Intestinal Microbiota and Glycemic Control

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Objectives

• Describe conditions that influence the composition of the intestinal microbiota
• Discuss the differences in the composition of the intestinal microbiota observed in individuals with and without diabetes
• Discuss interventions that impact glycemic control and diabetes through their effects on microbial balance
• Evaluate prebiotics, probiotics, specific nutrients, and dietary plans that impact glycemic control and diabetes through their effects on the intestinal microbiota

Microbiota

• Variable
  – Inter-individual
  – Intra-individual
• Predominantly bacteria
• Increased richness & diversity: better health

• Influenced by:
  – Mode of birth
  – Feeding after birth
  – Environment
  – Diet
• Influences health
Intestinal microbiota

- Metabolism of nutrients
- Synthesis of vitamins
- Conversion of bile acids
- Modulation of immune system
- Defense against opportunistic pathogens
- Regulation of intestinal barrier function
- Xenobiotic metabolism

Predominant bacterial phyla in the human body

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Bacilli; Clostridia</td>
<td>Lactobacillus; Ruminococcus; Clostridium; Staphylococcus; Enterococcus; Faecalibacterium</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidetes</td>
<td>Bacteroides; Prevotella</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria; Betaproteobacteria</td>
<td>Escherichia; Pseudomonas</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Actinobacteria</td>
<td>Bifidobacterium; Streptomyces; Nocardia</td>
</tr>
</tbody>
</table>

GI Microbiota: Type 2 Diabetes Mellitus (T2DM) vs Healthy Individuals
Overview of Differences in GI Bacteria
T2DM vs. Healthy Individuals

<table>
<thead>
<tr>
<th>Phylum/Firmicutes</th>
<th>Intestinal Levels of Bacteria in T2DM vs. Healthy Individuals</th>
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</thead>
<tbody>
<tr>
<td>Bacteroidetes</td>
<td>↓</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>↑</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>↓</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>↓</td>
</tr>
<tr>
<td>Eubacterium rectale</td>
<td>↓</td>
</tr>
<tr>
<td>Roseburia intestinals</td>
<td>↓</td>
</tr>
<tr>
<td>Akkermansia muciniphila</td>
<td>↓</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>↓</td>
</tr>
<tr>
<td>Eggertella lentis</td>
<td>↓</td>
</tr>
<tr>
<td>Clostridum clusters</td>
<td>↓</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>↓</td>
</tr>
<tr>
<td>Vernomicrobium</td>
<td>↓</td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>↑</td>
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</tbody>
</table>

Differences in GI Bacteria, T2DM vs. Healthy:
Phyla Bacteroidetes and Firmicutes

- T2DM: Significant decrease in number of organisms from Phylum Firmicutes
- Elevated number of organisms from Phylum Bacteroidetes
  - Bacteria of this phylum are known to increase after consumption of fatty foods
- Bacteroidetes/Firmicutes (B/F) ratio significantly and positively correlates with reduced glucose tolerance


Differences in GI Bacteria, T2DM vs. Healthy:
Short Chain-Fatty Acid producing bacteria

- T2DM: marked decrease in number of Short-Chain Fatty Acid (SCFA) producing bacteria, including:
  - Eubacterium rectale
  - Roseburia intestinals
  - Akkermansia muciniphila
  - Faecalibacterium prausnitzii
- Short Chain Fatty Acids:
  - Decrease pro-inflammatory cytokine concentration and mucosal inflammation
  - Improve beta cell function
  - Stimulate GLP-1 release

Differences in GI Bacteria, T2DM vs. Healthy:
SCFA producing bacteria (*Akkermansia muciniphila*)

- T2DM: *Akkermansia muciniphila*, is decreased in GIT\(^1\)
- *A. muciniphila* functions in\(^1,2,3\):
  - Maintaining integrity of the colon epithelia
  - Stimulating production of anti-inflammatory lipids
  - Regulating production of peptides that are involved in glucose regulation (GLP-1 and GIP)


Differences in GI Bacteria, T2DM vs. Healthy:
Pathogenic Bacteria

- T2DM: increased presence of pathogenic and opportunistic bacteria\(^1\)
  - *Clostridium* clusters
  - *Eggerthella lenta*
    - Gram positive organism associated with abdominal sepsis
  - *Escherichia coli*


Differences in GI Bacteria, T2DM vs. Healthy:
Sulfate Reducing Bacteria

- T2DM: increased presence of sulfate reducing bacteria, namely, *Desulfovibrio*\(^1,2\)
  - These bacteria form Hydrogen Sulfide (H\(_2\)S)
    - Cytotoxic to GI mucosal cells
    - Decrease colonic secretions
    - Decrease ulcer wound healing
    - Decrease release of GLP-1

T2DM: An Overview

- T2DM: characterized by elevated plasma glucose, deficit in secretion/action of insulin and incretins, and low level inflammation\(^1,^2\)
  - Moderate excess in systemic cytokine levels\(^1\)
  - Cytokines activate Toll-like Receptors on peripheral tissues
    - Results in phosphorylation of proteins regulating normal signaling through the insulin receptor\(^1\)
    - Leads to peripheral insulin resistance


T2DM: An Overview...continued

- Key molecule involved in triggering inflammation → Lipopolysaccharide (LPS)\(^1\)
- Phylum Bacteroidetes: increased in those with T2DM
- LPS from these Gram negative organisms is shed
  - LPS translocates across the intestinal mucosa and triggers release of pro-inflammatory cytokines
  - Innate inflammatory process is triggered
    - Leads to the beta cell damage and insulin resistance


How does dysbiosis inside the intestine affect health/disease pathogenesis? A possible mechanism

- T2DM: increased intestinal permeability
  - Impairment of tight junctions\(^3\)
  - Results in translocation of bacterial endotoxin (LPS) paracellularly\(^4\)
    - Mechanism producing low grade inflammation characteristic of T2DM\(^1\)
- Modulating gut microbiota composition with prebiotics:\(^1\)
  - Improved gut permeability
  - Decreased inflammation
  - Improved glucose tolerance

Drugs &
the microbiota

Alterations in
intestinal microbiota:
How medications used to treat T2DM affect the microbiota

Metformin

Known MOA1

Activates AMPK
↓ glucose production in the liver
↑ insulin sensitivity
Enhances glucose utilization

Mouse studies:
There may be more
Metformin modulates the gut microbiota towards a more beneficial state

**Akkermansia spp. population in lean vs obese mice**

Lean Mouse  
Obese/T2DM Mouse

**VS**

Greater abundance of *Akkermansia spp.*  
Decreased abundance of *Akkermansia spp.*

Presence of *Akkermansia spp.* inversely correlates with body weight in rodents & humans.

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**Effect of Metformin on the gut microbiota of HFD-fed diabetic mice**

Metformin

High Fat Diet (HFD)-fed, diabetic mouse  
↑ abundance of *Akkermansia spp.*

Correlated with improved metabolic parameters:  
- Amelioration of glucose intolerance
- Reversed metabolic endotoxemia & insulin resistance

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**Significance:**

These findings suggest that:

- Aside from its well-know MOA, metformin may also be achieving its efficacy via its modulation of the gut microbiota towards a more beneficial state.
- Pharmacologic manipulation of the gut microbiota in favor of *Akkermansia spp.* may be a potential treatment for T2DM.
- *Akkermansia spp.* has a potential role as a probiotic with antidiabetic effects.

*These were findings from mouse studies; still need human studies.*
Effect of Liraglutide on the microbiota of hyperglycemic mice

Liraglutide

Hyperglycemic mouse

Changed overall structure of mouse’s microbiota by leading to:

↓ obesity-related phylotypes
AND
↑ lean-related phylotypes

May be reason behind Liraglutide’s weight loss effect


Effect of Saxagliptin on the microbiota of hyperglycemic mice

In T2DM:

Saxagliptin

administration to hyperglycemic mice

↑ F to B ratio
(possibly correcting some of the dysbiosis characteristic of T2DM)

Decreased F to B ratio
(Dysbiosis)

↑ Bacteroides
↓ Firmicutes


Effect of Sitagliptin on the microbiota of obese & T2DM rats

Sitagliptin

Obese/T2DM Rat

↑ Roseburia
(SCFA-producing bacteria)

↑ Bifidobacterium

Improved insulin sensitivity

Improved glucose tolerance & low-grade inflammation

Alterations in intestinal microbiota:

How medications that affect the gut microbiota may impact the risk of T2DM/Obesity

Effect of antibiotics on the gut microbiota: overview

- Marked short-term disturbances in the human gut microbiota
- Significantly reduce bacterial diversity in the gut
- Linked with development of obesity & T2DM
- Use in children associated with obesity later in life
- Often incomplete recovery of gut flora to its initial composition
- Broad > Narrow-spectrum

Intestinal bacterial diversity in obese vs lean individuals

Obese

Low intestinal bacterial diversity

Lean

Greater intestinal bacterial diversity

Intestinal bacterial diversity in obese vs lean individuals

- More marked overall adiposity, insulin resistance & dyslipidemia
- Low gut bacterial richness
- Risk factor for progression to adiposity-associated co-morbidities: obesity & T2DM

Low vs high bacterial richness:
- More pronounced inflammatory phenotype

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Broad-spectrum antibiotic use & its connection to obesity

- ↑ use of broad-spectrum antibiotics may be contributing to obesity trends
- Ceftriaxone (broad-spectrum Abx)
- Amoxicillin (narrow-spectrum Abx)
- Significantly ↓ bacterial diversity in the gut
- Strong correlation with obesity rate
- Weak correlation with obesity rate

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Antibiotic use in childhood & obesity later in life

- Various observational studies:
- Abx use in children associated with obesity later in life
- Exposure to broad-spectrum abx at ages 0-23 months is associated with early childhood obesity; not with narrow
- Early-life abx use was associated with a 20% increased risk of being overweight at age 7 years
- Abx exposure during the first 12 months was associated with ↑ed BMI in boys aged 5–8 years

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Conclusion

- Overall, these studies highlight the importance of implementing more restricted use of antimicrobial agents.
- Antibiotic use may damage the gut flora and lead to dysbiosis that may contribute to the development of obesity & T2DM.
- Implementation of appropriate antibiotic stewardship programs is critical, and ensures that:
  - Broad-spectrum antibiotics are only used when absolutely necessary.
  - Regimens are promptly de-escalated when cultures and sensitivities (C&S) are available.
  - Duration of therapy with broad-spectrum agents is limited.

Food & the microbiota

Different Diets & the Microbiota

<table>
<thead>
<tr>
<th>Diet</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strict Vegetarian Diet¹</td>
<td>• Improved impaired glucose intolerance</td>
</tr>
<tr>
<td></td>
<td>• Corrected Firmicutes-to-Bacteroides ratio</td>
</tr>
<tr>
<td></td>
<td>• ↓ abundance of Enterobacteriaceae</td>
</tr>
<tr>
<td>Ma-Pi 2 Macrobiotic Diet²</td>
<td>• High fiber diet</td>
</tr>
<tr>
<td></td>
<td>• Fermentation stimulates bacterial growth</td>
</tr>
<tr>
<td></td>
<td>• Bacterial growth releases SCFAs = protective</td>
</tr>
<tr>
<td></td>
<td>to gut</td>
</tr>
<tr>
<td>WTP Diet (Whole Grain, Traditional Chinese Medicinal Foods, Probiotics)³</td>
<td>• Improvements in gut composition</td>
</tr>
<tr>
<td></td>
<td>• ↑ in Actinobacteria, ↓ in Proteobacteria</td>
</tr>
<tr>
<td></td>
<td>• ↑ in Bifidobacteriaceae, ↓ in Enterobacteriaceae</td>
</tr>
</tbody>
</table>

Strict Vegetarian Diet

6 obese subjects
TZDM +/- HN

SVD 1 month

△ A1c
△ Improved FBG & postprandial levels
△ Body weight
△ TG, TC
△ LDL
△ Corrected Firmicutes-to-Bacteroidetes ratio
△ abundance of Enterobacteriaceae

Food supplements & the microbiota

<table>
<thead>
<tr>
<th>Food Supplement</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial Sweeteners&lt;sup&gt;4&lt;/sup&gt;</td>
<td>• Over-representation of Bacteroides genus, under-representation of Clostridiales • Induced glucose intolerance by functionally altering the gut microbiota</td>
</tr>
<tr>
<td>Dietary Calcium&lt;sup&gt;5&lt;/sup&gt;</td>
<td>• Reduced colonic permeability and strengthened integrity of colonic epithelium</td>
</tr>
<tr>
<td>Chromium Malate&lt;sup&gt;6&lt;/sup&gt;</td>
<td>• Found in foods like Brewer’s yeast, meats, potatoes (skin), cheeses, molasses, spices, whole grain breads, fruits and vegetables • Involved in glucose transport and uptake • △ FBG &amp; insulin resistance • △ gram-positive bacteria</td>
</tr>
</tbody>
</table>

Artificial sweeteners

Control

Water + Glucose

BSA

Normal Microbiota

Normal BG levels

Altered Microbiota

Glucose Intolerance

NAD Mice

No differences in glucose homeostasis

Sachet

Glucose Intolerance

NAD Responsive Altered Microbiota

Glucose Intolerance

NAD Non-Responsive Altered Microbiota

Normal BG levels

NAD Normal Microbiota

Normal BG levels
**Conclusions**

- Diets that are low in calorie and high in fiber are most influential in improving glucose intolerance
- Try to avoid consistently using artificial sweeteners, especially saccharin (Sweet’N Low)
- Calcium, chromium malate, bitter melon, and dietary polyphenols can benefit an individual with T2DM
Good Sources of Probiotics & Prebiotics

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Prebiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt</td>
<td>Garlic</td>
</tr>
<tr>
<td>Kefir</td>
<td>Leeks</td>
</tr>
<tr>
<td>Cheese</td>
<td>Onions</td>
</tr>
<tr>
<td>Kimchi</td>
<td>Whole wheat</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>Legumes</td>
</tr>
<tr>
<td>Kombucha</td>
<td>Chicory root</td>
</tr>
<tr>
<td>Supplements: Culturelle, VSL#3</td>
<td>Green bananas</td>
</tr>
</tbody>
</table>

Effects of Prebiotics

*Live microorganisms* (bacteria or yeast) that, when administered in adequate amounts, confers a *health benefit* on the host.¹

*Selectively fermented ingredients* that allow specific changes, both in the composition and/or activity of the gastrointestinal microbiota that confer *benefits* upon host well-being and health.²

### Effects of Probiotics

#### VSL #3
- 8 beneficial lactic acid bacteria¹
  - *B. longum*, *B. infantis*, *B. breve*, *L. acidophilus*, *L. casei*, *L. delbrueckii* ssp. *bulgaricus*, *L. plantarum* and *Streptococcus salivarius* ssp. *thermophilus*
- ↓ inflammatory mediators: TNF-alpha, IL-6, and hs-CRP¹
  - Compromise intestinal integrity
  - Complicate metabolic diseases
- Prevented ↑ in serum insulin concentration and rise in insulin resistance²

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<table>
<thead>
<tr>
<th>Prebiotics</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Performance Insulin¹</td>
<td>• ↑ concentration of bifidobacteria and lactobacilli</td>
</tr>
<tr>
<td></td>
<td>• ↓ body weight, BMI, fasting blood glucose, and HbA1c</td>
</tr>
<tr>
<td></td>
<td>• ↓ multiple systemic inflammation markers: interleukin 6 (IL-6), Tumor Necrosis Factor – alpha (TNF-alpha), and lipopolysaccharides (LPS)</td>
</tr>
<tr>
<td>Psyllium Fiber²</td>
<td>• ↓ fasting blood glucose and HbA1c when ingested before meals</td>
</tr>
<tr>
<td>High-amylose maize resistant starch type 2³,⁴</td>
<td>• ↓ area under the curve for glucose</td>
</tr>
<tr>
<td></td>
<td>• assist with blood glucose homeostasis</td>
</tr>
</tbody>
</table>

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⁴ Maziarz MP et al. Nutrition Journal. 2017;16(1)
⁵ Jafarnejad S et al. Journal of Nutrition and Metabolism. 2016;1-8
Fecal microbiota transplantation (FMT)

- Transfer of intestinal microbiota
- From healthy donor to patient
- To introduce or restore a stable microbial community in the gut
- Manipulation of altered intestinal microbiota
- Reinstall depleted bacterial species associated with disease

FMT: potential for increasing insulin sensitivity

- Double blind, randomized controlled trial
- 18 M, metabolic syndrome
- Half received fecal microbiota infusion from lean male donors (allogenic group), vs self-collected feces (control)
- After 6-wk infusion of microbiota from lean donors, marked increase:
  - Insulin sensitivity
  - Levels of butyrate-producing intestinal microbiota
- No significant changes found in control group
FMT: potential for increasing insulin sensitivity

- 38 M, metabolic syndrome, BMI ≥ 30
- Fecal microbiota infusion from lean M donors, BMI < 25 (allogenic), vs own (autologous = control)
- 18 wks after FMT: microbiota similar to baseline; no significant effects on insulin sensitivity; no wt change
- 6 wks after FMT: responders vs non-responders
  - Altered microbiota composition
  - Peripheral & hepatic insulin sensitivity increased
  - Significant increase in Akkermansia muciniphila
  - Metabolic response dependent on decreased fecal microbial diversity at baseline


Thank You!

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