Gut Check: Understanding the Microbiome

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National Geographic’s: “Life Is Your Best Medicine,” “Healthy At Home,” and “Fortify Your Life”

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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 microbes in particular environment
- **Microbiota**—community of microorganisms themselves.
- **Core microbiome** similar for all individuals; **Variable microbiome** different between individuals.
- **Lower diversity** marker of **dysbiosis**: associated with autoimmune disease, obesity, and metabolic conditions.

Valdes AM, et al. BMJ 2018;361:k2179
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 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 associated with increased prevalence of C. difficile, Bacteroides fragilis, and E. coli and decreased prevalence of Bifidobacteria.

Probiotics and Birth Mode

- Mothers given probiotic (Bifidobacterium breve, Propionibacterium freudenreichii subsp. shermanii JS, Lactobacillus rhamnosus Lr705, and L. rhamnosus GG).
- **Probiotic group** (N = 168 breastfed and 31 formula-fed), or **placebo supplement** (N = 201 breastfed and 22 formula-fed) given during pregnancy, infants received same.
- **Placebo group**: both **birth mode and antibiotic use**, significantly associated with altered microbiota composition/function, particularly reduced *Bifidobacterium*.
- **Probiotic group**: effects of antibiotics/birth mode either completely eliminated or reduced.


Birth to 3 Years

- Within weeks, **microbial specialization** occurs. Different populations in mouth, gut, skin, etc.
- Microbial populations in infant **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 and diversity decrease.
- 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
- Provide nutrients for intestinal cells, maintaining tight junctions, reducing 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.
- **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.

Oral Microbiota Among Most Diverse

- Despite different etiologies, periodontitis and caries driven by feedforward loop between microbiota and host that favors emergence and dysbiosis.
- Disturbance in oral microbiota may impact diabetes, CVD and certain cancers.


**Table 1 Distribution of dominant microorganisms in oral cavity**

From: The oral microbiota – a mechanistic role for systemic diseases

<table>
<thead>
<tr>
<th>Section</th>
<th>Dominant microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard palate</td>
<td><em>Streptococcus, Uncl. Pasteurellaceae, Veillonella, Prevotella, Uncl.Lactobacillales</em></td>
</tr>
<tr>
<td>Tongue dorsum</td>
<td><em>Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Actinomycyes</em></td>
</tr>
<tr>
<td>Saliva</td>
<td><em>Prevotella, Streptococcus, Veillonella, Uncl. Pasteurellaceae</em></td>
</tr>
<tr>
<td>Palatine tonsils</td>
<td><em>Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Fusobacterium</em></td>
</tr>
<tr>
<td>Throat</td>
<td><em>Streptococcus, Veillonella, Prevotella, Uncl. Pasteurellaceae, Actinomycyes, Fusobacterium, Uncl. Lactobacillales</em></td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td><em>Streptococcus, Uncl. Pasteurellaceae, Gemella</em></td>
</tr>
<tr>
<td>Keratinised gingiva</td>
<td><em>Streptococcus, Uncl. Pasteurellaceae</em></td>
</tr>
<tr>
<td>Supragingival plaque</td>
<td><em>Streptococcus, Capnocytophaga, Corynebacterium, Uncl. Pasteurellaceae, Uncl. Neisseriaceae</em></td>
</tr>
<tr>
<td>Subgingival plaque</td>
<td><em>Streptococcus, Fusobacterium, Capnocytophaga, Prevotella, Corynebacterium</em></td>
</tr>
<tr>
<td>Dentures</td>
<td><em>Staphylococcus epidermidis, Streptococcus</em></td>
</tr>
<tr>
<td>Lips</td>
<td><em>Streptococcus, Candida albicans</em></td>
</tr>
</tbody>
</table>
Oral Microbiota and Blood Pressure

- Upon interaction with oral bacteria, nitrate is reduced to nitrite, swallowed and absorbed, raising 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. actinomycetemcomitans* in gingival sulcus significantly higher than that 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 participate in regulation of fatty acid tissue composition.
- Increase in *Firmicutes* in relation to *Bacteroidetes*, increases absorption of calories from food, increasing weight and fat mass.
- **Dysbiosis** with antibiotic use, especially during first 3 years life.
- *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.0b013e32832fa5e4

Child Weight Gain Trajectories Linked To Oral Microbiota Composition

- Gut and oral microbiota of 226 two-year-olds analyzed with gene sequencing.
- Weight/length measured 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.

American children on average:

- By 2 years age: 3 full doses of antibiotics
- By 10 years age: 10 full doses of antibiotics
- By 20 years age: 17 full doses antibiotic
- Four or more courses of antibiotics given before 3 years age independently associated with obesity at age 5. (OR: 1.6).


Antibiotics and Microbes

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

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 cancer death
• 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/t**urn on cancer growth genes. Linked with colorectal cancer.
- Have specific surface molecules that allow them to invade 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?

Fan X, et al. *Gut* 2018; 67(1): 120-7    Graphic from Getty Images
Oral Inflammation = Systemic Inflammation

- Severe periodontitis 6th most prevalent disease worldwide with an overall prevalence 11.2% and ~ 743 million people affected.
- 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.


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LPS and Neuroinflammation

- LPS enter circulation due to decreased barrier function
- Highly immunogenic, bind TLR-4, trigger systemic inflammation and degrade 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.

Over the last 50 years, the percentage of obese adults in the U.S. has risen steadily. During that time, the average amount of sleep we get each night has dropped. Could our sleep loss be contributing to the obesity epidemic?

Low bacterial diversity in human gut microbiota correlates with Westernization.

Clemente et al., Science Advances 2015
It’s the Fiber Folks!

- Diets high in fiber, low in sugar increase *Bifidobacteria*, decreasing intestinal permeability.
- Prebiotics: un-digestible plant fiber acts as food for microbiota.
- Bananas, onions, garlic, leeks, Jerusalem artichoke, apple skin, chicory root, dandelion greens, beans, wheat flour all prebiotics.

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 = too few *Bifidobacteria*, can lead to obesity.
- Diet high in sugar, simple carbs, and saturated fat encourages 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.


Vienna Low Doc, M.D.

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THE BENEFITS OF FERMENTED FOODS

BY APAGE

WHY EAT FERMENTED FOODS?

- **Enzymes**: Increased enzyme content helps you absorb nutrients, reducing the need for vitamins and supplements.
- **Probiotics**: These good bacteria help restore balance in the gut and aid digestion and immune health.
- **Safety**: The lactic acid created during the fermentation process kills *E. coli*, making it safer to consume than raw vegetables.
- **Preservation**: The fermentation process increases the nutritional value by enriching certain nutrients.

https://irishhealthstores.com/news-events/fermented-foods/

Vienna Low Doc, M.D.
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* supplementation *may* improve stress response.


<table>
<thead>
<tr>
<th>Dietary element</th>
<th>Effect on gut microbiome</th>
<th>Effect on health outcomes mediated by gut microbiome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low FODMAP diet</td>
<td>Low FODMAP diet increased Actinobacteria; high FODMAP diet decreased abundance of bacteria involved in gas consumption</td>
<td>Reduced symptoms of irritable bowel syndrome</td>
</tr>
<tr>
<td>Cheese</td>
<td>Increased <em>Bifidobacteria,</em> which are known for their positive health benefits to their host through their metabolic activities. Decrease in <em>Bacteroides</em> and <em>Clostridia,</em> some strains of which are associated with intestinal infections</td>
<td>Potential protection against pathogens, increased production of SCFA and reduced production of TMAO</td>
</tr>
<tr>
<td>Fibre and prebiotics</td>
<td>Increased microbiota diversity and SCFA production</td>
<td>Reduced type 2 diabetes and cardiovascular disease</td>
</tr>
<tr>
<td>Artificial sweeteners</td>
<td>Overgrowth of Proteobacteria and <em>Escherichia coli,</em> <em>Bacteroides,</em> <em>Clostridia,</em> and total aerobic bacteria were significantly lower, and faecal pH was significantly higher</td>
<td>Induced glucose intolerance</td>
</tr>
<tr>
<td>Polyphenols (eg, from tea, coffee, berries, and vegetables such as artichokes, olives, and asparagus)</td>
<td>Increased intestinal barrier protectors (<em>Bifidobacteria</em> and <em>Lactobacillus,</em> butyrate producing bacteria (<em>Faecalis</em> and <em>Roseburia</em>) and <em>Bacteroides vulgatus</em> and <em>Akermansia muciniphila.</em> Decreased lipopolysaccharide producers (<em>E. coli</em> and <em>Enterobacter cloacae</em>)</td>
<td>Gut micro-organisms alter polyphenol bioavailability resulting in reduction of metabolic syndrome markers and cardiovascular risk markers</td>
</tr>
<tr>
<td>Vegan</td>
<td>Very modest differences in composition and diversity in humans and strong differences in metabolomic profile compared with omnivore diet in humans</td>
<td>Some studies show benefit of vegetarian over omnivore diet, others fail to find a difference</td>
</tr>
</tbody>
</table>


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.


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**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 diarrhea amongst those taking PPI.

• PPI users have 5 times the risk of developing GI infections compared to non-users.


https://choosingwiselycanada.org/heartburn-gerd-ppi/
Role for Probiotics

- 2017 Cochrane systematic review/meta-analysis
  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 17 RCTs (2,102 children): significant reduction in duration of diarrhea with probiotic use (20 fewer hours).

- Meta-analysis 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>Outcome</th>
<th>Reference</th>
<th>No. of participants</th>
<th>Evidence of benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium difficile-associated diarrhea in adults and children</td>
<td>Goldenberg et al (2017)</td>
<td>39/9955</td>
<td>Yes</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>Al Fahim et al (2014)</td>
<td>17/5338</td>
<td>Yes</td>
</tr>
<tr>
<td>Probiotics for preventing acute upper respiratory tract infections</td>
<td>Hao et al (2016)</td>
<td>12/3720</td>
<td>Yes</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>Schweniger et al (2015)</td>
<td>9/735</td>
<td>No</td>
</tr>
<tr>
<td>Prevention of allergic asthma and atopy in infants</td>
<td>Aced et al (2013)</td>
<td>6/1364</td>
<td>No</td>
</tr>
<tr>
<td>Prevention of eczema in infants and children</td>
<td>Mannfield et al (2013)</td>
<td>16/2797</td>
<td>Yes</td>
</tr>
<tr>
<td>Prevention of necrotizing infections</td>
<td>Manzanares et al (2013)</td>
<td>30/2972</td>
<td>Yes</td>
</tr>
<tr>
<td>Treutamental rotavirus diarrhea in infants and children</td>
<td>Ahmad et al (2015)</td>
<td>14/1149</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Prevention and treatment of Crohn's disease and ulcerative colitis
Saed Jami et al (2015) | 14/192 | Yes |
Prevention of type 2 diabetes (fasting glucose, glycated hemoglobin test)
Amanathan et al (2016) | 9/275 | Yes |
Prevention of type 2 diabetes (insulin resistance, insulin level)
Albani et al (2015) | 3.3/805 | Yes |
Prevention of enterocolitis in preterm neonates with focus on Lactobacillus reuteri
Zhang et al (2016) | 7/425 | Yes |
Prevention of enterocolitis in preterm neonates with type 2 diabetes
Athalee-Jago et al (2016) | 6/1778 | Yes |
Reduction of serum concentration of reactive protein
Maselli et al (2017) | 19/935 | Yes |
Reduction of total cholesterol and low density lipoprotein (cooperative cholesterol) depression, anxiety, and cognitive functioning
Zhang et al (2017) | 15/976 | Yes |
Reduction of total cholesterol and low density lipoprotein (cooperative cholesterol) depression, anxiety, and cognitive functioning
Wu et al (2017) | 6/1060 | Yes |


Clinical Resource Tool: www.usprobioticguide.com
Click next to brand name to see evidence......

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


• 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