The Microbiome
What is the Microbiome- A Big Picture

● Definition:
  ○ The collection of genomes of the microbes (bacteria, bacteriophages, fungi, protozoa, and viruses) that live inside a human body
Are we Human? Or are we Microbe?

- **10% Human?**
  - 10 trillion human cells
  - 100 trillion bacterial cells

- **1% Human?**
  - 20,000 human genes
  - 2 million to 20 million genes

Let’s Zoom In!

- Planktonic Bacteria really only occur during infections
- The public thinks bacteria live all alone in this world, but in reality they live in communities

Planktonic Bacteria!
Biofilms

In reality, bacteria live in complex communities, allowing them to interact with each other and various co-inhabiting species.
We can think of a biofilm as an army.
Each species has a specific role.
If one is missing, the whole system notices.

The Army Inside Us

**TO PROTECT AND SERVE!**
The Bacteria Community

Sophora flavescens

E. coli

Mycobacterium vaccae

Candida albicans.

F. prausnitzii
So What’s the Microbiome?

What does it mean to be a Microbiome?

Let’s Zoom Out!
Levels of Ecology

- Organism
- Population
- Community
- Ecosystem
- Biome
Biomes around the world vs the Biomes in us

Where we find most of the micro-diversity in our body
Where do Your Microbes Come From?

- Early Life
  - Vaginal fluids
  - Skin
  - Playing in the dirt

- Life
  - Foods!
    - Fresh vegetables
    - Fermented foods
BBB and Stress, Why stress?

- The Blood Brain Barrier becomes more permeable with stress
  - The severity of the stress and the duration of the stress cause varying levels of permeability of the BBB
  - “Entry of several restricted elements from the blood to the brain compartment after breakdown of the BBB results in immunological, biochemical and pathological reaction causing brain edema formation and cell injury.”

- We can use the permeability BBB and stress to be analogous to the our gut and its response to various stresses
  - Leaky Gut- When the mucosal lining becomes compromised and contents from our intestine, including bacteria, leak into other parts of the body
What Happens When Things Go Wrong?

- The Side Effects
  - Obesity
  - IBD (Inflammatory Bowel Disease)
  - Insulin Resistance
  - Autism???

- Many of the neurological disorders are now being linked to gut microbiota!!

- What are the Causes?
  - Inflammation is a huge player
Inflammation- the ROS Pathway

● What am I?
  ○ Reactive Oxygen Species
    ■ Hydrogen Peroxide (H2O2)
    ■ Hydroxyl Radical (OH-)
    ■ Superoxide Anion (O2-)

● How am I formed?
  ○ Unavoidable byproduct of cellular respiration (making energy)
  ○ The byproducts of some enzyme reactions

● What do I do?
  ○ Reaction with any organic molecule to stabilize
  ○ Modify or change the shape of proteins- now they are no longer useful!
  ○ React with lipids, dissolving them, AND creating another ROS in the process
Stressors

- Aging/Senescence
- Wounding
- Xenobiotics
- Radiation/Light
- Heat & Cold
- Pathogens
- Biotoxins
- Drought
- Heavy Metals
- Air Pollutants
  \( (O_3;SO_2) \)
- Hormones

Oxidative STRESS

Molecular Damage

- Lipids & Fatty Acids
- Amino Acids
- Proteins
- Nucleic Acids
- Pigments

Cellular Effects

- Membrane Damage
- Loss of Organelle Functions
- Reduction in Metabolic Efficiency
- Reduced Carbon Fixation
- Electrolyte Leakage
- Chromatid Breaks
- Mutations

Cell DEATH
Inflammation- Oxidative Stress

● What am I?
  ○ This deadly reaction starts with free radicals (ex. O3)
  ○ Oxidative Stress is when our bodies are unable to stop the chain reaction of molecules stealing electrons from one another
  ○ The damage left behind by this chain reaction and cause stress on the body causing inflammatory disease, cancer, etc

● How am I stopped?
  ○ Antioxidants!
    ■ They stop the chain reactions of the free radicals
Inflammation- Cytokines

- **Who am I?**
  - Proteins, peptides, and glycoproteins

- **What do I do?**
  - Regulate the inflammatory response

### Table 3. Inflammatory biomarkers, LBP, and gut permeability of the obese subjects at baseline, 9, and 23 weeks after the intervention

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Baseline (-30 day)</th>
<th>Phase I (9 week)</th>
<th>Phase II (23 week)</th>
<th>Medical reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg L⁻¹, n = 67)</td>
<td>6.60 (5.10–8.20)</td>
<td>4.90** (4.20–6.20)</td>
<td>5.93†† (4.69–6.85)</td>
<td>0–10</td>
</tr>
<tr>
<td>LBP (µg mL⁻¹)</td>
<td>23.21 (15.54–35.50)</td>
<td>19.98** (14.15–30.83)</td>
<td>23.08†† (11.51–36.99)</td>
<td>–</td>
</tr>
<tr>
<td>IL-1β (pg mL⁻¹)</td>
<td>0.07 (0.03–0.12)</td>
<td>0.07 (0.05–0.15)</td>
<td>0.06 (0.04–0.12)</td>
<td>–</td>
</tr>
<tr>
<td>IL-6 (pg mL⁻¹)</td>
<td>2.28 (1.79–3.12)</td>
<td>2.02* (1.62–2.62)</td>
<td>1.68†† (1.27–2.46)</td>
<td>–</td>
</tr>
<tr>
<td>TNF-α (pg mL⁻¹)</td>
<td>1.07 (0.87–1.49)</td>
<td>1.03 (0.81–1.40)</td>
<td>1.04* (0.82–1.50)</td>
<td>–</td>
</tr>
<tr>
<td>Adiponectin (µg mL⁻¹)</td>
<td>3.57 (2.56–5.22)</td>
<td>3.82** (2.90–5.90)</td>
<td>4.23†† (3.06–6.17)</td>
<td>–</td>
</tr>
<tr>
<td>L/M ratio (n = 76)</td>
<td>0.026 (0.020–0.031)</td>
<td>0.022** (0.019–0.026)</td>
<td>0.023* (0.019–0.026)</td>
<td>–</td>
</tr>
</tbody>
</table>

LBP, lipopolysaccharide-binding protein; IL, interleukin; TNF-α, tumor necrosis factor-α; L/M ratio, lactulose/mannitol ratio. Results were expressed as median (interquartile range).

Significantly different from baseline, *P < 0.05, **P < 0.01; Significantly different from Phase I, †P < 0.05, ††P < 0.01 (two-tailed test).
What Happens in a World without Germs?

- Now we are coming to accept that certain bacteria are highly beneficial for proper development and health.
- Until very recently we associated bacteria with negative connotations.
- Evolution has selected these for this microbe-host relationship. So by creating germ free environments, we cause imbalances in our microbiome, which can have deleterious effects.
Germ Free Mice

- Gnotobiotic mice have only been around since 2008! Not even a decade!
- Mice Health problems
  - Gut organs were underdeveloped
  - Immune system expression is reduced
- Conclusions
  - Gut Microbes have a role in the organ development of our gut and are needed to kick start our immune system

### Table: Intestinal organ development

<table>
<thead>
<tr>
<th>Small Intestine</th>
<th>Site</th>
<th>Phenotype in Germfree mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peyers Patches</td>
<td>fewer, less cellular</td>
</tr>
<tr>
<td></td>
<td>Lamina propria</td>
<td>thinner, less cellular</td>
</tr>
<tr>
<td></td>
<td>Germinal centers</td>
<td>fewer plasma cells</td>
</tr>
<tr>
<td></td>
<td>Isolated lymphoid follicles</td>
<td>smaller, less cellular</td>
</tr>
</tbody>
</table>

| Mesenteric Lymph nodes | Germinal centers | fewer plasma cells |

### Table: Cellular Defects

<table>
<thead>
<tr>
<th>Intestinal epithelial lymphocytes</th>
<th>CD8+ T cells</th>
<th>fewer, reduced cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamina propria lymphocytes</td>
<td>CD4+ T cells</td>
<td>proportional decrease in number</td>
</tr>
<tr>
<td></td>
<td></td>
<td>decreased Th17 cells (Small intestine)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>increased Th17 cells (Colon)</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>CD4+CD25+ T cells</td>
<td>reduced expression of Foxp3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reduced suppressive capacity</td>
</tr>
</tbody>
</table>

### Table: Molecular immune deficiencies

<table>
<thead>
<tr>
<th>Paneth Cells</th>
<th>Molecule</th>
<th>Phenotype in Germfree mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Angiogenin-4</td>
<td>reduced expression</td>
</tr>
<tr>
<td></td>
<td>RegIIIy</td>
<td>reduced expression</td>
</tr>
<tr>
<td>B cells</td>
<td>Secretory IgA</td>
<td>reduced production</td>
</tr>
<tr>
<td>Intestine</td>
<td>ATP</td>
<td>reduced</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestinal epithelial cells</th>
<th>Molecule</th>
<th>Phenotype in Germfree mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHC class II</td>
<td>reduced expression</td>
</tr>
<tr>
<td></td>
<td>TLR 9</td>
<td>reduced expression</td>
</tr>
<tr>
<td></td>
<td>IL-25</td>
<td>elevated</td>
</tr>
</tbody>
</table>
Bacteria from Humans to Germ Free Mice

- Bacteria from the normal weight person
- Bacteria from an obese person

Germ Free mice
We Are Trying To Create A Germ Free World

● How?
  ○ Voluntary C-section births
  ○ Overuse of Antibacterial soaps and gels
  ○ Over prescribing antibiotics
    ■ Wipes out healthy gut flora and creates resistant strains in natural systems (ie. resistance plasmids/mobilomes in sewers, lakes and soil)

● Results
  ○ Higher risks of
    ■ IBD (Inflammatory Bowel Disease)
    ■ Obesity
    ■ Autism?
    ■ Insulin Resistance
    ■ Weaker Immune Systems
    ■ Asthma
What does your microbiome do?

- Helps digest food
- Synthesize Vitamins
- Metabolize drugs
- Detoxify carcinogens
- Stimulates the renewal of the intestinal lining
- Activate and support the immune system
- Influence hormone levels
The Brain Element: Enteric Nervous System (ENS)

- 500 million nerves embedded in the wall of the intestines
- 90% of the information goes FROM the gut TO the brain, not vice versa
- Form of Communication?
  - Vagus Nerve
- What are they saying?
  - 90% of the serotonin in our bodies is produced in our GUT
How do the Gut and the Brain Talk to One Another?

- **Proposed Mechanisms of Action.**
  - “There are a variety of proposed mechanisms, including both humoral and neural routes, through which the microbiota can modulate signaling along the gut-brain axis.
  - For example, recent studies suggest a role for both the vagus nerve and modulation of systemic tryptophan levels in relaying the influence of both resident and exogenous microflora along this bidirectional communication axis.”
Microbes in the gastrointestinal tract are under selective pressure to manipulate host eating behavior to increase their fitness, sometimes at the expense of host fitness. Microbes may do this through two potential strategies: (i) generating cravings for foods that they specialize on or foods that suppress their competitors, or (ii) inducing dysphoria until we eat foods that enhance their fitness. We review several potential mechanisms for microbial control over eating behavior including microbial influence on reward and satiety pathways, production of toxins that alter mood, changes to receptors including taste receptors, and hijacking of the vagus nerve, the neural axis between the gut and the brain. We also review the evidence for alternative explanations for cravings and unhealthy eating behavior. Because microbiota are easily manipulatable by prebiotics, probiotics, antibiotics, fecal transplants, and dietary changes, altering our microbiota offers a tractable approach to otherwise intractable problems of obesity and unhealthy eating.
Bacteria can rule our minds - like a puppetmaster:

“Like microscopic puppetmasters, microbes may control the eating behavior of hosts through a number of potential mechanisms including microbial manipulation of reward pathways, production of toxins that alter mood (shown in pink, diffusing from a microbe), changes to receptors including taste receptors, and hijacking of neurotransmission via the vagus nerve (gray), which is the main neural axis between the gut and the brain.”

Nutrient deprivation is not sufficient to explain unhealthy eating

A similar hypothesis proposes that cravings result from nutrient shortage [84]. For instance, fruit flies seek out specific nutrients after deprivation [107]. However, this hypothesis does not explain many findings regarding cravings in humans. Food cravings strike even in times of plenty [108, 109], and often foods that would satisfy a supposed nutrient shortage are not the ones that are craved [110]. Furthermore, fasting reduces cravings [111–113] rather than increasing them, as would be expected from the nutrient shortage hypothesis. The same pattern holds for cravings of non-food items such as clay and earth [114]. Young and colleagues subjected geophagy (earth-eating) to a systematic review and concluded that human geophagy is not driven by nutrient scarcity [114].
Dr. Ian Carroll

“We’re not able to say a gut bacterial imbalance causes the symptoms of anorexia nervosa, including associated symptoms, such as anxiety and depression. But the severe limitation of nutritional intake at the center of anorexia nervosa could change the composition of the gut microbial community. These changes could contribute to the anxiety, depression, and further weight loss of people with the disorder. It’s a vicious cycle, and we want to see if we can help patients avoid or reverse that phenomenon. We want to know if altering their gut microbiota could help them with weight maintenance and mood stabilization over time.”

https://www.med.unc.edu/gi/news/
Gut microbiome and anorexia nervosa (AN)

Disease: dietary and mental components

The study:
Comparison between microbiomes of patients before and after treatment for AN.

Results:
Patients’ microbiomes lacked microbiome diversity compared with control population.

Before treatment Clostridia was absent in AN patients; after treatment Clostridia population rebounded.

Researchers found an inverse relationship between Ruminococcaceae bacterial family and negative mental health.

Your Microbiome and PTSD

- Mice were exposed to “social defeats”
- They had elevated levels of different inflammation
  - High levels of cytokines and bacterial components including LPS and peptidoglycan
- Stress can lead to a “leaky gut”
The Future of Microbes

- We will make better our efforts to feed our microbes
  - We want to increase the microdiversity in our bodies
- Make beers/cheeses/wines with certain microbes to cure certain diseases?