Objectives

1. Identify examples of how diet, lifestyle, and the environment influence the human microbiome.
2. Discuss the relationship between the microbiota and disease.
3. Identify how certain medications, such as proton pump inhibitors and antibiotics, impact oral and gut microbiota.
4. Describe the role of diet, dietary fiber, prebiotics and probiotics in optimizing the microbiota.
Definitions

- **Microbiome**—collective genomes of microorganisms in particular environment
- **Microbiota**—community of microorganisms themselves.
- Lower diversity is marker of *dysbiosis* (microbial imbalance) and is associated with autoimmune disease, obesity, and metabolic conditions.

---

Birth

- Babies born *vaginally* covered in microbial film as they pass through birth canal.
- Babies born by *C-section* are colonized by *skin microbes*—very different species.
- Babies acquire microbes from everyone and everything they touch.
- Where the baby is born, what type of delivery, if breastfed or bottle fed — all these impact the microbiome for months or years after birth.
Breast milk contains numerous genera of microbes, and prebiotic human milk oligosaccharides, which support growth of Bifidobacterium spp., important for inhibiting pathogenic organisms, modulating mucosal barrier function, and promoting immunological and inflammatory responses.

**Neonatal Microbiome**

- Greatest insults to the natural assembly of neonatal microbiome: C-section delivery, antibiotic use, and formula feeding.
- Differences in specific microbial species observed between C-section and vaginally delivered babies up to 7 years after birth.
- Intrapartum antibiotic use associated with lower abundance of Lactobacilli and Bifidobacteria in neonatal gut.
- Formula feeding has been associated with increased prevalence of C. difficile, Bacteroides fragilis, and E. coli and decreased prevalence of Bifidobacteria.

**Probiotics and Birth Mode**

- Mothers given probiotic, consisting of Bifidobacterium breve (2 × 10^8 cfu) Propionibacterium freudenreichii subsp. shermanii JS (2 × 10^9 cfu), Lactobacillus rhamnosus Lc705 (5 × 10^9 cfu) and L. rhamnosus GG (5 × 10^9 cfu).
- Probiotic group (N = 168 breastfed and 31 formula-fed), or placebo supplement (N = 201 breastfed and 22 formula-fed) during pregnancy, infants received same.
- In probiotic group, effects of antibiotics and birth mode were either completely eliminated or reduced.

Birth to 3 Years

• Within weeks, microbial specialization occurs. Different populations in mouth, gut, skin, etc.
• Microbial populations in infants similar to people they live with. Microbiota dramatically altered by new foods, antibiotics, proton-pump inhibitor use, etc. These shifts can last many, many years.
• Number and types of species increase and change with age. Example: babies have more folate-producing microbes – adults have more folate-harvesting microbes.

Age 3 to Old Age

• Microbiome becomes stable. Even with disruptions (medications, disease, dietary changes) – usually returns to baseline.
• Large shifts occur with onset of puberty (skin changes), pregnancy (vaginal microbiome), menopause, etc.
• After age 65, microbe populations decrease and species become more similar.
• Climate, geography, diet, hygiene, medication use, etc. all impact microbiome.

Microbiota

• Train and modulate immune system (e.g., skin, gut)
• Convert skin oils to compounds that keep skin supple and lower pH
• Block adhesion and suppress growth of pathogenic bacteria
• Break down carbs and make butyrate, energy for intestinal cells but also crucial for maintaining tight junctions to reduce permeability.
• Make ARA and DHA, signal brain cells to divide (infants). Gut and brain neurons communicate. Gut microbes make serotonin, melatonin, GABA, and others.
• Produce vitamins and assist in building amino acids.
• Help maintain blood pressure (complex carbs formate impact salt processing).
• Many dietary, lifestyle and medications can dramatically impact the microbiome and ultimately impact human health.


Oral Microbiome
• Extensively studied as part of the Human Microbiome Project.
• Core microbiome similar for all individuals and comprised of predominant species at different sites of healthy body.
• Variable microbiome is different between individuals in response to unique lifestyles and phenotypic and genotypic determinants.


Oral Microbiota Among Most Diverse
• 700 microbial species: bacteria, fungi, viruses, archaea and protozoa form complex ecological community. Oral microbiota generally exist as biofilm.
• Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria most significant for oral health.
• Despite different etiologies, periodontitis and caries driven by feedforward loop between microbiota and host (inflammation and dietary sugars, respectively) that favors emergence and persistence of dysbiosis.
• Disturbance in oral microbiota may impact diabetes, CVD and certain cancers.

Oral Microbiota and Blood Pressure

- Upon interaction with oral bacteria, nitrate is reduced to nitrite, swallowed and then absorbed, increasing plasma nitrite levels.
- Endogenous nitrite reductases in circulation reduce plasma nitrite further to bioactive NO, which then acts as vasodilator.


Mouthwash, Tongue Cleaning and BP

- In healthy volunteers, chlorhexidine increased systolic BP ~ 5 mm/Hg, equivalent to manipulation of dietary salt intake
- Those who cleaned tongue twice daily, had greatest increase in systolic BP after using chlorhexidine.

Pregnancy

- Early stages of pregnancy, total number of microbes increase significantly.
- *P. gingivalis, A. actinomyctemcomitans* in gingival sulcus significantly higher than that in non-pregnant women.
- During late pregnancy, *Candida* is more frequently detected.


Periodontitis and Preterm Birth

- Pre-term birth (PB): delivery taking place before 259 days gestation.
- PB accounts for 75-80% perinatal mortality and for most neurological and respiratory complications in neonates.
- Periodontitis associated with PB, low birth weight, pre-eclampsia.
- *P. gingivalis* associated with shorter gestations and C-section delivery.
- Periodontal treatment associated with fewer PB.


Microbes: Energy and Inflammation

- Microbiota can increase energy production from diet and take part in the regulation of fatty acid tissue composition.
- Increase in *Firmicutes* in relation to *Bacteroidetes*, increases absorption of calories from food, supplying larger amounts of fat to host with concomitant increase in both weight and fat mass.
- Dysbiosis seen with antibiotic use, especially during first 3 years of life.
- LPS-containing *Firmicutes* significantly increase plasma LPS; activating TLR4 and upregulating expression of pro-inflammatory cytokines.


Fessler MB, et al. *Curr Opin Lipidol* 2009; DOI: 10.1097/MOL.0b013e32832fa5c4
Child Weight Gain Trajectories Linked To Oral Microbiota Composition

- Gut and oral microbiota of 226 two-year-olds analyzed with gene sequencing.
- Weight and length measured at 7 time points to identify children with rapid weight gain (strong risk factor for childhood obesity).
- Rapid weight gain associated with less diversity and higher ratio of Firmicutes-to-Bacteroidetes in oral microbiota.


Antibiotics and Obesity

- American children up to 2 years of age, on average receive 3 full doses of antibiotics: up to 10 years of age received 10 full doses, and 17 full doses antibiotic by age 20.
- Four or more courses of antibiotics given between ages 2 to 3 years independently associated with obesity at age 8. (OR: 1.6).


Antibiotics and Microbes

- Disrupt existing microbiota; linked to antibiotic-associated diarrhea, pseudomembranous colitis, and increased susceptibility to subsequent disease.
- Extent of change depends on antibiotic type, duration and dose.
- Azithromycin, amoxicillin, clindamycin, and ciprofloxacin decrease oral microbiota diversity.


Source: L Segal & Tim Blaser, Ann Am Thor Sci 2014

Observational data
Antibiotic Prophylaxis

- UIC study: 80% of antibiotics prescribed by dentists for prophylaxis unnecessary.
- Amoxicillin 69% of scripts
- Clindamycin next most prescribed (dentists are highest frequency prescribers) – strongly associated with *C. difficile*.


Esophageal Cancer

- Sixth leading cause of cancer death worldwide
- *P. gingivalis* detected in 61% of cancerous tissues, 12% adjacent tissues, and 0% of normal esophageal mucosa.
- Eradication of common oral pathogen might help reduce the burden of esophageal cancer.


Colorectal Cancer

- *Fusobacteria* cause excessive immune responses/turn on cancer growth genes. Linked with colorectal cancer.
- *Fusobacteria* have specific surface molecules assisting them to attach and invade colorectal cancer cells.
- *F. nucleatum* associated with periodontitis, abundant in oral cavity, thought to originate there.

Pancreatic Cancer and Gum Disease

- 10-year study: bacterial contents in mouthwash samples from 361 Americans who later developed pancreatic CA + 371 matched controls were analyzed.
- P. gingivalis and Aggregatibacter actinomycetemcomitans associated with > 50% increased risk of pancreatic cancer.
- Screening tool? Prevention?

Oral Inflammation = Systemic Inflammation

- Severe periodontitis 6th most prevalent disease worldwide with an overall prevalence of 11.2% and around 743 million people affected.
- Bacteria can enter bloodstream from periodontitis, untreated carious lesions.
- Oral pathogenic bacteria including F. nucleatum, P. gingivalis, and A. actinomycetemcomitans have been detected in a multitude of extra-oral tissue sites, including the lung, heart, gut, placenta, and inflamed joints.
- Oral Treponema spirochetes found in brains of those with Alzheimer's dementia and in branches of the trigeminal nerves.

LPS and Neuroinflammation

- LPS enter circulation due to decreased barrier function
- Highly immunogenic, bind TLR-4, trigger systemic inflammation and degrades BOTH intestinal and blood brain barriers.
- TLR-4 expressed on microglia and neurons: once activated, produce pro-inflammatory cytokines (TNF-α, IL-1β, NO).
- LPS induces cognitive impairment, anxiety, depression in animal models.
- Systemic inflammation/infection can change microglial phenotype and disrupt BBB integrity in absence of precipitating neuronal damage/infection.


Brain-Gut Axis

- Human studies/animal models of depression show increased inflammatory mediators in both periphery and CNS.
- Healthy oral and gut microbiota plus adequate dietary fiber help prevent disruption of intestinal lining and blood-brain barrier.

It's the Fiber Folks!

- Diets high in fiber and low in sugar increase *Bifidobacteria*, preventing toxins from passing through intestinal wall into bloodstream.
- Prebiotics: undigestible plant fiber acts as food for microbiota.
- Bananas, onions, garlic, leeks, Jerusalem artichoke, apple skin, chicory root, dandelion greens, beans, wheat flour just a few examples of prebiotic foods.

Too Little Fiber, Too Much Sugar

Canadians average daily sugar intake:
- 101 grams (24 tsp) children 1-8 years
- 115 grams (27 tsp) children 9-18 years
- 85 grams (20 tsp) for adults - lower due to increase intake “diet” sodas.

Obesity and Microbiota?

- Early disruption of gut microbiota (C-section, antibiotics) = too few *Bifidobacteria*, can lead to obesity.
- Diet high in sugar, simple carbs, and fat encourages growth of microbes better at extracting energy from food, signaling body to store energy as fat.
- Bacteria transplanted from overweight mice to thin mice make the thin mice gain weight.

Sugar Substitutes

- Sugar substitutes frequently 1000 times sweeter than sucrose.
- Despite GRAS status by regulatory agencies, sugar substitutes can have negative effects on gut microbiota.
- Sucralose, saccharin and stevia all shown to disrupt balance and diversity of gut microbiota.

The Polyols (Sugar Alcohols)

- Erythritol, mannitol and sorbitol have no effect on gut microbiota.
- Isomaltose and maltitol, increase *Bifidobacteria* and may have prebiotic actions.
Impact of Certain Diets

- 21 healthy people had substantially altered gut microbiota profiles after four weeks on gluten-free diet; significant reduction in key beneficial microbiota species.
- Low FODMAP diets lead to significant reduction in Bifidobacterium and profound changes in the microbiota and metabolome; duration and clinical relevance are not known.


---

Table 1 | Examples of foods, nutrients, and dietary patterns that influence human health linked to their effects on gut microbiome

<table>
<thead>
<tr>
<th>Dietary pattern</th>
<th>High FODMAP</th>
<th>Low FODMAP</th>
<th>Low FODMAP adapted (A)</th>
<th>Reduced symptoms of irritable bowel syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese</td>
<td>Increased</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced symptoms of irritable bowel syndrome</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>Increased</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced symptoms of irritable bowel syndrome</td>
</tr>
<tr>
<td>Artificial sweeteners</td>
<td>Overgrowth of Enterobacteria and Entamoeba histolytica</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced symptoms of irritable bowel syndrome</td>
</tr>
<tr>
<td>Yogurt</td>
<td>Increased</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced symptoms of irritable bowel syndrome</td>
</tr>
</tbody>
</table>

Sleep and Stress

- Disruption of circadian rhythm alters gut microbiome equilibrium. Microbes and humans share circadian clock.
- Emotional and physiological stress affect gut microorganisms; impacting immune and nervous systems.
- Lactobacillus, Bifidobacterium, and Enterococcus may improve stress response.


Chronic Stress

NIEHS researchers found chronic stress disturbs gut microbiome in mice, triggering an immune response and promoting the development of colitis, a chronic digestive disease characterized by inflammation of the inner lining of the colon.


Early exposure to microbes has important health effects, leading many researchers to examine the "hygiene hypothesis."
Allergies and Asthma: Hygiene Hypothesis

- Allergies are rare in developing countries but rates of asthma and seasonal allergies tripled in high income nations since 1980s.
- Our genes haven’t changed.
- Early exposure to environmental microbes train immune system.
- Hand sanitizers, antibacterial soaps, air filters, “clean living” may negatively impact this training.

Randomized placebo-controlled trial of *L. rhamnosus* HN001 given from 35 weeks gestation to 6 months postpartum to women who were breastfeeding and 2 years for all infants.

- At 2 years and 11 years: 54% reduction in eczema, 27% reduction hay fever, and 29% reduction in atopic sensitization to food and aeroallergens.


Medications: Proton Pump Inhibitors

- Millions take PPIs for heartburn when not indicated or for too long. PPIs dramatically disrupt gut microbiota.
- Meta-analysis 23 studies (n=300,000): 65% increase risk *C. difficile* associated diarrheas amongst those taking PPI.
- PPI users have 5 times the risk of developing GI infections compared to non-users.

Role for Probiotics

- 2017 Cochrane systematic review/meta-analysis of 31 RCTs: moderate certainty evidence that probiotics are effective for preventing *C. difficile* associated diarrhea in both adults and children.
- Why are they not recommended?


Acute Infectious Diarrhea

- Strong evidence for probiotics in acute infectious diarrhea, which is common for those traveling, kids going to daycare, etc.
- Meta-analysis of 17 RCTs (2,102 children): significant reduction in duration of diarrhea with probiotic use (20 fewer hours).
- Meta-analysis of 8 RCTs (1,229 children): *L. reuteri* reduced duration of diarrhea (25 fewer hours), increased cure rate days 1 and 2.

Summary of Systematic Review Analyzing the Role of Probiotics on Clinical Outcomes

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Study Location</th>
<th>Study Design</th>
<th>Participants</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em></td>
<td>China</td>
<td>RCT</td>
<td>120 children</td>
<td>10 days</td>
<td>Reduces duration of diarrhea by 20 hours</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em></td>
<td>USA</td>
<td>RCT</td>
<td>100 children</td>
<td>7 days</td>
<td>No effect on duration of diarrhea</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>Europe</td>
<td>RCT</td>
<td>80 children</td>
<td>5 days</td>
<td>Increases cure rate by 10%</td>
</tr>
<tr>
<td><em>Saccharomyces boulardii</em></td>
<td>Brazil</td>
<td>RCT</td>
<td>120 children</td>
<td>10 days</td>
<td>Reduces duration of diarrhea by 25 hours</td>
</tr>
</tbody>
</table>

Evidence is ranked using grading system of I, II, III. You can then see the references for your review.


Accessed January 17, 2019
• IT IS ALL CONNECTED...
  • Eat a diet rich in whole plant foods, prebiotics, and fiber.
  • Limit sugar intake and use of sugar substitutes.
  • Include fermented foods/drinks.
  • Consider probiotics – be species and strain specific.
  • Find healthy ways to manage your stress and get adequate sleep.
  • Good dental hygiene and regular dental visits.

“When we try to pick out anything by itself, we find it hitched to everything else in the universe.”

John Muir