Gut Microbiome-Targeted Treatment for Diabetes:
What’s Your Gut Telling You?

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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. Evaluate microbiome-targeted therapies as potential interventions to prevent and treat type 2 diabetes mellitus (T2DM)
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 and function of microbes
   b. Scope
      i. Body’s bacteria would circle the Earth 2.5 times
      ii. Weighs up to 1 to 2 kg
      iii. 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

Table 1. Tools for Analyzing Microbiome

<table>
<thead>
<tr>
<th>Approach</th>
<th>Data</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA gene sequencing</td>
<td>Community composition</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>Metagenomics</td>
<td>Whole genome sequencing</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>Metatranscriptomics</td>
<td>Gene expression</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>Metaproteomics</td>
<td>Protein expression</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Metabolic productivity</td>
<td>Mass spectrometry</td>
</tr>
</tbody>
</table>

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

d. 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, provetellaceae)
         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 functions¹
   a. Mature and train the immune system
   b. Inhibit invasion by pathogens
   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. Associations between gut dysbiosis and human disease²
    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
       x. Alcohol consumption
    b. Disruption of microbial communities associated with a host of chronic and acute diseases

Figure 1. Dominant Bacterial Taxa by Body Site⁴
Table 2: Influence of Gut microbiome Communities on Health

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

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

Gut Dysbiosis and Diabetes Mellitus

I. Overview of diabetes mellitus
   a. Disease prevalence
      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
      iii. Local prevalence
          1. San Antonio
              a. 14.2% of population diagnosed with type 1 diabetes mellitus (T1DM) or T2DM
              b. T2DM in San Antonio prevalence varies by race
                  i. Whites: 8%
pert     ii. Blacks: 12%
pert     iii. Hispanics: 16%
pert 2. Bexar county: prevalence 13%
pert 3. Texas: prevalence 10%
pert 4. United States: prevalence 9%
pert   b. Morbidity and mortality
      i. Absolute number of deaths due to diabetes increased by 93% from 1990 to 2010
      ii. In 2012 estimated annual cost of diabetes $245 billion
II. Pathophysiology: Egregious Eleven

1. Pancreatic β-Cells
   - ↓ β-Cell function
   - ↓ β-Cell mass
   - ↓ Insulin

FINAL COMMON DENOMINATOR

2. ↓ Incretin effect
3. α-Cell defect

HYPERGLYCEMIA

4. Adipose
   - Increased glucose reabsorption

5. Muscle
   - Increased glucose production
   - Decreased peripheral muscle uptake

6. Liver
   - Increased glucose production

7. Brain
   - Increased appetite
   - Decreased morning dopamine surge
   - Increased sympathetic tone

8. Colon/biome
   - Abnormal microbiota; possible decreased GLP-1 secretion

9. Immune dysregulation/inflammation

10. Stomach/small intestine
    - Increased rate of glucose absorption

11. Kidney
    - Increased glucose reabsorption

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

a. Describes pathways that contribute to development of diabetes
b. 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
      1. Glucagon-like peptide-1 (GLP-1) diminished in diabetes
      2. GLP-1 aids in glucose disposal as well as inhibition of HGP
   vi. α-cell: overproduction of glucagon in diabetes patients, contributing to increased basal HGP
   vii. Kidney: increased sodium-glucose cotransporter-2 (SGLT2) threshold
   viii. Brain: delayed satiety in response to increases in insulin
   ix. Stomach/small intestine: increased glucose absorption
   x. Immune dysregulation/inflammation: macrophage and interleukin-1 (IL-1) recruitment to pancreas results in β-cell apoptosis
   xi. Colon/microbiome: influences host metabolism in three main ways that can affect multiple other facets of Egregious Eleven
1. Increased production of lipopolysaccharides (LPS)\textsuperscript{14,15}
   a. LPSs shed from Gram-negative bacterial cell walls (i.e., \textit{E. coli})
      i. Bind to toll-like receptor-4 (TLR4)/CD14 complex
      ii. TLR4 activates innate immune system, resulting in pro-inflammatory response
      iii. Decrease expression of tight junction proteins and increase mucosa integrity
   b. Decreased integrity of intestinal mucosa increases release of LPS into bloodstream\textsuperscript{14}
      i. Higher plasma LPS levels in DM patients than healthy counterparts
2. Decreased short-chain fatty acids (SCFAs) production\textsuperscript{15}
   a. SCFAs (butyrate, acetate, propionate) produced by bacterial fermentation of dietary fiber and resistant starches
      i. Main energy source for gut epithelium (mainly butyrate)
      ii. Bind G-protein coupled receptors (GPCRs) 41 and 43 in intestinal mucosa, immune cells, liver, and adipose tissues
         1. Intestinal mucosa: SCFAs bind to GPCRs on enterohepatic L-cells in colon \(\rightarrow\) increase GLP-1 secretion
         2. Immune cells: inhibit NF-KB activation; decrease TNF-\(\alpha\) and IL-6 suppression and decreased inflammation
3. **Bile acids**\(^{16}\)
   a. Gut bacteria convert primary bile acids to secondary bile acids via bile salt hydrolases
   b. Secondary bile acids act as signaling molecules to induce GLP-1 secretion from small intestine L-cells
   c. Gut microbes implicated in specific mechanisms of dysbiosis
      i. LPS production by Gram-negative bacteria\(^ {14}\)
         1. *E. coli*
         2. *Salmonella*
         3. *Shigella*
         4. *Pseudomonas*
         5. *Neisseria*
         6. *H. influenzae*
         7. *Bordetella pertussis*
         8. *Vibrio cholerae*
      ii. Beneficial SCFA producers:\(^ {17,18}\)
         1. Mainly species in the Firmicutes phyla
            a. *Roseburia sp.*
            b. *Faecalibacterium prausnitzii*
            c. *Eubacterium hallii*
            d. *Eubacterium rectale*
      iii. Microbiota with beneficial bile salt hydrolases\(^ {16}\)
         1. *Lactobacillus*
         2. *Bifidobacterium*
         3. *Firmicutes*
         4. *Enterococcus*
         5. *Clostridium*
         6. *Bacteroides*

III. American Diabetes Association (ADA) acknowledged importance of the relationship between microbiome and diabetes\(^ {9}\)
   a. 2014 ADA and JDRF Research Symposium: Diabetes and the Microbiome
      i. First gathering of experts that focused on the link between the pathophysiology of the microbiome of diabetes
      ii. Symposium made several recommendations to guide future diabetes and microbiome research

The Gut Microbiome in Patients with Diabetes

I. Microbiome studies: associations with metabolic (dys)function
   a. Historically, studies have yielded diverse results\(^ {19-21}\)
   b. Several recent robust studies demonstrated differences between T2DM patients and controls as well as complex relationships between bacterial taxa\(^ {17,18}\)
Discordant findings due to differences in:  
1. Diet
2. Age
3. Birth (Caesarian section versus vaginal delivery)
4. Host genotype
5. Physical activity
6. Smoking
7. Alcohol consumption
8. Medications
9. Geographic location

**Microbiome-Targeted Therapies for the Prevention and Treatment of Diabetes**

I. Personalized nutrition:
   a. Individualized dietary plan based on an individual’s distinctive characteristics
   b. Link between microbiome composition and post-prandial glucose response (PPRG)
      i. Identified microbiome as integral component in formulating a personalized nutrition plan to optimize PPRG

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**Figure 4: Microbiota Trends in Metabolic (Dys)function**

**Figure 5. Illustration of Experimental Design**
c. Study design: three-part study
   i. Part 1: Created PPG prediction algorithm based on profiling of 800 Israeli participants (54% overweight, 22% obese), which included:
      1. Continuous glucose monitoring (CGM)
      2. Real time diary: food, sleep, physical activity
      3. Gut microbiome analysis (16S rRNA and metagenomic analysis)
      4. Blood tests (HbA1c%, lipid levels)
      5. Anthropometrics
      6. Lifestyle, medical history
   ii. Part 2: Algorithm validation in second cohort of Israeli participants
   iii. Part 3: Dietary intervention
      1. 26 new participants randomized to algorithm-produced diet plan (intervention) or dietician-produced diet plan (comparator) after week-long profiling period
         a. Two-week intervention included one week of “good” diet (e.g., foods associated with lower PPGR) and one week of “bad” diet (e.g., food associated with higher PPGR)
         b. PPGR highly variable between individuals
   d. Analyzed partial dependence plots (PDPs) to better understand the role of various factors in the algorithm’s predictions
      i. PDPs illustrate how individual variables contribute to model predictions
      ii. Values greater than 0 indicate positive contribution and values less than 0 indicate negative contribution
      iii. Meal carbohydrate weight most significant contributor to PPGR
      iv. Relative abundance (RA) of multiple bacterial taxa contributed to PPGR as well

Figure 6: Microbiome PDP²³
e. Post-intervention microbiota alterations
   i. Most significant microbiota changes were inter-individual pre- and post-intervention
   ii. Several bacterial taxa were significantly changed due to diet type in all participants

![Figure 7: Heatmap of Taxa Changes in RA between “Good” and “Bad” Diet Weeks](image)

### iii. Increase in *Roseburia inulinivorans* during “good” diet week and decrease during “bad” diet week
   1. Low levels of *Roseburia* genera consistently associated with T2DM
iv. Rapid microbial response to dietary change

Figure 9: Significant Changes in RA of Taxa and CGM During “Good” and “Bad” Diet Weeks$^{23}$

f. Conclusions

   i. Gut microbiome independently contributes to PPGR
   ii. Changes in the gut microbiome in response to diet indicate a role for the microbiome in mediating metabolism and glycemic control
   iii. Further research needed to better characterize role of different microbial taxa to identify a specific microbiome composition to optimize metabolic health

II. Metformin

   g. Mechanism of action

      i. Activates adenosine monophosphate-activated protein kinase (AMPK) in the liver
         1. Reduces hepatic glucose production (HPG)
         2. Insulin sensitivity (IS) improvement
      ii. Reaches high concentrations in intestine when administered orally
      iii. Activates AMPK in intestinal mucosa, aids in maintaining barrier integrity$^{24,25}$
1. Decreased leakage of LPS from gut resulting in improved IS and decreased inflammation

![Figure 10: Metformin Mediation of LPS](image)

h. Impact on gut microbiome
   i. Metformin treatment results in microbiome similar to non-diabetic subjects
   ii. Increase in *Escherichia* and *Intestinibacter* genera
   iii. Increase in *Bifidobacterium*
   iv. Increased RA of *A. muciniphila*
   v. Directly promotes growth of *Bifidobacterium adolescentis*

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>Condition</th>
<th>Treatment</th>
<th>Microbiota</th>
<th>Host phenotype</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-diabetic</td>
<td>No treatment</td>
<td>Healthy</td>
<td>Healthy</td>
<td>No treatment</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetic</td>
<td>No treatment</td>
<td>Dysbiotic</td>
<td>Hyperglycaemia</td>
<td>Metformin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>“Normalised”</td>
<td>Controlled glycaemia</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 11. Gut microbiota in patients with T2DM with and without metformin versus healthy controls](image)
i. Are metformin’s effects on microbiome a result of influence on blood glucose or direct effects on the microbiome?

![Diagram showing the cyclical mechanism of metformin's effects on glycemic control and healthy microbiome.]

**Figure 12. Metformin’s Cyclical Mechanism**

III. Fecal microbiota transplantation (FMT)
   a. Wu et al. transferred fecal samples from T2DM patients before (M0) and 4 months after (M4) initiation of metformin treatment to germ-free mice.26
   i. Mice who received M4 fecal transplants demonstrated better glucose tolerance compared to M0 recipients
      1. Glycemic control by metformin partly due to changes in microbiome
   b. Vrieze et al. investigated short-term safety and efficacy of FMT from lean donors to treat metabolic syndrome.28
      i. Prospective, blinded, randomized controlled study

**Table 3: Study Subjects**

<table>
<thead>
<tr>
<th>Inclusion criteria:</th>
<th>Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian males with treatment naïve metabolic syndrome</td>
<td>Lean Caucasian males (BMI &lt;23 kg/m²)</td>
</tr>
<tr>
<td>Use of any medication, probiotics, and/or antibiotics in previous 3 months</td>
<td>Matched by age to recipients</td>
</tr>
</tbody>
</table>

ii. Randomized to receive allogenic or autologous microbiota infusion

iii. Outcomes
   1. Primary: change in insulin sensitivity with lean donor FMT
   2. Secondary: change in specific small and large gut microbiota and SCFAs
   3. Differences between treatment groups at 6 weeks with p-values corrected for multiple comparisons

iv. Results
   1. Baseline characteristics similar between autologous and allogeneic infusion groups (Appendix 1)
Table 4: Insulin Sensitivity in Recipients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Basal state</th>
<th>Clamp (step 1)</th>
<th>Clamp (step 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allogenic</td>
<td>Autologous</td>
<td>Allogenic</td>
</tr>
<tr>
<td>Baseline</td>
<td>EGP(^a)</td>
<td>10.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Day 60</td>
<td>EGP</td>
<td>9.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Baseline</td>
<td>Rd(^a)</td>
<td>****</td>
<td>11.6</td>
</tr>
<tr>
<td>Day 60</td>
<td>Rd(^a)</td>
<td>****</td>
<td>13.6</td>
</tr>
</tbody>
</table>

EGP: endogenous glucose production, Rd: rate of glucose disposal, \(^a\)units: mcmol/kg/min, *EGP completely suppressed

Table 5: Insulin Sensitivity in Donors

<table>
<thead>
<tr>
<th></th>
<th>Basal state</th>
<th>Clamp (step 1)</th>
<th>Clamp (step 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGP(^a)</td>
<td>13.0</td>
<td>2.2</td>
<td>*</td>
</tr>
<tr>
<td>Rd(^a)</td>
<td>---</td>
<td>22.5</td>
<td>65.0</td>
</tr>
</tbody>
</table>

EGP: endogenous glucose production, Rd: rate of glucose disposal, \(^a\)units: mcmol/kg/min, *EGP completely suppressed

1. No significant changes in gut microbiota of autologous infusion subjects: 184 ± 71 species before transplantation compared to 211 ± 50 species after
2. Significant gut microbiota changes in allogenic infusion subjects
   a. 178 ± 62 species before transplantation compared to 234 ± 40 species after (\(P < 0.05\))
   b. Specific taxa closely related to *Roseburia* sp., indicating a role in butyrate production

Table 6: Specific Bacteria Taxa Changes

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Bacterial taxa</th>
<th>Fold change after/before transplantation</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td><em>Dorea Formicigenerans</em></td>
<td>1.92</td>
<td>0.02</td>
</tr>
<tr>
<td>Firmicutes</td>
<td><em>Clostridium sphenoides</em></td>
<td>1.95</td>
<td>0.02</td>
</tr>
<tr>
<td>Firmicutes</td>
<td><em>Coprobacillus cateniformis</em></td>
<td>1.65</td>
<td>0.02</td>
</tr>
<tr>
<td>Firmicutes</td>
<td><em>Ruminococcus lactaris</em></td>
<td>2.47</td>
<td>0.02</td>
</tr>
<tr>
<td>Firmicutes</td>
<td><em>Clostridium nexile</em></td>
<td>2.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Preoteobacteria</td>
<td><em>Oxalobacter formigenes</em></td>
<td>1.70</td>
<td>0.02</td>
</tr>
</tbody>
</table>

v. Conclusions
1. Results indicate possible role for FMT in prevention and/or treatment of metabolic disorders (e.g., diabetes)
2. Long-term follow-up needed to assess treatment longevity and effects on weight, HbA1c, blood pressure, lipids, and other markers of metabolic health
3. Future studies examining FMT specifically in DM needed
IV. Potential microbiome targeted DM treatments
   a. Probiotics
      i. Living bacteria or fungi that confer a health benefit for the host
      ii. Modes of action: antimicrobial activity, improved intestinal integrity, immunomodulation
      iii. Example: *Lactobacillus plantarum*
   b. Prebiotics
      i. Nondigestible compounds that lead to favorable changes in intestinal microbiota which stimulate growth of selective and beneficial gut bacteria
      ii. Often designed to increase abundance of *lactobacilli* and *bifidobacteria*
      iii. Compounds in development: transgalactooligosaccharides, inulin, oligofructose, xylooligosaccharide
   c. Physical activity
      i. Animal studies show aerobic exercise contributes to improved intestinal integrity, increased microbial diversity, and reduced inflammation
      ii. Only observational human studies

V. Future directions & research gaps
   a. Development of treatment modalities targeting the gut microbiome will depend on further data collection in order to define the optimal microbiome composition
   b. Need for RCTs assessing diet, prebiotics, physical activity, and microbiota replacement therapies for diabetes treatment and prevention
   c. ADA and JDFR recommend further research on the role of the gut microbiome in DM
      i. Need to define the distinction between the microbiomes of metabolically healthy obese individuals and obese individuals who develop diabetes
Research Project: The Microbiome as a Potential Mediator of Diabetes Health Disparities

Research Question
Is Mexican American ethnicity a predictor of gut microbiome composition among patients with diabetes independent of other factors commonly associated with health disparities in this population?

Methods

Design
Prospective study of volunteers from San Antonio, TX from June 1, 2017 to May 31, 2018

Population
Subjects to enroll: 50

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ≥ 18 years of age</td>
<td>• Prior gastrointestinal surgery that has altered the anatomy of the esophagus, stomach, or small/large intestine</td>
</tr>
<tr>
<td>• Self-identify as Mexican American</td>
<td>• Chronic daily use of any medications that could alter gastrointestinal secretory or motor function (e.g., prokinetic agents, narcotic analgesics, laxatives, anticholinergics, anti-diarrheals)</td>
</tr>
<tr>
<td></td>
<td>• Use of antibiotics, gastric-acid suppressing medications, probiotics, within two months of the stool sample collection</td>
</tr>
</tbody>
</table>

Protocol
- Subjects to be divided into two groups: T2DM versus no T2DM
  - T2DM defined as having been diagnosed and currently receiving treatment
- Subject recruitment and pre-screening (See Appendix 1)
- Data collection
  - Survey items: country of birth, country of birth of parents and grandparents, age, sex, socioeconomic status, height and weight, chronic comorbidities, medication use
  - Food diary to be completed over first three study days
  - Stool collection kit to be used on study day four
- Sample processing and sequencing
  - DNA extraction with MoBio powerlyzer kit
  - Amplify 16S rDNA V4 region
  - 16S rRNA sequencing with Illumina MiSeq machine
- Microbiome analysis
  - Process sequences with software
  - Classify sequences into operational taxonomic units (OTUs) using Mothur’s Bayesian Classifier and referenced to Greengenes database

Summary
- Gut microbiome plays a major role in human health, especially with respect to metabolic health
- Have identified multiple mechanisms through which gut microbiome plays a role in diabetes pathophysiology
- Various individual taxa implicated in gut dysbiosis contributing to diabetes, but conflicting study results indicate complex relationship exists between gut microbiota, diet, age, physical activity, genotype, age, and medication usage
- Novel treatment modalities targeting gut microbiota, including personalized dietary algorithm and lean donor FMT, associated with beneficial effects on glycemic control and metabolic syndrome
- Randomized control trials of microbiome-targeted therapies needed as well as further microbiome studies to distinguish healthy from dysbiotic gut microbiomes
Appendix 1: Baseline Characteristics

Supplementary Table 1. Characteristics of Study Subjects at Baseline and After 6 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Allogenic group (N = 9)</th>
<th>Autologic group (N = 9)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Age, y</td>
<td>47 ± 4</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Length, cm</td>
<td>185 ± 2</td>
<td>178 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>123 ± 6</td>
<td>113 ± 7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>35.7 ± 1.5</td>
<td>35.6 ± 1.4</td>
</tr>
<tr>
<td>Body fat mass, %</td>
<td>40 ± 1</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.7 ± 0.2</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>Glycated hemoglobin, mmol/mol</td>
<td>39 ± 1.1</td>
<td>38 ± 1.2</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.5 ± 0.4</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>HDLc</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>LDLc</td>
<td>3.1 ± 0.4</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma free fatty acid, mmol/L</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>138 ± 3</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>85 ± 2</td>
<td>83 ± 5</td>
</tr>
</tbody>
</table>

NOTE. Values are expressed as mean ± standard error of the mean. The body mass index is the weight in kilograms divided by the square of the height in meters. No significant differences in clinical variables were found between baseline and 6 weeks in both treatment groups. HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TG, triglycerides.

Appendix 2. Prescreening Questions for Potential Subjects

<table>
<thead>
<tr>
<th>Question</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you at least 18 years of age?</td>
<td>Exclude</td>
<td></td>
</tr>
<tr>
<td>Do you consider yourself Mexican American?</td>
<td>Exclude</td>
<td></td>
</tr>
<tr>
<td>Do you have a history of major gastrointestinal surgery?</td>
<td>Exclude</td>
<td></td>
</tr>
<tr>
<td>Do you have any use in the past two months of any of the following medications:</td>
<td>Exclude</td>
<td></td>
</tr>
<tr>
<td>Acid reflux medications (e.g., Tums®, Zantac®, Prilosec®, Nexium®, Prevacid®)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics (e.g., Keflex®, Bactrim®, minocycline, amoxicillin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probiotics (except dietary probiotics, like yogurt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-diarrhea medications (e.g., Imodium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laxatives (e.g., Ex-Lax)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-depressants (e.g., Zoloft®, Celexa®, Effexor®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-anxiety medications (e.g., Xanax®, Ativan®, Klonopin®)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narcotic pain medications (e.g., hydrocodone, codeine, morphine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you been diagnosed with Type 2 Diabetes and are you currently using a diabetes medication?</td>
<td>Non-T2D group</td>
<td>T2D group</td>
</tr>
</tbody>
</table>