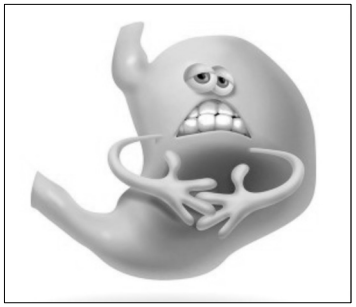
Gut Check: *Understanding the Microbiome*



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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 oral microbiota and health.
3. Discuss the relationship between the gut microbiota and health.
4. Identify how certain medications, such as proton pump inhibitors and antibiotics, impact the microbiota.
5. Describe the role of diet, dietary fiber, prebiotics, and probiotics in optimizing the microbiota.

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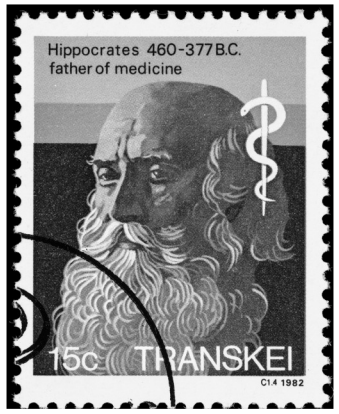

All disease begins in the gut.

Hippocrates

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Human Microbiome Project

- **Massive research initiative of NIH** cataloging the microorganisms living in and on our body starting in 2007.
- Led to *rapidly growing appreciation* for **incredible and diverse impact** these organisms have on our **health and well-being.**
- Gut bacteria produce **vitamins** and break down our food; their presence or absence has been linked to **obesity**, **inflammatory bowel disease**, **IBS**, **anxiety**, **depression**, **food allergies**, **neuroinflammation**, **GI infections**, **high blood pressure**, **diabetes**, **metabolic syndrome,** and more.
- **Our resiliency**—our ability to recover quickly from stressors—may be a function of ***which bacteria inhabit or don't inhabit our gut***.

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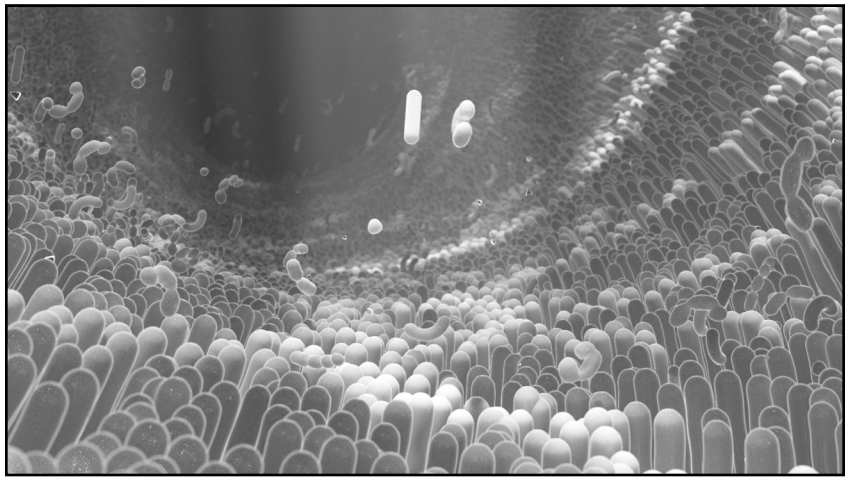

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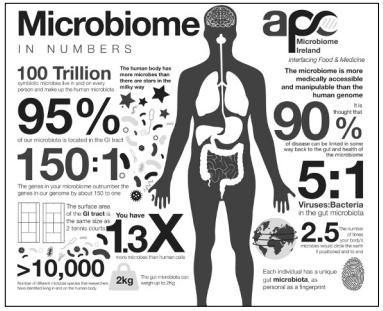
Definitions

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



Valdes AM, et al. *BMJ 2018;361:k2179*

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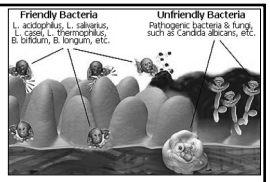
Microbiota…



- **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 n-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)

Wilkins T, et al. Probiotics for Gastrointestinal Conditions: A Summary of the Evidence. *Am Fam Physician* 2017 Aug 1;96(3):170-178.

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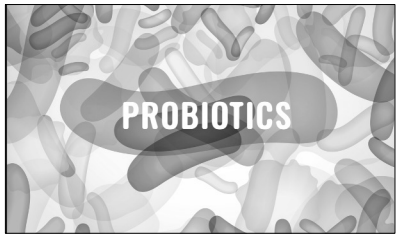
Probiotics

- **Live microorganisms when** administered in adequate amounts confer a *health benefit to the host.*
- Probiotics **support the immune response** via enhanced **phagocytic** capacity and activity, stimulation of specific **immunoglobulins**, and enhancement of **intestinal barrier function.**



Arunachalam K, Gill HS, Chandra RK. Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). *Eur J Clin Nutr.* (2000) 54:263–7.

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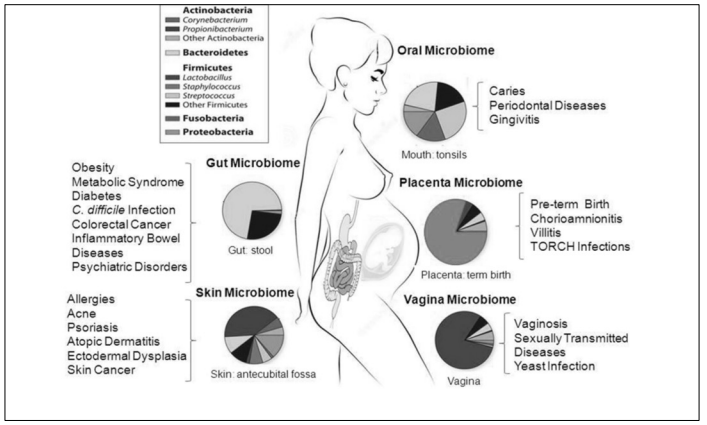

Graphic from: Belizario, JE, et al. *Front Microbiol* 2015; October 6

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Health consequences			Balanced immune and metabolic development	Normal growth and immune function
Normal microbiota development		Transfer of maternal microbes	High abundance of bifidobacteria	Gradually increasing abundance of clostridia
Factors promoting healthy microbiota	Maternal health, wellbeing, healthy diet	Natural birth	Breastfeeding, probiotics, prebiotics	Healthy diet, probiotics, prebiotics
Developmental stage	Pregnancy	Birth	Infancy	Childhood
Factors promoting microbiota imbalance	Maternal antibiotic use, stress, unhealthy diet	C-section, intrapartum & perinatal antibiotics	Antibiotics, formula, early introduction of solid foods	Antibiotics, unhealthy diet
Abnormal microbiota development		Lack of maternal microbes, increased abundance of pathogens	Low abundance of bifidobacteria	High abundance of Gram-negatives
Health consequences			Inflammation, infection, abdominal symptoms	Immune diseases, growth impairment, overweight

Korpela K, de Vos WM. Infant gut microbiota restoration: state of the art. *Gut Microbes.* 2022 Jan-Dec;14(1):2118811.

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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 **impacts the microbiome** for **months or years after birth.**

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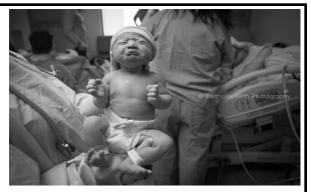
Neonatal Microbiome



- Greatest insults to the natural assembly of neonatal microbiome: C-section delivery, antibiotic use, and formula feeding.
- Differences in microbial species observed between C-section and vaginally delivered babies *up to 7 years after birth.*[1]
- Intrapartum antibiotics associated with **lower abundance of good bacteria (*Lactobacilli* and *Bifidobacterium)*** in neonatal gut.[2]
- Formula feeding associated with **increased** prevalence of pathogenic bacteria (*C. difficile*, *Bacteroides fragilis*, *E. coli)* and **decreased** prevalence of ***Bifidobacterium*.**[3]

1. Salminen S, et al. *Gut.* 2004;53:1388–1389; 2. Aloisio I, et al. *Appl Microbiol Biotechnol.* 2014;98:6051–6060.
3. Mueller NT, et al. *Trends Mol Med* 2015; 21(2): 109-17

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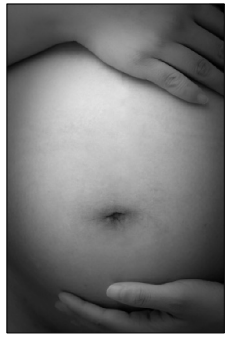
Probiotics & Birth Mode

- **Mothers given probiotic** or placebo during pregnancy and then **infants** given same.
  - **Placebo group:** birth mode and/or antibiotics significantly altered microbiota composition and function, reducing *Bifidobacterium.*
  - **Probiotic group:** effects of birth mode and/or antibiotics either *completely eliminated or dramatically reduced.*

(Probiotic: *Bifidobacterium breve, Propionibacterium freundenreichii subsp. shermanii* JS, *Lactobacillus rhamnosus L705,* and *L. rhamnosus GG)*



Korpela K, et al. Probiotic Supplementation Restores Normal Microbiota Composition and Function in Antibiotic-Treated and in Caesarean-Born Infants. *Microbiome* 2018; 6(1): 182

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Breast Milk

- Prospective, **12-month** longitudinal study, **bacterial composition** identified in **breast milk, areolar skin, and infant stool samples** of **107 healthy mother-infant** pairs in Los Angeles, California and St Petersburg, Florida.
- During **first 30 days of life**, infants who obtained **75% or more of daily milk intake** from breastmilk received **27.7% of the bacteria from breast milk** and **10.3% from areolar skin – almost 40% of bacteria was from mother to infant.**
- **Infant gut microbial communities** more closely related to **infant's mother's milk and skin** compared with random mother ($P < .001$).
- **Bacterial diversity** ($p=0.003$) and **composition changes** associated with proportion of daily **breast milk intake in a dose-dependent manner**, even after the introduction of solid foods.
- Breastfeeding confers **protection against respiratory and gastrointestinal tract infections** and **allergic diseases**, and **reduces risk of chronic diseases** (e.g., diabetes, obesity, and inflammatory bowel disease). Likely through modulation of microbiome.

Bittinger K, et al. *JAMA Pediatr.* 2017 Jul 1;171(7):647-654.

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Birth to 3 Years

- Within weeks, **microbial specialization** occurs. Different populations in mouth, gut, skin, etc.
- Microbial populations in infant are **similar to people they live with.**
- Microbiota dramatically altered by **new foods, antibiotics, PPI use**, etc.
- **Number and types of species increase and change with age**.
  - Example: babies have more folate *producing* microbes—adults have more folate *harvesting* microbes.

Azad MB, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Can Medical Association Journal,* 2013; 185(5), 385-394.

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Probiotics: Long Term Follow-Up of Moms and Infants

- **316 mother infant pairs: *L. rhamnosus* HN001 (6 billion cfu) or probiotic**
- Pregnant mothers supplemented daily **from 35 wks. gestation to 6 months post-partum** if nursing. **Infants** supplemented from birth until **2 years old.**
- At **2, 4, and 6 years of age**, prevalence of **eczema** and **allergic sensitization** determined by clinical diagnosis and skin prick (results following slide).
- **Prevalence** at **11 years** follow-up:
  - **29% reduced risk of atopic sensitization**
  - **42% reduced risk of eczema**
  - **24% reduced risk of wheeze**

Wickens K, et al. *Clin Exp Allergy* 2013; 43(9):1048-57.
Wickens K, et al. *Pediatr Allergy Immunol* 2018; 29(8): 808-14

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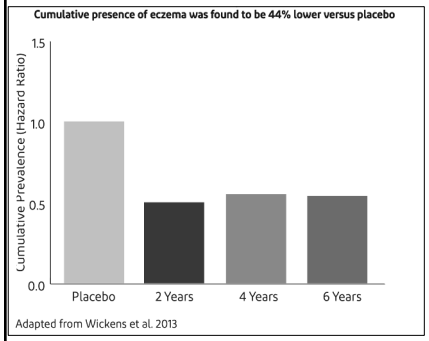
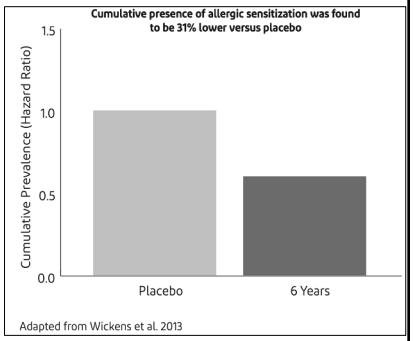
Impact of Probiotics at 2, 4, and 6 Years on Eczema and Allergic Sensitization





Wickens K, et al. *Clin Exp Allergy* 2013; 43(9):1048-57.

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Age 3 to Old Age

- **Microbiome becomes stable by age 3-5**.
- Even with disruptions (medications, disease, dietary changes)—**it usually *returns to baseline.***
- **After age 65, microbe populations decrease, species** become more similar (dysbiosis), may explain/contribute to some diseases of aging.

Yatsunenko T, et al. Human gut microbiome viewed across age and geography. *Nature* 2012; 486:222-228.

The Human Microbiome Project Consortium (2012). Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 86, 207-214.

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Obesity & Microbiota?

- **Diets high in sugar, simple carbs, and saturated fat** encourages growth of microbes better at ***extracting*** energy from food, signaling body to **store energy as fat**.
- **Early disruption** of gut microbiota leads to **low levels *Bifidobacteria* and obesity.**
- **Obesity** during middle age (40–60 years) consistently associated with higher risk of **dementia later in life.**

Federico A, et al. Gut microbiota, obesity and metabolic disorders. *Minerva Gastroenterol Dietol* 2017;63(4):337-344

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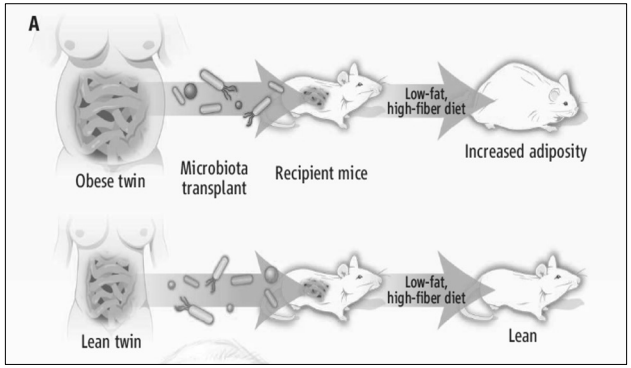
Ridaura, V.K., et al., *Gut microbiota from twins discordant for obesity modulate metabolism in mice.* Science, 2013.



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Microbes: Energy & Inflammation

- **Microbiota** can **increase energy** production from diet and take part in the regulation of the **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.**[1]
- **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.**

Duranti S, et al. Obesity and microbiota: an example of an intricate relationship. *Science* 2017; 12:18. doi: 10.1186/s12263-017-0566-2.

Fessler MB, et al. *Curr Opin Lipidol* 2009; DOI: 10.1097/MOL.0b013e32832fa5e4

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Title of the study	Year	Subjects of the study	Final result(s) gathered	Reference
Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics.	2011	28354 mother-child	Antibiotics in infancy influences the risk of overweight in later childhood	Ajslev et al., 2011
Infant antibiotic exposures and early-life body mass.	2013	11532 children	Exposure to antibiotics during the first 6 months of life was associated with increases in body mass.	Trasande et al., 2013
Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study.	2014	74946 children	Exposure to antibiotics during the first 12 months of life is associated with a small increase in BMI in boys aged 5–8 years	Murphy et al., 2014
Infant antibiotic exposure and the development of childhood overweight and central adiposity	2014	1047 children	Antibiotic use in the first year of life was associated with overweight	Azad et al., 2014
Association of antibiotics in infancy with early childhood obesity.	2014	64580 children	Repeated exposure to broad-spectrum antibiotics was associated with early childhood obesity	Bailey et al., 2014
Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity.	2015	436 mother-child dyads	Exposure to antibiotics in the second or third trimester of pregnancy were associated with higher risk of childhood obesity.	Mueller et al., 2015
Prenatal exposure to systemic antibacterials and overweight and obesity in Danish schoolchildren: a prevalence study.	2015	9886 children	Prenatal exposure to systemic antibacterials was associated with an increased risk of overweight and obesity at school age	Mor et al., 2015
Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life.	2015	6114 boys and 5948 girls	Antibiotic exposure before 6 months was associated with increased body mass	Saari et al., 2015
Early Life Antibiotic Exposure and Weight Development in Children.	2016	979 children	Repeated exposure to antibiotics early in life, especially β-lactam agents, is associated with increased weight and height.	Mbakwa et al., 2016
Antibiotic Use and Childhood Body Mass Index Trajectory.	2016	142824 children	Body Mass Index increase	Schwartz et al., 2016
Administration of Antibiotics to Children Before Age 2 Years Increases Risk for Childhood Obesity.	2016	21714 children	Administration of 3 or more courses of antibiotics before age of 2 years was associated with an increased risk of early childhood obesity	Scott et al., 2016

Del Fiol FS, et al. Obesity: A new adverse effect of antibiotics? *Front Pharmacol* 2018; https://doi.org/10.3389/fphar.2018.01408

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Antibiotics & 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.**[1]
- **Four or more courses** of antibiotics given between ages 2 to 3 years independently **associated with obesity at age 5**. (OR: 1.6).[2]

1. Cox LM. Antibiotics in early life and obesity. *Nat. Rev. Endocrinol 2015;* 11, 182–190.

2. Kelly D, et al. Antibiotic use in early childhood and risk of obesity: longitudinal analysis of a national cohort. World J Pediatrics 2019;15(4):390-397.

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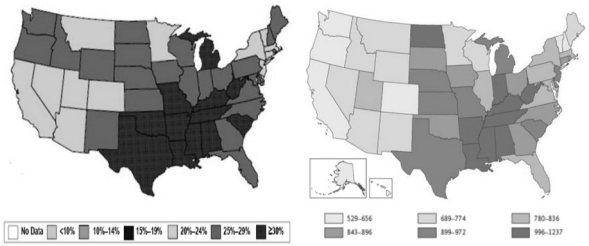
Comparisons between the geography of obesity and antibiotic use, 2010



Antibiotic prescriptions per 1000 persons, 2010

L Segal & MJ Blaser, *Ann Am Thor Soc* 2014

Observational data

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Antibiotics and Microbes

- **Disrupt existing microbiota**; linked to **antibiotic-associated diarrhea**, **pseudomembranous colitis**, and increased **susceptibility** to subsequent disease.[1]
- Extent of change depends on **antibiotic type, duration, and dose.**
- **Systematic review:** changes in gut microbiome from **metronidazole and clarithromycin** lasted longest (**4 years**), **clindamycin** (**2 years**), and **ciprofloxacin** (**1 year**).[2]

1. Abeles SR, et al. Microbial diversity in individuals and their household contacts following typical antibiotic courses. *Microbiome* 2016; 4: 39–51.
2. Zimmermann P, Curtis N. The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. *J Inf Secur.* 2019;79(6):471–89.

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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*.

Suda KJ, et al. *JAMA Network Open* 2019;2(5):e193909.

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Oral Health & Systemic Disease

- **1891:** first oral microbiologist Willoughby D. Miller put forward theory of **oral focal infections**, suggesting that **oral microbial infection** can affect other parts of the body, related to a **variety of systemic diseases.**
- **1912:** Frank Billings **speculated that infection of the teeth** may be the cause of **rheumatoid arthritis, nephritis, endocarditis, and other diseases.**
- **Periodontal inflammation** leads to loss of connective tissues/bones. **Extensive inflammatory cell infiltration** appears in connective tissue near periodontal pocket epithelium. This **low-grade inflammation may disturb the health of body or worsen other systemic diseases.**

Miller WD. The human mouth as a focus of infection. *Lancet* 1891; 138, 340–342

Billings F. Chronic focal infections and their etiologic relations to arthritis and nephritis. *Arch. Intern. Med* 1912; IX, 484–498 (1912).

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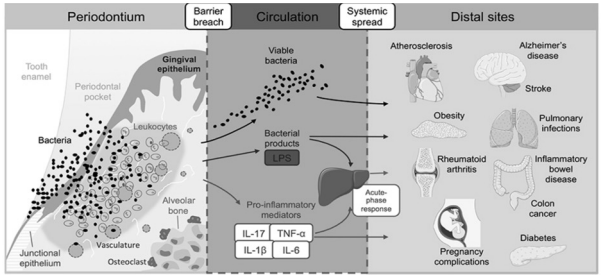
Oral Microbiota Among Most Diverse

- **700 microbial species**: bacteria, fungi, viruses, archaea, and protozoa form complex ecological community. Oral microbiota generally exist as **biofilm.**
- 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.**[1]
- Increasing evidence suggests an association with dysbiosis of **oral ecosystem**, and development of **diabetes, CVD,** and certain **cancers**.[2]

1. Lamont RJ, et al. The oral microbiota: dynamic communities and host interactions. *Nature Reviews Microbiology* 2018; 16: 745-59

2. Zhang Y, et al, Human oral microbiota and its modulation for oral health, *Biomedicine & Pharmacotherapy* 2018; 99:883-93



- **Severe periodontitis affects 743 million people worldwide.**
- Bacteria can enter bloodstream and translocate to extra-oral tissue: **lung, heart, gut, placenta, brain inflamed joints, etc.** Study found **100%** of patients with CVD **had *P. gingivalis* arterial colonization**, found in **brains** of those with **AD.**

From: Konkel JE, et al. Distal Consequences of Oral Inflammation *Front. Immunol* 2019; https://doi.org/10.3389/fimmu.2019.01403

Oral Microbiota & Gut Inflammation

- Adults produce >1000 mL/d of **saliva**, carrying **oral microbes to the GI tract**. Bacteria can also enter GI tract via **bloodstream.**
- Inflammation caused by ***P. gingivalis*** in oral cavity can **alter intestinal microbial communities, disrupt intestinal barrier, induce endotoxemia, and trigger a systemic inflammatory response.**
- ***F. nucleatum*** can migrate to intestine, **inhibiting the immune response mediated by T cells, and promoting progression of IBD.**

Peng X, et al. *International Journal of Oral Science* 2022; 14, 14.

Oral Microbiota & Cardiovascular Disease

- Cross-sectional studies, case analyses, epidemiological investigations: **periodontitis is important risk factor for CVD**.
- Periodontal disease-related bacteria stimulate cells to produce inflammatory factors (e.g., IL-1β, IL-6, TNF-α) and **enter circulation** from damaged periodontal tissue, resulting in **inflammation** and **vascular** endothelial **damage &** formation of atherosclerotic **plaques**.
- After periodontal treatment, **CRP and other inflammatory markers are significantly reduced, further linking oral health to CVD.**

Herrera D, et al. *Periodontology* 2020; 83: 66–89

Schoffer C. *J. Periodontol 2021;* 92: 793–802

Oral Microbiota & Cancer

- Oral cavity **one of largest microbial storage areas** in human body and **microbial variations** may be **highly linked with malignancies.**
- Anaerobic oral bacteria, especially ***Fusobacterium nucleatum*** **and** ***Porphyromonas gingivalis*****,** have **close relationships with various types of carcinomas.**
- **Some aerobic bacteria (**e.g., *Parvimonas)* are linked to tumorigenesis. **Human papillomavirus, oral fungi, and parasites** also linked to oropharyngeal carcinoma.

Sun J, et al. Role of the oral microbiota in cancer evolution and progression. *Cancer Med.* 2020 Sep;9(17):6306-6321.

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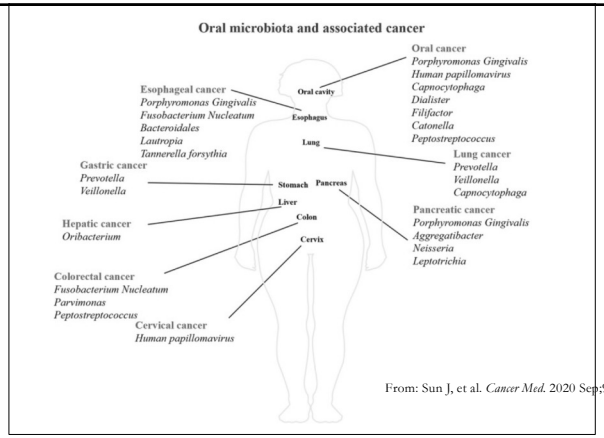

From: Sun J, et al. *Cancer Med.* 2020 Sep;9(17):6306-6321

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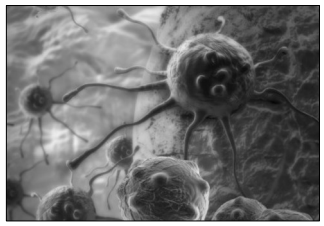
Esophageal Cancer



Gao, S, et al. *Infect Agent Cancer* 2016; 11: 3–12.
Chen, X. et al. *PLoS ONE* 2015; 10: e0143603.

- **Esophageal squamous cell carcinoma** closely related to **tooth loss and tooth brushing frequency**. Incidence of **metastasis** in **periodontitis patients significantly higher** than that in non-periodontal patients
- ***P. gingivalis*** detected
  - **61% cancerous tissues**
  - **12%** adjacent tissues
  - **0% of normal** esophageal mucosa.
- ***F. nucleatum*** is also a promotor.

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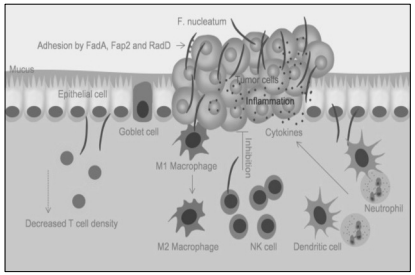
Colorectal Cancer



Nosho K, et al. *World J Gastroenterol* 2016; 22: 557–566
Liu Y, et al. *J Gastroenterol* 2019 Jan;54(1):33-41

- ***F. nucleatum:*** **pivotal promoting factor** in colorectal cancer.
- ***Fusobacteria*** cause excessive immune response, **turning on cancer growth genes in CR cancer.**[1]
- **Have specific surface molecules allowing them to attach and invade** colorectal cells.[1]
- **Higher levels** *F. nucleatum* DNA in tumor, associated with **poorer survival.**
- May contribute to **chemo-resistance in GI cancers.**[2]

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Pancreatic Cancer

- **Meta-analysis** 8 studies: RR **for periodontitis and pancreatic cancer** was **1.74** (95% CI 1.41-2.15) and **1.54** for **edentulism** (95% CI 1.16-2.05). **No heterogeneity or publication bias**. Reports from 3 **continents, association is generalizable.**
- Associations between **pancreatic cancer** and abundance of ***P. gingivalis* in oral wash** samples, and pancreatic cancer and increased levels of antibodies against *P. gingivalis* have been well-documented.

Maisonneuve P, et al. Periodontal disease, edentulism, and pancreatic cancer: a meta-analysis. *Ann Oncol* 2017 May 1;28(5):985-995
Chang, JS, et al. Investigating the association between periodontal disease and risk of pancreatic cancer *Pancreas* 2016; 45:134–141.
Fan X et al. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut.* 2018 Jan;67(1):120-127.

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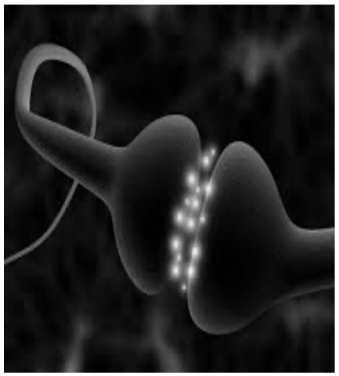

Gut-Brain Communication

- **Gut bacteria** produce neurotransmitters: **dopamine**, **serotonin**, **norepinephrine**, **GABA**, **acetylcholine**, **melatonin**; critical for mood, sleep, anxiety, concentration, reward, and motivation. Acts in many ways like an endocrine-like organ.
- Gut microbiota can cause changes in how our brains react to events/stressors
- Serotonin associated with **depression and happiness**; *90%* is made in the digestive tract—not the brain.
- **Gut microbiota** regulate brain function through gut-brain axis, **and *dysbiosis*** may trigger **anxiety** and **depression.**

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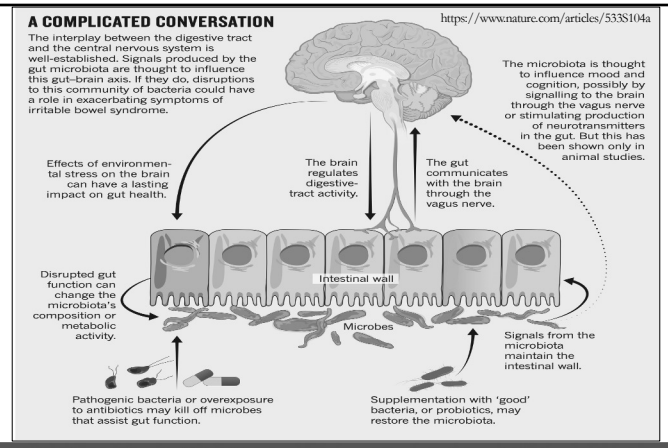

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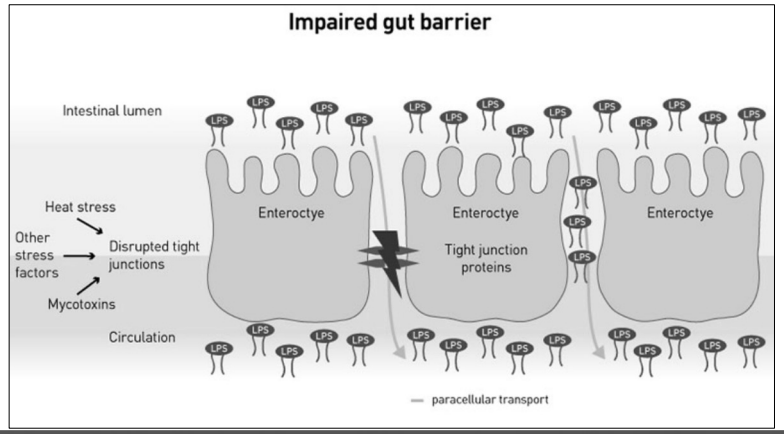

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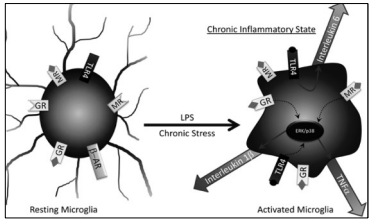
Neuro-inflammation



- LPS highly immunogenic, **bind TLR-4,** trigger **systemic inflammation** and **degrades BOTH intestinal and blood brain barriers.**
- LPS induces **cognitive impairment, anxiety, depression in animal models.**
- ***Systemic* inflammation** can **change microglial phenotype** and **disrupt BBB integrity** in absence of precipitating neuronal damage/infection.

Zhao J, et al. *Sci Rep* 2019; 9:5790 doi:10.1038/s41598-019-42286-8

Kure C, et al. *Front Pharmacol* 2017; doi.org/10.3389/fphar.2017.00117

Vieraona Low Dog, M.D.

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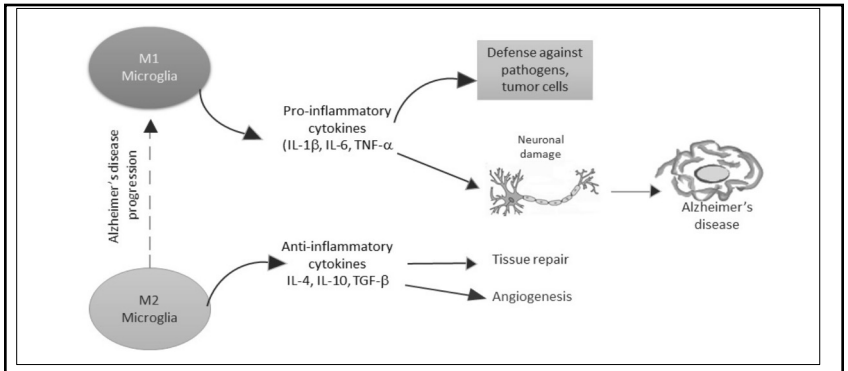

Chronic neuroinflammation *linked to* **anxiety, depression, bipolar disorder, and Alzheimer's disease.**

From: Chandra Mohan at www.sigmaaldrich.com/technical-documents/articles/biology/microglia-in-neuroinflammation.html. Accessed 12/22/20

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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.**

Carlessi AS, et al. *Eur J Neurosci 2019*; doi: 10.1111/ejn.14631.

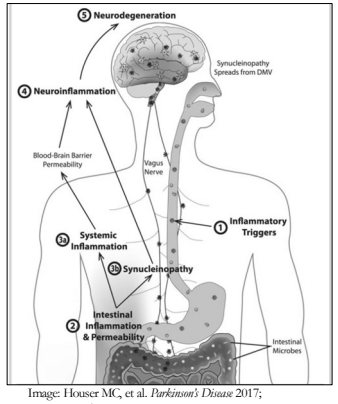

Image: Houser MC, et al. *Parkinson's Disease* 2017; doi:10.1038/s41531-016-0002-0

Vieraona Low Dog, M.D.

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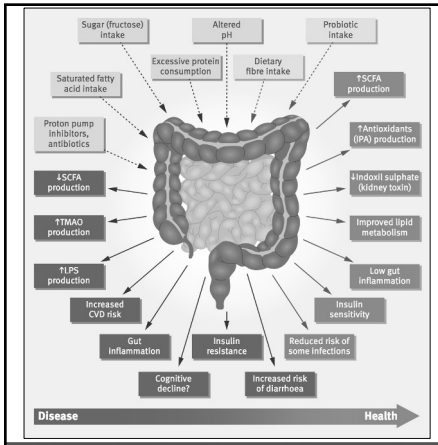

Diet, lifestyle, and medications can **dramatically impact** the microbiome and ultimately impact human health.

From: Valdes AM, et al. Role of gut microbiota in nutrition and health. *British Medical Journal* 2018;361:j2179

More Fiber, Less Sugar

- Diets **high in fiber** and **low in sugar** increase *Bifidobacteria,* preventing toxins from passing through intestinal wall into bloodstream.
- Strong evidence for optimizing intestinal barrier function with **dietary fiber.**
  - **Aim for 25-35 grams/d**

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 microbe 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.

Bonder MJ, et al. The influence of a short-term gluten-free diet on the human gut microbiome. Genome Med 2016;8:45
McIntosh K, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut* 2017;66:1241-51.

Table 1 | Examples of foods, nutrients, and dietary patterns that influence human health linked to their effe

Dietary element	Effect on gut microbiome	Effect on health outcomes mediated by gut microbiome
Low FODMAP diet	Low FODMAP diet increased Actinobacteria; high FODMAP diet decreased abundance of bacteria involved in gas consumption[58]	Reduced symptoms of irritable bowel syndrome[16]
Cheese	Increased *Bifidobacteria*,[97 98] which are known for their positive health benefits to their host through their metabolic activities.[99] Decrease in *Bacteroides* and *Clostridia*, some strains of which are associated with intestinal infections[98]	Potential protection against pathogens.[100] Increased production of SCFA and reduced production of TMAO[99]
Fibre and prebiotics	Increased microbiota diversity and SCFA production[22 101 102]	Reduced type 2 diabetes[22] and cardiovascular disease[103]
Artificial sweeteners	Overgrowth of Proteobacteria and *Escherichia coli*.[104] *Bacteroides*, *Clostridia*, and total aerobic bacteria were significantly lower, and faecal pH was significantly higher[47]	Induced glucose intolerance[105]
Polyphenols (eg, from tea, coffee, berries, and vegetables such as artichokes, olives, and asparagus)	Increased intestinal barrier protectors (*Bifidobacteria* and *Lactobacillus*), butyrate producing bacteria (*Faecalibacterium prausnitzii* and *Roseburia*) and *Bacteroides vulgatus* and *Akkermansia muciniphila*.[107] Decreased lipopolysaccharide producers (*E coli* and *Enterobacter cloacae*)[106]	Gut micro-organisms alter polyphenol bioavailability resulting in reduction of metabolic syndrome markers and cardiovascular risk markers[108]
Vegan	Very modest differences in composition and diversity in humans and strong differences in metabolomic profile compared with omnivore diet in humans[50]	Some studies show benefit of vegetarian over omnivore diet,[109] others fail to find a difference[110]

Valdes AM, et al. Role of gut microbiota in nutrition and health. *British Medical Journal* 2018;361:j2179

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, stevia**—all shown to **disrupt balance and diversity** of gut microbiota.
- **Erythritol, mannitol, sorbitol** have no adverse effect.

Nettleton JE, et al. Reshaping the gut microbiota: Impact of low calorie sweeteners and the link to insulin resistance? *Physiol Behav* 2016;164(Pt B):488-93.
Ruiz-Ojeda FJ, et al. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials, *Adv Nutr* 2019; 10(1): S31-48

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Erythritol & CVD

- As a sweetener, levels > 1,000-fold greater than levels found naturally in foods.
- Increasing blood erythritol levels speeds up blood clot formation and artery blockage in mice.
- NIH-funded research: people with highest erythritol levels (top 25%) were twice as likely to have cardiovascular events over three years of follow-up as those with the lowest (bottom 25%).
- Blood erythritol levels measured in 8 healthy volunteers after drinking a beverage sweetened with erythritol. Erythritol levels increased 1,000-fold and remained substantially elevated for several days. For at least two days, levels high enough to trigger changes in platelet function.

Witkowski, M, *et al. Nature Medicine* 2023 https://doi.org/10.1038/s41591-023-02223-9

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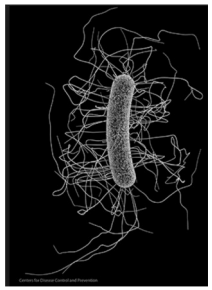
PPIs, Dysbiosis, and Infection

- Stomach acid **directly destroys** harmful **pathogens.**
- When **acid is shut down**, ~**50% of salivary and ingested bacteria survive** by slipping past this "gastric acid trap."
- **Translocated bacteria** disrupt gut microbiota, leading to **dysbiosis, SIBO, and dyspepsia.**
- **70% of immune system resides in** GI tract: **critical line of defense.**
- By ***altering balance between beneficial and pathogenic microbes***, the ***risk for infection*** is increased.



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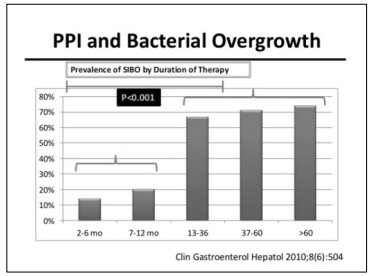
Dysbiosis & SIBO

- Meta analysis 19 studies (n=7055): **statistically significant association between increased risk of SIBO and PPI use** (OR 1.71).[1]
- Dysbiosis and SIBO increase **intestinal permeability,** allowing **bacteria** and other substances to pass directly through the intestinal mucosa **into the blood stream.**
- **PPIs may have more prominent effect on microbiota composition** on **population basis** than any other drug.[2]



1. Su T, et al. *J Gastroenterol* 2018; Jan;53(1):27-36
2. Imhann F, et al. *Gut.* 2016; 65: 740–748

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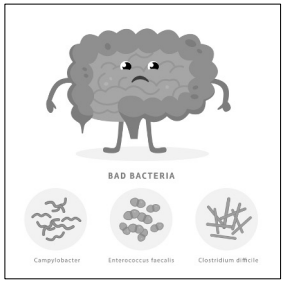
PPI & C. difficile Infection

- **FDA** warning 2012: **PPIs increase risk** of ***C. diff*** **infection (CDI)** which can cause life-threatening inflammation of the colon and diarrhea.
- Review 56 studies (n=356,000): **double the odds** of **CDI** if taking PPIs compared to non-users.



Trifan A, et al. *World J Gastroenterology* 2017

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Probiotics for Preventing C. difficile: Adults & Children

Outcome	Risk with Control	Risk with Probiotics (95% CI)	Relative Effect (95% CI)	Number of Participants (studies)	Certainty of Evidence
Antibiotic Associated Diarrhea in Children	271 per 1000	103 per 1000 (79-133)	RR: 0.38 (0.29-0.49)	1141 (6 RCTs)	Moderate

Benefits in NNT	Harms in NNT
1 in 42 for preventing C. difficile–associated diarrhea (1 in 12 in high risk patients)	No significant harm

From: Goldenberg JZ, et al. **Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children**. *Cochrane Database Syst Rev* 2017 Dec; 2017(12): CD006095

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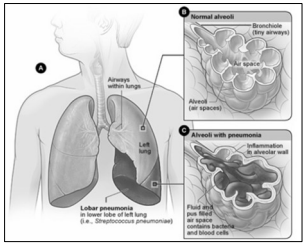
PPI & Community Acquired Pneumonia

- Without adequate stomach acid, there can be **overgrowth of oropharyngeal bacteria**, which can increase the risk for infection.
- **Review of 26 studies:** 1.5-fold increase in risk for community-acquired pneumonia, with the highest risk occurring within 30 days of starting PPI.



Lambert AA, et al. *PloS One* 2015

55

PPIs & SARS-CoV-2

- **GI tract** significant portal of entry for virus. Bind to widely expressed **ACE-2 receptors** in intestine, replicating rapidly.
- **No stomach acid means *no viral inactivation.***
- Korean study: **current use of PPIs** conferred 79% greater risk of **severe clinical outcomes;** risk climbed to **90% if PPI use started within 30 days** of confirmed Covid 19 infection.[1]
- US study: **mortality** from COVID-19 **was 2.3 times higher in PPI users,** compared to non-users.[2]

1. Lee SW, et al. *Gut* 2021

2. Ramachandran P, et al. *Eur J Gastroenterol Hepatol* 2021

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PPI & Infection Risk in Children

- **Review 14 studies:** significant association between acid-suppressive therapies and risk of **GI infection, sepsis, and pneumonia** in infants.[1]
- **~70–85 % infants** experience regurgitation/reflux within first two months of life; **resolves on its own in 95 % of babies** by 1st birthday.[2]
- Pediatric GI guidelines strongly recommend ***against*** **acid suppression** for **GER.** (*2-4 weeks elimination of cow's milk dramatically reduces reflux~ 42-58% of infants with GERD.)[2]

1. Chung EY, et al, *Hospital Pediatrics* 2013; 2. Czinn SJ, et al. *Paediatr Drugs* 2013

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PPIs + Probiotics in Children

- 128 children with GERD randomized to **12 weeks PPI** (esomeprazole 1 mg/kg/d; max 40 mg) **plus probiotics** (*Lactobacillus reuteri* DM 17938) or identical **placebo**. Control: 120 healthy age-matched children.
- After 12 weeks, **dysbiosis** occurred in **56.2%** of group receiving **placebo** versus **6.2%** of those taking **probiotics** ($p<0.001$).
- Probiotics + PPI significantly **decreased prevalence of small intestinal bacterial overgrowth (SIBO),** compared to PPI and placebo ($P < 0.001$).

Belei O, et al. *J Neurogastroenterol Motil.* 2018 Jan 30;24(1):51-57.

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Prevention Cold & Flu-Like Symptoms

- **DBPCT 326 children** (3-5 years age) randomized to placebo (N = 104), *L. acidophilus* NCFM (N = 110), or *L. acidophilus* NCFM + *Bifidobacterium lactis* Bi-07 (N = 112). Children treated 2 x daily for 6 months.
- **Compared to placebo:**
  - Single and combination probiotics reduced ***incidence*** of **fever** 53% (P = .0085) and 72.7% (P = .0009), **coughing** 41.4% (P = .027) and 62.1% (P = .005), **rhinorrhea** 28.2% (P = .68) and 58.9% (P = .03), respectively.
  - **Fever, coughing, and rhinorrhea *duration*** decreased significantly by 32% (single strain; P = .0023) and 48% (combination; P < .001).
  - ***Antibiotic use incidence*** reduced 68.4% (single; P = .0002) and 84.3% (combination; P < .0001).

Leyer GJ, et al. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* 2009 Aug;124(2):e172-9.

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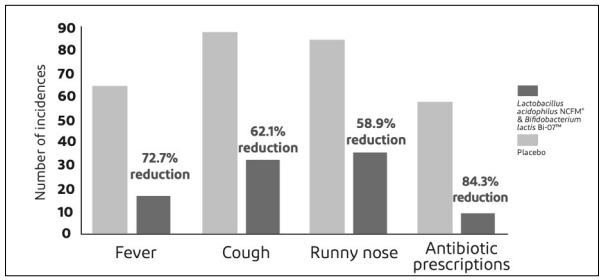
Reduction in Symptoms, Antibiotic Use with L. acidophilus NCFM + B. lactis Bi-07



Leyer, GJ, et al. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* 2009; 124(2): e172-179.

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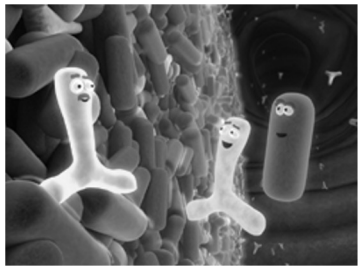
Acute Infectious Diarrhea

- **High quality evidence support** probiotics in **acute infectious diarrhea**, common for those **traveling,** kids going to **daycare,** etc. Note: start probiotics first sign of diarrhea and 1-2 weeks beyond; if traveling, start 2 days before travel and duration of trip.[1]
- Meta-analysis **17 RCTs** (2,102 children): significant **reduction in duration** of diarrhea with *S. boulardii* use (20 fewer hours).[2]
- Meta-analysis **8 RCTs** (1,229 children): *L. reuteri* DSM 17938 reduced duration of diarrhea (25 fewer hours), increased cure rate on days 1 and 2.[3]



1. https://www.aafp.org/afp/2017/0801/p170.html. Accessed December 22, 2020
2. Feizzadeh S, et al. Efficacy and safety of *Saccharomyces boulardii* for acute diarrhea. *Pediatrics*. 2014;134(1):e176–e191.
3. Urbańska M, et al. Systematic review with meta-analysis: *Lactobacillus reuteri* DSM 17938 for diarrhoeal diseases in children. *Aliment Pharmacol Ther*. 2016;43(10):1025–1034.

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

Outcome	Reference	No of studies/ participants	Evidence of benefit?
Clostridium difficile associated diarrhoea in adults and children	Goldenberg et al (2017)[111]	39/9955	Yes
Necrotising enterocolitis	Al Faleh et al (2014)[112] Rees et al (2017)[113]	17/5338	Yes
Antibiotic associated diarrhoea in children	Goldenberg et al (2015)[114]	26/3898	Yes
Probiotics for preventing acute upper respiratory tract infections	Hao et al (2015)[115]	12/3720	Yes
Urinary tract infections	Schwenger et al (2015)[116]	9/735	No
Prevention of asthma and wheeze in infants	Azad et al (2013)[117]	6/1364	No
Prevention of eczema in infants and children	Mansfield et al (2014)	16/2797	Yes
Prevention of invasive fungal infections in preterm neonates	Agrawal et al (2015)[119]	19/4912	Unclear
Prevention of nosocomial infections	Manzanares et al (2015)[120]	30/2972	Yes
Treatment of rotavirus diarrhoea in infants and children	Ahmadi et al (2015)[121]	14/1149	Yes
Prevention and treatment of Crohn's disease and ulcerative colitis	Saez Lara et al (2015)[122]	14/821 ulcerative colitis 8/374 Crohn's disease	Yes
Pulmonary exacerbations in children with cystic fibrosis	Ananathan et al (2016)[123]	9/275	Yes
Type 2 diabetes (fasting glucose, glycated haemoglobin test)	Akbari et al (2016)[124]	13/805	Yes
Type 2 diabetes (insulin resistance, insulin levels)	Zhang et al (2016)[125]	7/425	Yes
Necrotising enterocolitis in pre-term neonates with focus on *Lactobacillus reuteri*	Athalye-Jape et al (2016)[126]	6/1778	Yes
Reduction of serum concentration of C reactive protein	Mazidi et al (2017)[127]	19/935	Yes
Cardiovascular risk factors in patients with type 2 diabetes	Hendijani et al (2017)[128]	11/641	Yes
Reduction of total cholesterol and low density lipoprotein cholesterol	Wu et al (2017)[129]	15/976	Yes
Depressive symptoms	Wallace and Milev (2017)[79,130]	6/1080	Yes
Vulvovaginal candidiasis in non-pregnant women	Xie et al (2018)[131]	10/1656	Yes

From: Valdes AM, et al. Role of gut microbiota in nutrition and health. *BMJ* 2018;361:j2179

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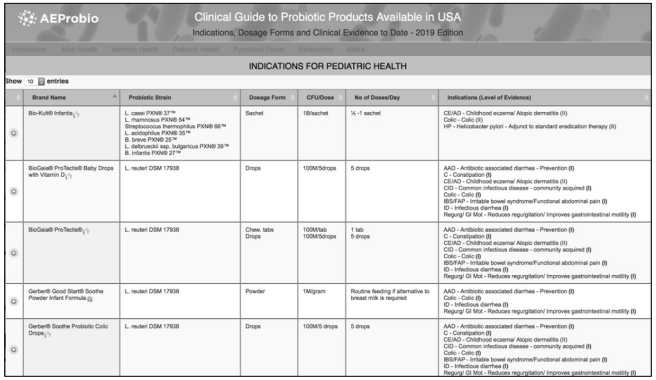
Clinical Resource Tool: www.**usprobioticguide**.com



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Click next to brand name to see evidence…

Colic - Colic	I	82. Savino, F., E. Pelle, E. Palumeri, R. Oggero, and R. Miniero. "Lactobacillus reuteri (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study." Pediatrics 119.1 (2007): e124-e130. 85. Chau, K., E. Lau, S. Greenberg, S. Jacobson, P. Yazdani-Brojeni, N. Verma, and G. Koren. "Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating Lactobacillus reuteri DSM 17938." The Journal of pediatrics 166.1 (2015): 74-78. 84. Sung, V., H. Hiscock, M.L.K. Tang, F.K. Mensah, M.L. Nation, C. Satzke, R.G. Heine, A. Stock, R.G. Barr, and M. Wake. "Treating infant colic with the probiotic Lactobacillus reuteri: double blind, placebo controlled randomised trial." BMJ 348 (2014): g2107. 83. Savino, F., L. Cordisco, V. Tarasco, E. Palumeri, R. Calabrese, R. Oggero, S. Roos, and D. Matteuzzi. "Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial." Pediatrics 126.3 (2010): e526-e533.

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

http://www.usprobioticguide.com/PBCPediatricHealth.html?utm_source=pediatric_ind&utm_medium=civ&utm_campaign=USA_CHART Accessed January 17, 2019

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Level 1 Evidence for Probiotics in Adults

- **Antibiotic Associated Diarrhea/*C. diff* Prevention**
  - Bio-K, Culturelle, Dan Active Actimel, Florastor and FlorastorMax, Schiff Digestive Advantage Advanced Probiotics, Ultra Flora Restore
- **Constipation**
  - Activia, Bio-Gaia Protectis (*also for GERD*)
- **IBS**
  - Activia, Align, Bio-K, BioKult, GoodBelly (*also for GERD*), Ideal Bowel Support, Ultra Flora Intensive Care (*also for GERD*)

http://usprobioticguide.com/PBCAdultHealth.html?utm_source=adult_ind&utm_medium=civ&utm_campaign=USA_CHART

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Level 1 Evidence for Probiotics in Children

- **Antibiotic Associated Diarrhea/*C. diff* Prevention**
  - BioGaia® Protectis, Culturelle Kids, FlorastorKids, Gerber Good Start Grow Toddler Probiotic + Gentle Infant Formula + Soothe Vitamin D and Probiotic Drops, Pedia-Lax® Probiotic Yums
- **Constipation**
  - BioGaia® Protectis, Gerber Good Start Grow Toddler Probiotic + Gentle Infant Formula + Soothe Vitamin D and Probiotic Drops, Pedia-Lax® Probiotic Yums
- **IBS/Functional Abdominal Pain**
  - BioGaia® Protectis, Culturelle Kids, Gerber Good Start Grow Toddler Probiotic + Gentle Infant Formula + Soothe Vitamin D and Probiotic Drops, Pedia-Lax® Probiotic Yums, Visbiome + Extra Strength

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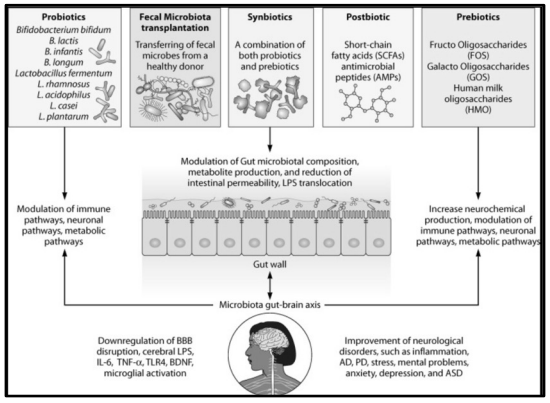

Image From: Sorboni SG, et al. Clin Microbiol Rev. 2022 Jan 19;35(1):e0033820. doi: 10.1128/CMR.00338-20.

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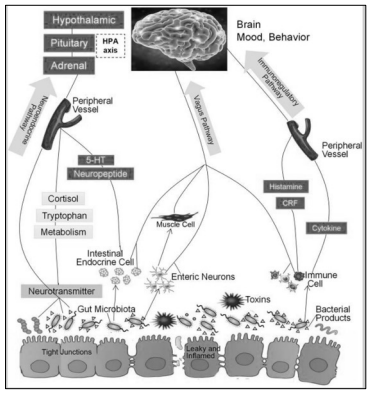

Sleep & Stress

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

Farre N, et al. Sleep and circadian alterations and the gut microbiome: associations or causality. *Current Sleep Med Reports* 2018; 4(1):50-57

Li, Y, et al. The role of microbiome in insomnia, circadian disturbance and depression. *Front Psychiatr* 2018; doi: 10.3389/fpsyt.2018.00669

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Melatonin & Sleep

- Melatonin secreted by **pineal gland** crucially important for **sleep-wake cycle**. **Suppressed by blue light, released with increasing darkness**.
- Strong evidence melatonin supplementation is **highly effective for shortening sleep latency**, especially those whose sleep is **delayed two or more hours** beyond 'normal' bedtime.[1]
- Melatonin should be taken 1.5–2 hours *before* desired bedtime. If you **want to fall asleep at 10 PM**, **take melatonin at 8 PM**.

1. Auld F, et al. *Sleep Med Rev* 2017

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- Melatonin gently **inhibits secretion of HCl**, stimulating release of gastrin, **increasing contraction of LES**.[1]
- Is important **mediator of gut-brain axis**, having protective effects against **stress-related damage to GI tract.**[1]
- Studies show it has **beneficial effect** on gut microbiome.[2]

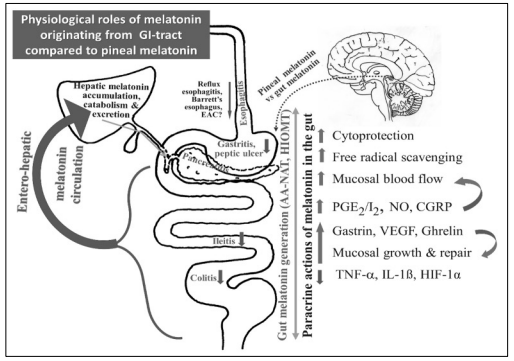

Image From: 1. Majka J. et al. *Int J Mol Sci* 2018
2. Yin J, et al. *J Pineal Res* 2018; Nov;65(4):e12524.

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IT IS ALL CONNECTED…

"When we try to pick out anything by itself, we find it hitched to everything else in the universe."
John Muir

- Eat a diet rich in **whole foods**, **whole plant foods**, **prebiotics**, **and fiber.**
- **Consider taking probiotics.**
- **Limit sugar and sugar substitutes.**
- Find healthy ways to **manage** your **stress** and get adequate **sleep**.
- Good **dental hygiene** & ***regular* dental visits.**

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