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (N = 37)</th>
<th>Diabetes (n = 14)</th>
<th>No diabetes (n = 23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>59 (48-68)</td>
<td>68 (59-72)</td>
<td>55 (38-61)</td>
<td>0.0032</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>27 (73)</td>
<td>9 (64)</td>
<td>18 (78)</td>
<td>0.4537</td>
</tr>
<tr>
<td>BMI *, median (IQR), kg/m²</td>
<td>28.7 (26.6-34)</td>
<td>30 (26-36)</td>
<td>28 (27-31)</td>
<td>0.4653</td>
</tr>
<tr>
<td>Metformin, no. (%)</td>
<td>12 (33)</td>
<td>12 (86)</td>
<td>0</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Highest level of education, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.2893</td>
</tr>
<tr>
<td>High school or equivalent</td>
<td>8 (22)</td>
<td>2 (14)</td>
<td>6 (26)</td>
<td></td>
</tr>
<tr>
<td>Some college, no degree</td>
<td>14 (38)</td>
<td>8 (57)</td>
<td>6 (26)</td>
<td></td>
</tr>
<tr>
<td>Associate’s degree</td>
<td>6 (16)</td>
<td>3 (21)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Bachelor’s degree</td>
<td>4 (11)</td>
<td>0 (0)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>Master’s degree</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Trade/technical/vocational training</td>
<td>4 (11)</td>
<td>1 (7)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Employment status, no (%)</td>
<td></td>
<td></td>
<td></td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Retired</td>
<td>15 (41)</td>
<td>11 (79)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>Employed for wages</td>
<td>17 (46)</td>
<td>1 (7)</td>
<td>16 (70)</td>
<td></td>
</tr>
<tr>
<td>Out of work and looking of work</td>
<td>5 (14)</td>
<td>2 (14)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>17 (46)</td>
<td>9 (53)</td>
<td>5 (25)</td>
<td>0.0793</td>
</tr>
<tr>
<td>Dyslipidemia, no. (%)</td>
<td>10 (27)</td>
<td>5 (36)</td>
<td>5 (22)</td>
<td>0.3574</td>
</tr>
<tr>
<td>History of MI, no. (%)</td>
<td>1 (3)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>0.3784</td>
</tr>
<tr>
<td>History of cancer, no. (%)</td>
<td>1 (3)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>0.3784</td>
</tr>
<tr>
<td>Depression, no. (%)</td>
<td>1 (3)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>0.3784</td>
</tr>
<tr>
<td>IBS, no. (%)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

IQR, interquartile range; MI, myocardial infarction; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; PUD, peptic ulcer disease; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome

*BMI not reported by one subject
II. Shannon diversity
   a. No statistically significant difference in Shannon diversity (p = 0.034)
      i. Diabetic: 3.21
      ii. Non-diabetic: 3.07

![Shannon Diversity by Study Group](image)

**Figure 5: Shannon Diversity by Study Group**

III. Taxonomical differences in gut microbial composition in Mexican Americans with and without T2DM
   a. Significantly different RA of 16 OTUs (Table 5)

<table>
<thead>
<tr>
<th>Species</th>
<th>Fold change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ruminococcus torques</em></td>
<td>-3.910</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Streptococcus sp.</em></td>
<td>3.536</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Prevotella sp.</em></td>
<td>-2.047</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Clostridium sp.</em></td>
<td>-2.372</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Blautia sp.</em></td>
<td>-4.681</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Eubacterium dolichum</em></td>
<td>-4.259</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Ruminococcus sp.</em></td>
<td>2.274</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Lachnospiraceae sp.</td>
<td>5.754</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Pyramidobacter pscilos</em></td>
<td>-2.334</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>2.323</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Clostridiales sp.</td>
<td>-2.248</td>
<td>p = 0.0055</td>
</tr>
<tr>
<td><em>Clostridium citroniae</em></td>
<td>-3.755</td>
<td>p = 0.0058</td>
</tr>
<tr>
<td>SMB53 sp.</td>
<td>4.050</td>
<td>p = 0.0197</td>
</tr>
<tr>
<td>Veillonella dispar</td>
<td>4.711</td>
<td>p = 0.0278</td>
</tr>
<tr>
<td>Lachnospiraceae sp.</td>
<td>5.350</td>
<td>p = 0.0399</td>
</tr>
<tr>
<td>Lachnospira sp.</td>
<td>2.000</td>
<td>p = 0.0399</td>
</tr>
</tbody>
</table>
b. *Streptococcus* genus
   i. Median RA 3.5 times higher in non-diabetic subjects in this study (p = 0.046)

IV. Firmicutes:Bacteroidetes ratio

<table>
<thead>
<tr>
<th></th>
<th>Diabetic</th>
<th>Non-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.637:1</td>
<td>0.507:1</td>
</tr>
</tbody>
</table>

V. Community composition: microbiome similarity
   a. Bray-Curtis dissimilarities plotted using PCoA by diabetes status (Figure 6)
   b. No strong associations apparent
      i. Two distinct groups in lower left quadrant and lower right quadrant
      ii. Currently calculating healthy eating index (HEI) scores to further elucidate groupings

c. Most distinct coordinate subject 35
   i. Tricyclic antidepressant (TCA) use in subject 35
Table 7: *Blautia* Median RA

<table>
<thead>
<tr>
<th>Group</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 35</td>
<td>0.38</td>
</tr>
<tr>
<td>T2DM</td>
<td>0.007</td>
</tr>
<tr>
<td>Non-T2DM</td>
<td>0.007</td>
</tr>
</tbody>
</table>

ii. No published research regarding TCA modulation of the gut microbiome

**Discussion & Conclusions**

I. Baseline characteristics
   a. Previous studies with healthier control groups\textsuperscript{20-23,26}
   b. Use of metformin variable in previous studies\textsuperscript{20-23,26}
   c. Higher median BMI than previous studies\textsuperscript{21}

II. Alpha diversity
   a. Previous studies with increased alpha diversity in healthy subjects\textsuperscript{27}
   b. Some studies show no difference\textsuperscript{20}
   c. Possibly due to the metformin’s effects on the gut microbiome\textsuperscript{28}

III. Taxonomical differences
   a. Distinct from results of previous comparisons of diabetic versus non-diabetic gut microbiome
      i. No significant differences in RA:
         1. *Lactobacillus*
         2. *Bacteroides*
         3. Clostridia
         4. *Bifidobacterium*
         5. *Roseburia*
   b. *Streptococcus* genus
      i. Significantly higher in non-diabetic subjects in our study
      ii. Greater abundance in patients with atherosclerotic cardiovascular disease (ASCVD)\textsuperscript{29}
          1. Study did not report metformin use
          3. Positively associated with ASCVD-enriched Enterobacteriaceae cluster
      iii. Metagenomic linkage groups (MLGs) of *Streptococcus* enriched in patients with hypertension compared to healthy controls\textsuperscript{30}
          iv. Associated with trimethylamine N-oxide (TMOA) production\textsuperscript{31}
              1. TMAO: product of gut microbial metabolism of phosphatidylcholine, choline, and carnitine
              2. Increases platelet hyperreactivity and enhances thrombotic risk
   c. *Firmicutes*:Bacteroidetes ratio
      a. Our data conflicts with previous diabetes gut microbiome study\textsuperscript{22}
      b. Increased ratio implicated in increased energy harvest and obesity\textsuperscript{17}
      c. Cohorts with higher BMIs have higher *Firmicutes*:Bacteroidetes ratio\textsuperscript{24}
d. Firmicutes:Bacteroidetes ratio decreases with weight loss

V. Community composition: microbiome similarity
   a. Outlier subject 35 with high Blautia RA
      i. Blautia positively correlated with BMI\textsuperscript{15,32}
      ii. Blautia positively associated with higher levels of saturated and monosaturated fatty acids and negatively associated with unsaturated and polyunsaturated fatty acids
   b. Future step: examine PCoA using HEI scores

VI. Limitations
   a. Subject self-reporting
      i. Diabetes status: approximately 1/3 of diabetics in the United States are undiagnosed
      ii. Current medications
      iii. Comorbid conditions
   b. Small sample size: not powered to see significant differences in gut microbiome
   c. Potential confounding by BMI, comorbid conditions, and medication use

Conclusions and Future Implications

I. Besides diabetes status, age, and metformin use, the study groups had similar baseline characteristics, including BMI status
   a. All of these factors potentially influenced gut microbial composition

II. Alpha diversity was not different between diabetic and non-diabetic Mexican American subjects

III. Microbial composition was significant different between groups

IV. Future directions for research
   a. Metformin
      i. Investigating role in human health through effects on gut microbiome
      ii. Examining gut microbiota pre- and post-initiation of metformin in diabetes
   b. Studies assessing the role of TCAs in modulating the gut microbiome
   c. Microbiome-targeted therapies
      i. Personalized nutrition\textsuperscript{33}
         1. Individualized dietary plan based on an individual’s distinctive characteristics
         2. Studies have shown gut microbiome independently contributes to post-prandial glucose response (PPGR)
   x. Fecal microbiota transplant (FMT)
      1. Wu et al. transferred fecal samples from T2DM patients before (M0) and 4 months after (M4) initiation of metformin treatment to germ-free mice\textsuperscript{28}
         a. Mice who received M4 fecal transplants demonstrated better glucose tolerance compared to M0 recipients
         b. Glycemic control by metformin partly due to changes in microbiome
      2. Vrieze et al. investigated short-term safety and efficacy of FMT from lean donors to treat metabolic syndrome\textsuperscript{34}
a. Allogenic FMT recipients demonstrated significantly improved insulin sensitivity compared to autologous controls
b. Coincided with significant changes in allogenic FMT subjects
   i. 178 ± 62 species before transplantation compared to 234 ± 40 species after ($P < 0.05$)
   ii. Specific taxa closely related to *Roseburia* sp., indicating possible role in butyrate production

xi. **Metformin**$^{35,36}$
1. Metformin treatment results in microbiome similar to non-diabetic subjects
2. Increase in *Escherichia* and *Intestinibacter* genera
3. Increase in *Bifidobacterium*
4. Increased RA of *A. muciniphila*
5. Directly promotes growth of *Bifidobacterium adolescentis*
References
Appendix 1: Relative abundance by phylum
Appendix 2: Relative abundance by species