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THE GUT MICROBIOME: EFFECTS AND IMPLICATIONS IN PEDIATRIC HEALTH

Rebecca Heller, PhD, RD

Medical Science Liaison

Abbott Nutrition – Scientific and Medical Affairs

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DISCLOSURE

- The content of this program has met the continuing education criteria of being evidence-based, fair and balanced, and non-promotional
- This educational event is supported by Abbott Nutrition Health Institute, Abbott Nutrition
- I am an employee of Abbott Nutrition

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OBJECTIVES

- Review prebiotic and probiotic digestion and effect on the gastrointestinal tract
- Examine current literature on the microbiome
- Discuss the microbiome in gastrointestinal disorders
- Review the impact of dietary prebiotics and probiotics on gut health

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PREBIOTICS AND PROBIOTICS

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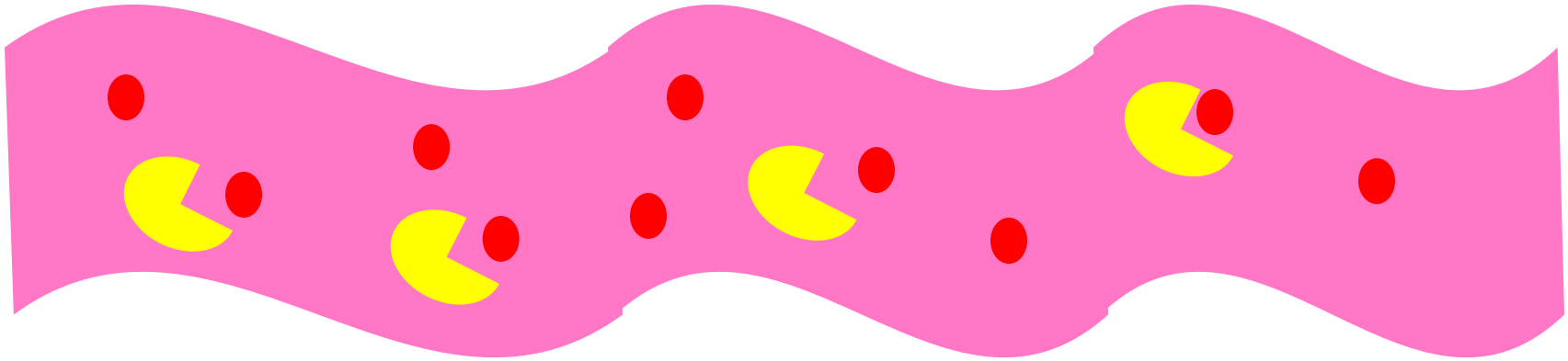
PREBIOTICS VS PROBIOTICS



- Prebiotic: “a substrate that is selectively utilized by host microorganisms conferring a health benefit” ***~International Scientific Association for Prebiotics and Probiotics (ISAPP)***
- Probiotic: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” ***~ISAAP and the Food and Agriculture Organization of the United Nations (FAO)***

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Gibson, et al. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Hill, C., et al. *Nature reviews Gastroenterology & hepatology*, 2014, *11*(8), 506-514.

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PREBIOTICS

Contain oligosaccharides

- Bananas
- Onions
- Garlic
- Leeks
- Asparagus
- Artichokes
- Whole wheat products
- Soybeans
- Chicory root
- Dandelion greens

Wolfram T. Prebiotics and probiotics: creating a healthier you. 2016. Retrieved from http://www.eatright.org/resource/food/vitamins-and-supplements/nutrient-rich-foods/prebiotics-and-probiotics-the-dynamic-duo

PREBIOTICS

- Contain oligosaccharides (including FOS, GOS, HMOs)
- Natural or synthetic sugars with varying degrees of polymerization
- Must be resistant to GI tract acidity and digestive enzymes as they must be fermented in the ileum and colon
- Fermentation produces short chain fatty acids (SCFA) and gasses including carbon dioxide, hydrogen, and methane. The longer the carbohydrate chain length, the longer it takes to ferment

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Eswaran, S., et al. *The American journal of gastroenterology*, 2013, *108*(5), 718.

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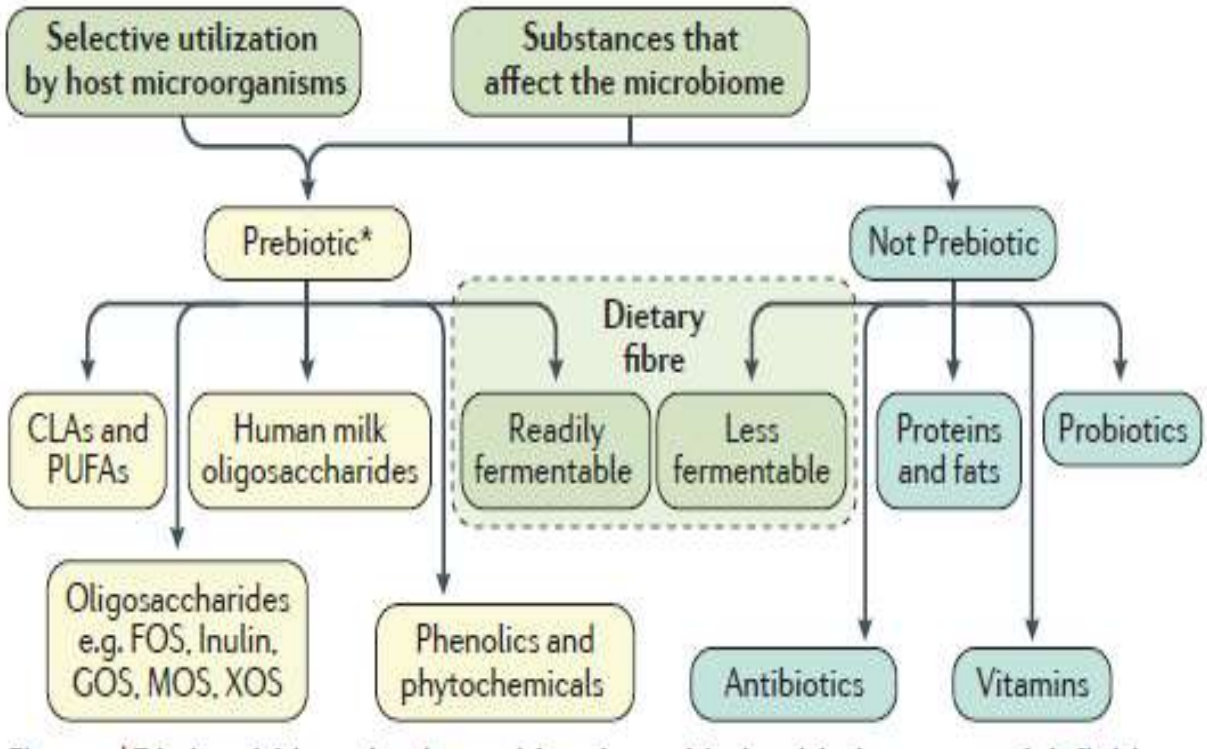
WHAT IS CONSIDERED A PREBIOTIC?

CLA= Conjugated linoleic acid
PUFA= polyunsaturated fatty acid
FOS= Fructooligosaccharide
GOS= Galactooligosaccharide
MOS= mannanoligosaccharide
XOS= xylooligosaccharide



*FOS and GOS are the most researched prebiotics

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Gibson, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491-502.

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SHORT CHAIN CARBOHYDRATES

- Highly soluble and fermentable oligosaccharides
- Metabolized by the gut microbiota into SCFAs in the colon
- scFOS is generally recognized as safe (GRAS) for healthy and compromised infants, children, and adults
  - Chain length: maximum 5 units
  - Consuming 1-5g/day of scFOS have been shown to have a positive effect on colonic microflora.
  - Stimulates growth of many bifidobacterial strains

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Eswaran S, et al. *The American journal of gastroenterology.* 2013;*108*(5):718.
Tokunaga T, et al. *Bifidus.* 1993;6:143-150.
Hidaka H, et al. *Bifidobacteria Microflora.* 1986;5(1):37-50.
Bouhnik Y, et al. *Nutr J.* 2007;6:42. doi: 10.1186/1475-2891-6-42.

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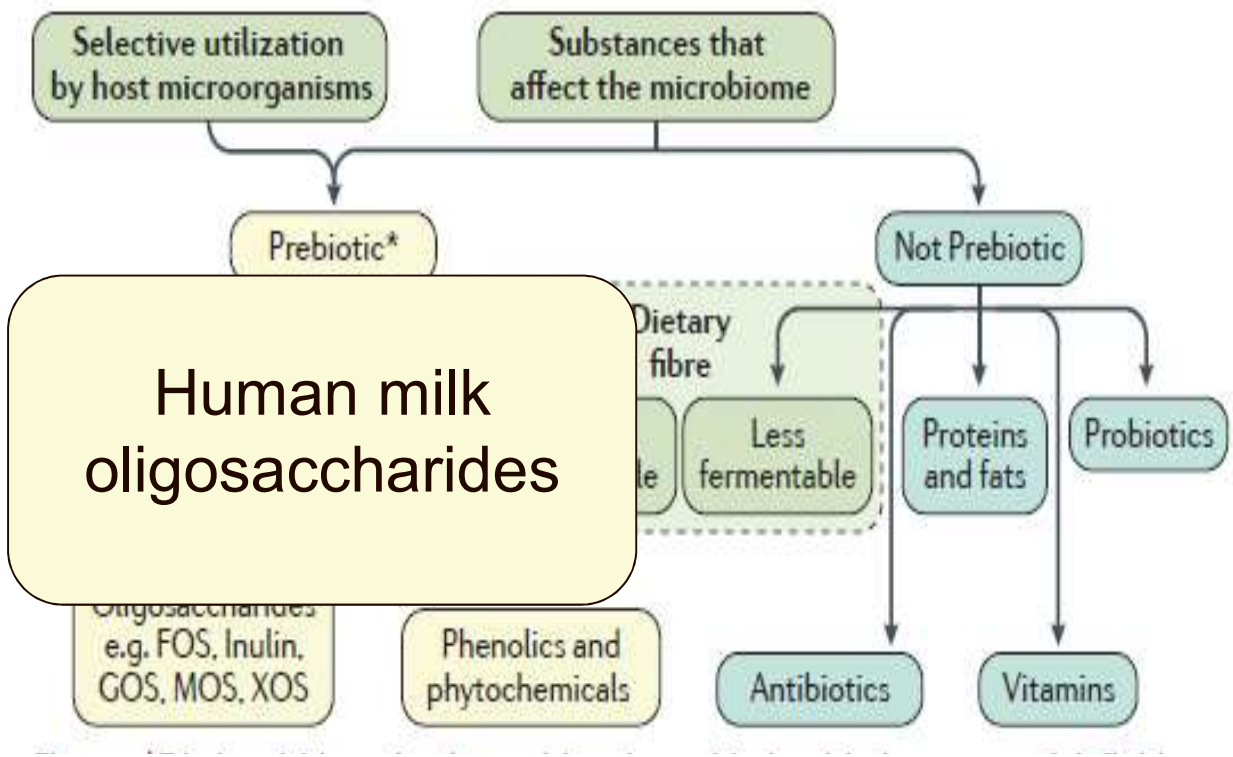

*FOS and GOS are the most researched prebiotics

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Gibson, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491-502.

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HUMAN MILK OLIGOSACCHARIDES

- Soluble and fermentable in colon
- Serve as metabolic substrate for beneficial bacteria in the colon, creating SCFAs
- Can enter circulation and travel through blood stream
- Complex oligosaccharide structures
  - Building blocks for HMOS: glucose, galactose, N-Acetyl-Glucosamine, fucose, and sialic acid
  - Vary in structure from 3-22 monosaccharide units
  - ~200 different HMO structures

Ayechu-Muruzabal, V., et al. (2018). Diversity of Human Milk Oligosaccharides and Effects on Early Life Immune Development. *Frontiers in pediatrics*, *6*, 239.
Ray, C., Kerketta, J., Rao, S., et al. (2019). Human Milk Oligosaccharides: The Journey Ahead, *International Journal of Pediatrics*, 2019, 2390240

CLASSIFICATION OF HMOS

Classification	Category	Structure	Percent of total HMOs
Neutral	Fucosylated	contain fucose at the terminal position	35-50%
Neutral	Non-fucosylated	contain N-acetylglucosamine at the terminal end	42-55%
Acidic	Sialylated	contain sialic acid at terminal end	12-14%

Plaza-Díaz, J., Fontana, L., & Gil, A. (2018). Human Milk Oligosaccharides and Immune System Development. *Nutrients*, *10*(8), 1038. doi:10.3390/nu10081038
Ray, C., Kerketta, J., Rao, S., et al. (2019). Human Milk Oligosaccharides: The Journey Ahead, *International Journal of Pediatrics*, 2019, 2390240

PROBIOTICS

Found in foods that have undergone fermentation

- Kefir
- Sauerkraut
- Yogurt
- Tempeh
- Apple cider vinegar
- Kimchi
- Kombucha
- Miso

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Wolfram T. Prebiotics and probiotics: creating a healthier you. 2016. Retrieved from http://www.eatright.org/resource/food/vitamins-and-supplements/nutrient-rich-foods/prebiotics-and-probiotics-the-dynamic-duo

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PROBIOTICS

- Participate in the conversion of prebiotics in to short chain fatty acids
- Contribute to bile acid metabolism
- Provide a barrier against pathogenic bacteria
- Modulate the innate and adaptive immune systems

Palma G, et al. *The Journal of Physiology.* 2014;592(14):2989-2997.

Prebiotics Feed Healthy Microbiota

PREBIOTICS
(HMOs, FOS, GOS)

PROBIOTICS
(Beneficial Bacteria)

Short Chain Fatty Acids

Support immune responses

Support metabolism

Promote digestive tract health

Support the developing mucosal barrier

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Bode L. Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology*. 2012;22:1147-1162.
Kleinman RE, Greer FR. *Pediatric Nutrition*. 7th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2014; 94-95.

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SCFA

- Acetate, propionate, butyrate, and valerate:
  - Preferred source of energy for colonic cells, helping maintain intestinal integrity
  - Enhance water and electrolyte absorption
  - Create an acidic environment that is unfavorable to pathogens
    - Decrease in intraluminal pH → increase in *Bifidobacteria*, *Lactobacilli*, and non-pathogenic *E. Coli* + a decrease in *Bacteriodaceae*

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Eswaran S, et al. *The American journal of gastroenterology*. 2013;*108*(5):718.

SCFA – BUTYRATE

- Plays major role in regulation of cell proliferation and differentiation in IBS
  - In IBS, deficit in butyrate causes tight junction lesions and impaired intestinal permeability
- Exerts effects on myenteric neurons and motility
- Maintains intestinal barrier
- Has been shown to suppress colonic inflammation by the inhibition of the IFN-γ / STAT1 signaling pathway

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Eswaran S, et al. *The American journal of gastroenterology*. 2013;*108*(5):718.
Cho SS, Finocchiaro T. (Eds.). *Handbook of prebiotics and probiotics ingredients: health benefits and food applications*. CRC Press. 2009.

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THE MICROBIOME – INFANCY THROUGH ADOLESCENCE

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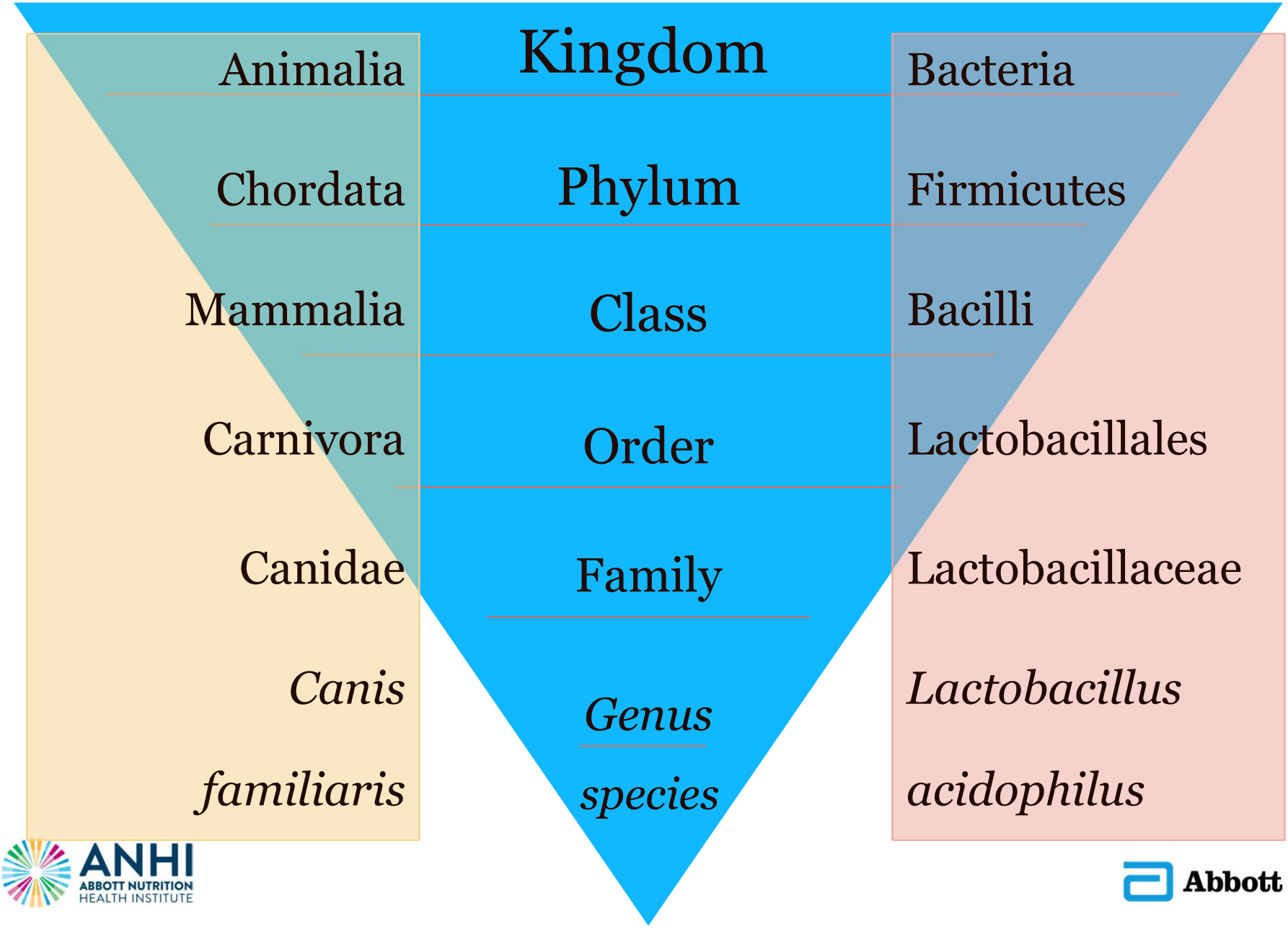
TAXONOMY



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DOMINANT PHYLA

FIRMICUTES
Clostridium
Roseburia
Faecalibacterium
F Prausnitzili
Blaua
Dorea
Lactobacillus
Peptostreptococcus
Eubacterium
Streptococcus
Staphylococcus
Butyrivibrio

BACTEROIDETES

Bacteroides
Prevotella

ACTINOBACTERIA

Bifidobacterium
Collinsella

PROTEOBACTERIA
Escherichia
Klebsiella
Desulfovibrio

ARCHAEA
Methanobrevibacter
M Smithii

VERRUCOMICROBIA
Akkermansia

FUSOBACTERIA
Fucobacteriaceae

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Shortt C, et al. *Eur J Nutr.* 2018;57(1):25-49. dio:10.1007/s00394-017-1546-4

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MAIN FUNCTIONS OF MICROBIOTA

Metabolic

- Fermentation
- Energy salvage
- Vitamin production (K, B12, etc.)
- Absorption Ca, Fe, etc.
- Epithelial cell proliferation & differentiation

Protective

- Pathogen displacement
- Nutrient & receptor competition
- Anti-microbial factors

Structural

- GI barrier fortification
- Support immune function
- Induction of IgA
- Maintain tight junctions

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Grenham S, et al. *Frontiers in Physiology.* 2011;2(94):1-15
Guarner F. *Digestion.* 2006;73(suppl 1):5-12

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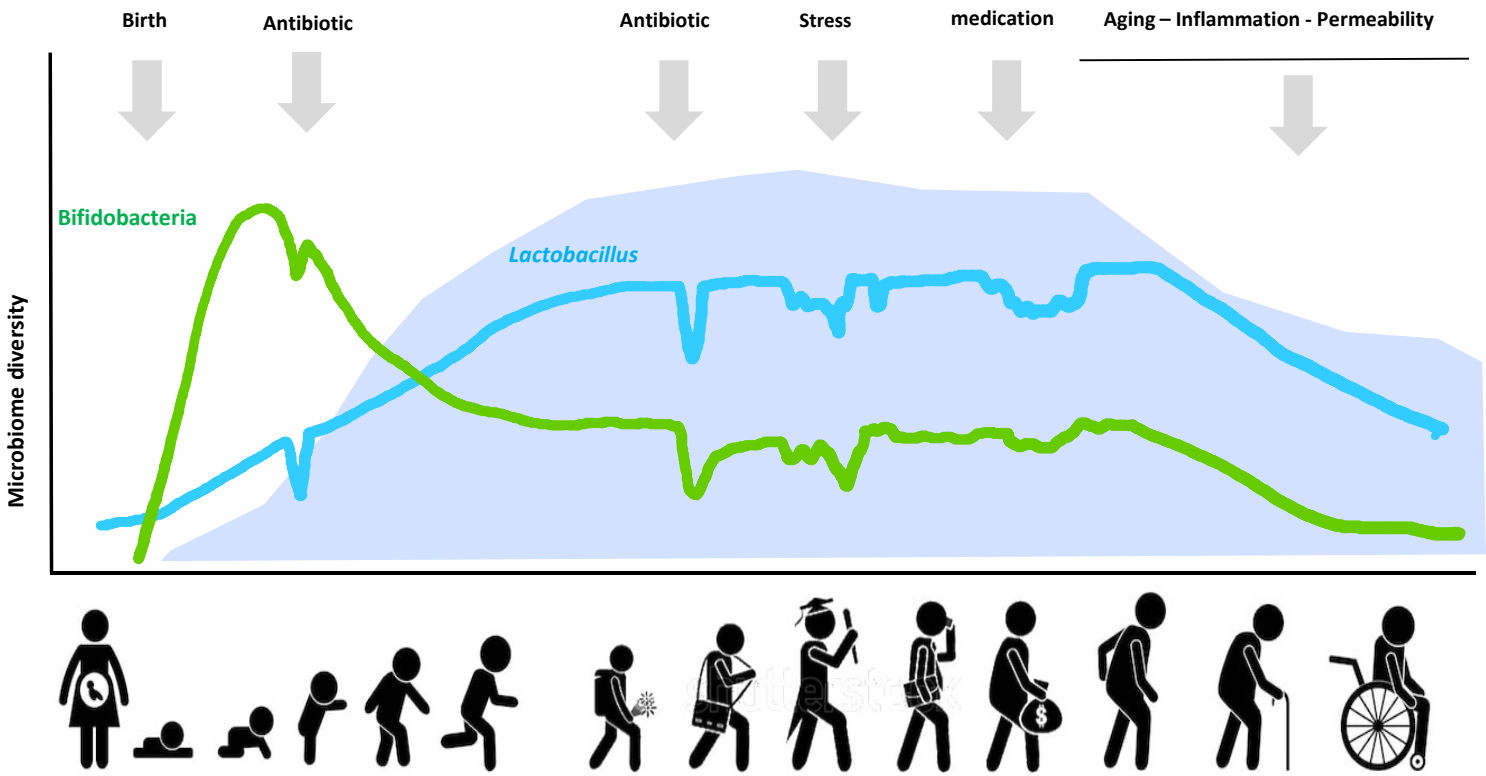
THE GASTROINTESTINAL (GI) MICROBIOME AND HEALTH ARE INTIMATELY CONNECTED THROUGH ALL LIFE STAGES…



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Odamaki T, et al. BMC Microbiol. 2016; 90 (16)

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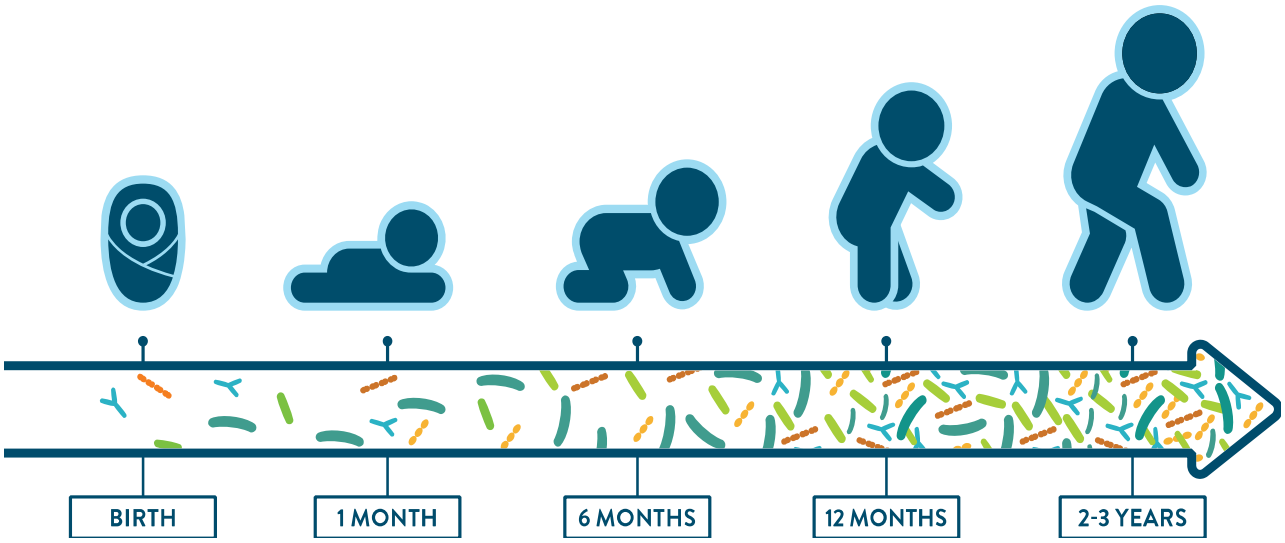
GUT MICROBIOTA AND EARLY LIFE

- Infancy is a critical window for microbiome development



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Laitinen K, *et al*. Impact of early nutrition on intestinal microbiome: effects on immunity and long-term health. In: Saavedra JM, Dattilo AM, eds. *Early Nutrition and Long-Term Health: Mechanisms, Consequences, and Opportunities*. Cambridge, UK: Elsevier; 2017:203-228.

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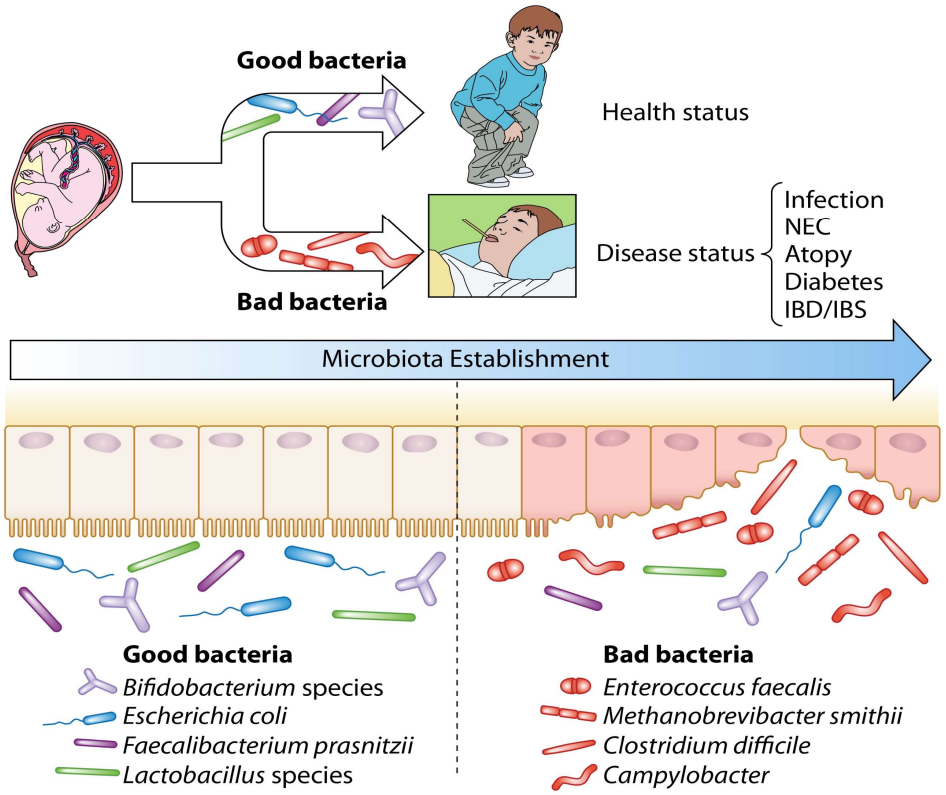
MICROBIOTA ESTABLISHMENT INFLUENCES INFANT HEALTH STATUS



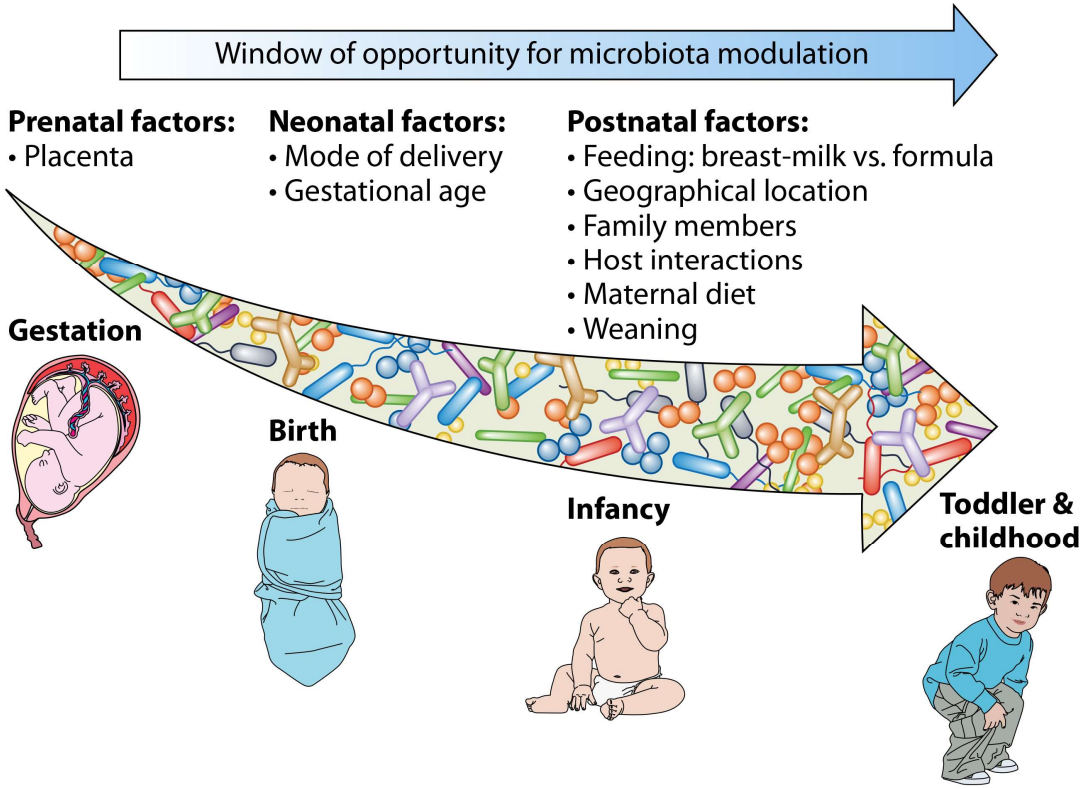
Milani C. *Micro and Mol Bio Rev*. 2017; 81 (4)

GUT HEALTH AND THE MICROBIOME

- Gastrointestinal tract contains a majority of the immune system
  - Intestinal bacteria
  - Immune cells
  - Mucosal-associated lymphoid tissue (MALT)
- Presence of beneficial bacteria and microbial profile
  - Decreases risk of infection and improves future health outcomes
- Microbiota in healthy full-term infants is primarily beneficial bacterial strains

Morowitz MJ, et.al. Strain-resolved community genomic analysis of gut microbial colonization in a premature infant. *Proceedings of the National Academy of Sciences.* 2011;108(3):1128-1133.
Butel et al. Conditions of Bifidobacterial Colonization in Preterm Infants: A Prospective Analysis. *Journal of Pediatric Gastroenterology and Nutrition.* 2007;44(5):577-82.

MODULATION OF GI MICROBIOTA FROM GESTATION TO CHILDHOOD



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Milani C. *Micro and Mol Bio Rev*. 2017; 81 (4)

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BIRTH THOUGH 6 MONTHS

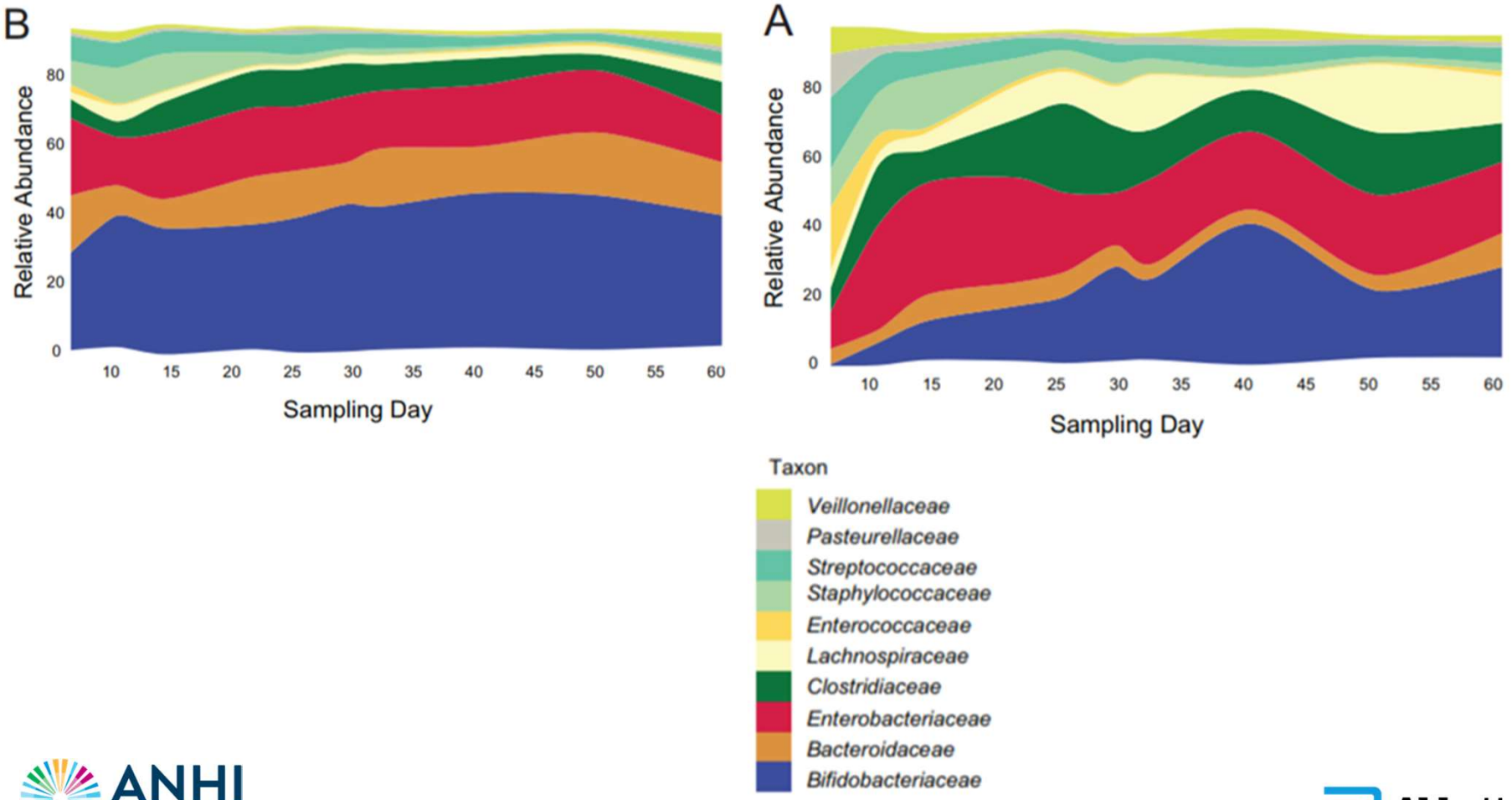
- The placenta was previously thought to be sterile, but now we know that the placenta has it's own microbiome
- **Method of delivery:**
  - **Vaginal:** Birth canal is heavily colonized by *Lactobacillus* and *Prevotella*. Found on infant's skin and mouth, and are present in meconium
  - **Cesarean Section:** Baby is colonized by microorganisms found on skin including *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*

Mueller CM, et al. *The ASPEN adult nutrition support core curriculum*. Silver Spring, MD: American Society for Parenteral and Enteral Nutrition; 2012.
Penders, J. *Early diet and the infant gut microbiome: how breastfeeding and solid foods shape the microbiome*. In Microbiota in health and disease: from pregnancy to childhood (Chapter 5:1281-1292). Wageningen Academic Publishers; 2017

PREDOMINANT BACTERIA BY MODE OF DELIVERY

Vaginal Delivery

Cesarean Section



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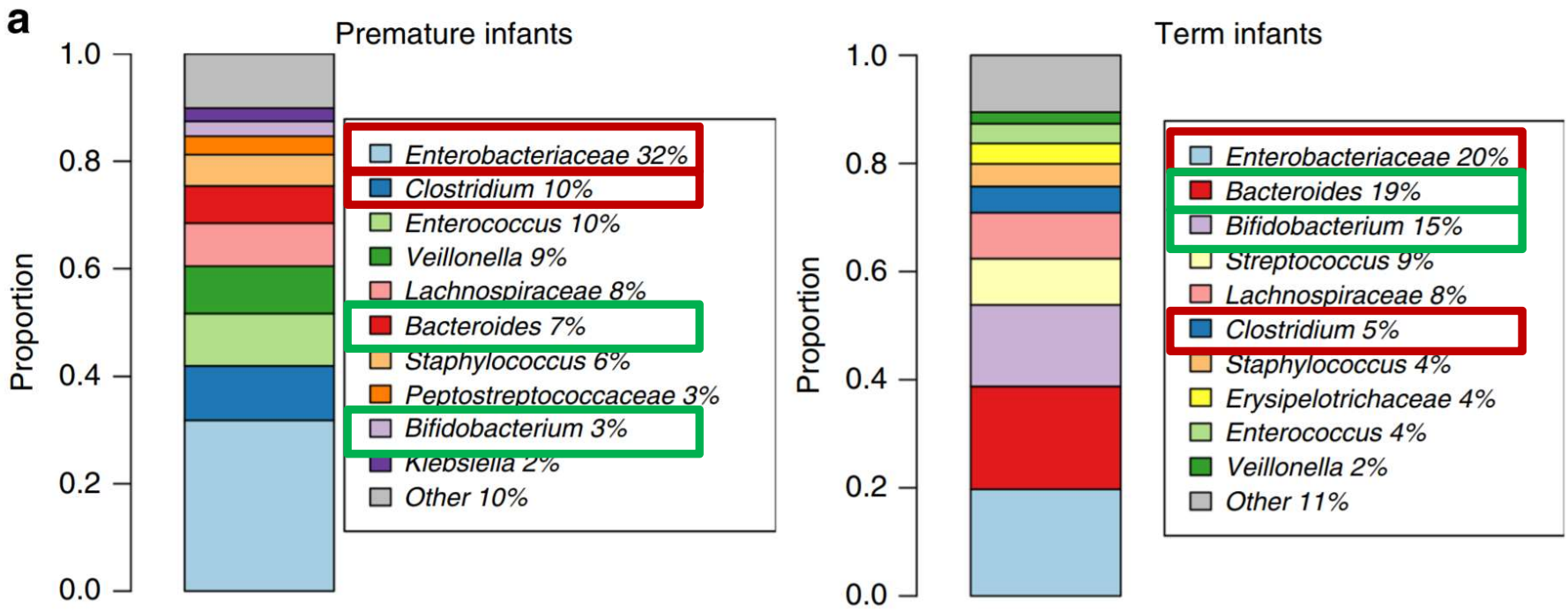
Frese SA, et al. *mSphere.* 2017;2(6): pii:e00501-e00517.

MICROBIOME OF PRETERM INFANTS

- Microbiota in preterm infants differs from healthy full-term infants
  - Antibiotic use
  - Underdeveloped immune system and gut epithelia

Chernikova DA, et al. The premature infant gut microbiome during the first 6 weeks of life differs based on gestational maturity at birth. *Pediatric Research.* 2018;84(1):71-79.
Cong et al. Gut Microbiome Developmental Patterns in Early Life of Preterm Infants: Impacts of Feeding and Gender. *PLOS ONE.* 2016; 11(4):e0152751.



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Chernikova DA, et al. *Pediatric Research.* 2018;84(1):71-79.

MICROBIOME OF PRETERM INFANTS

- Microbiota in preterm infants differs from healthy full-term infants
  - Antibiotic use
  - Underdeveloped immune system and gut epithelia
- Prebiotics can have positive health implications
  - HMOs in particular have been linked to improved microbiota profile
- Improving infant immunity
  - Microbiome
  - Gut health
  - Inflammatory responses

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Chernikova DA, et al. The premature infant gut microbiome during the first 6 weeks of life differs based on gestational maturity at birth. *Pediatric Research.* 2018;84(1):71-79.
Cong et al. Gut Microbiome Developmental Patterns in Early Life of Preterm Infants: Impacts of Feeding and Gender. *PLOS ONE.* 2016; 11(4):e0152751.

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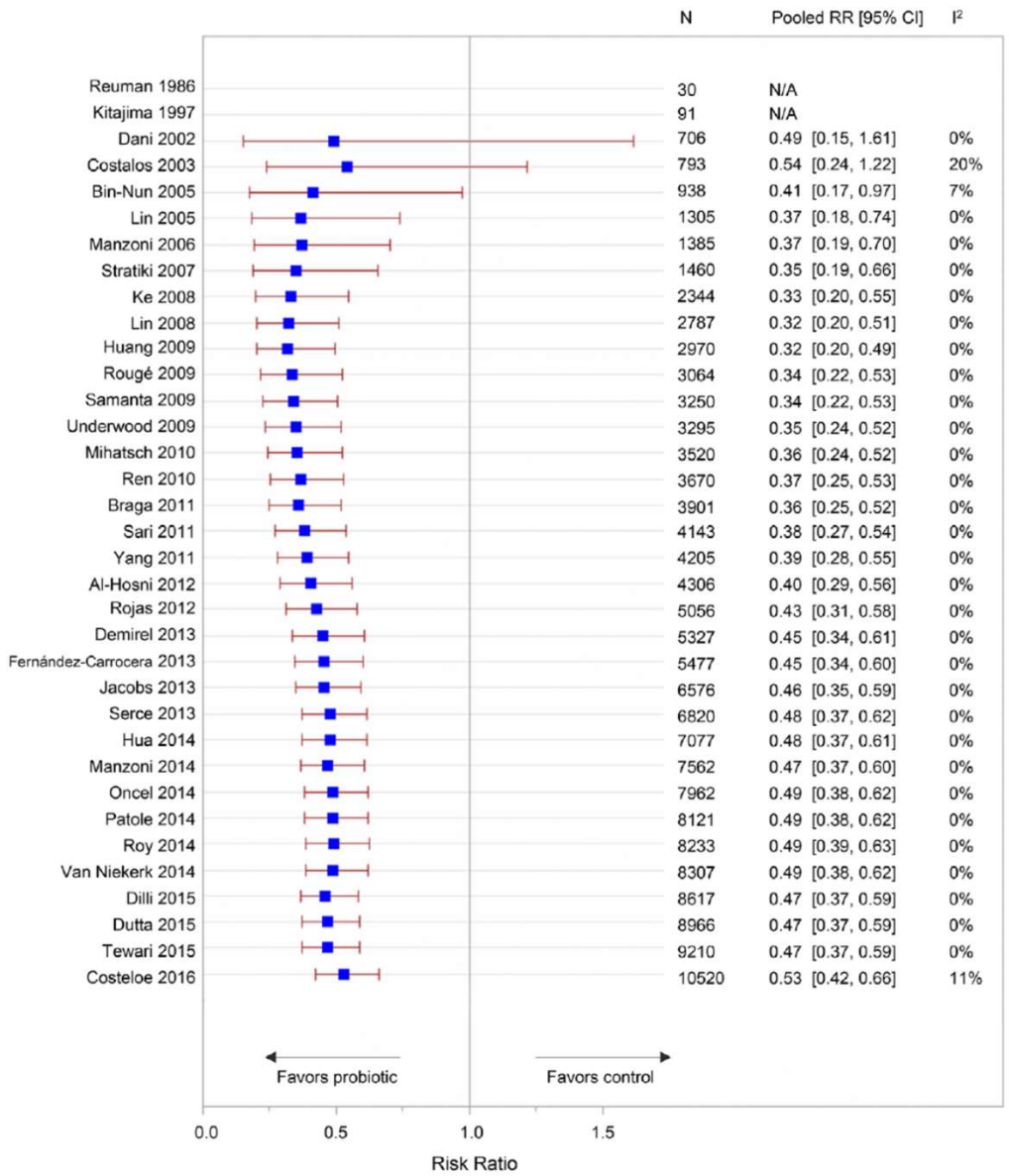
PROBIOTICS IN THE NICU



Patel, Underwood. *Semin Ped Surg*. 2018 Feb;27(1):39-46.

BIRTH THOUGH 6 MONTHS

- **Oral intake:**
  - **Breast milk fed:** Human milk has it's own microbiome. 150+ human milk oligosaccharides. 2'-Fucosyllactose is most abundant. High in certain strains of *Lactobacillus* and *Bifidobacteria.*
  - **Formula fed:** Microbiome is more diverse with higher levels of bacteroides, clostridia and *Enterobacteriaceae*

Mueller CM, et al. *The ASPEN adult nutrition support core curriculum*. Silver Spring, MD: American Society for Parenteral and Enteral Nutrition; 2012.
Penders, J. *Early diet and the infant gut microbiome: how breastfeeding and solid foods shape the microbiome*. In Microbiota in health and disease: from pregnancy to childhood (Chapter 5:1281-1292). Wageningen Academic Publishers; 2017.
Vangay P, et al. *Cell host & microbe.* 2015;*17*(5):553-564.

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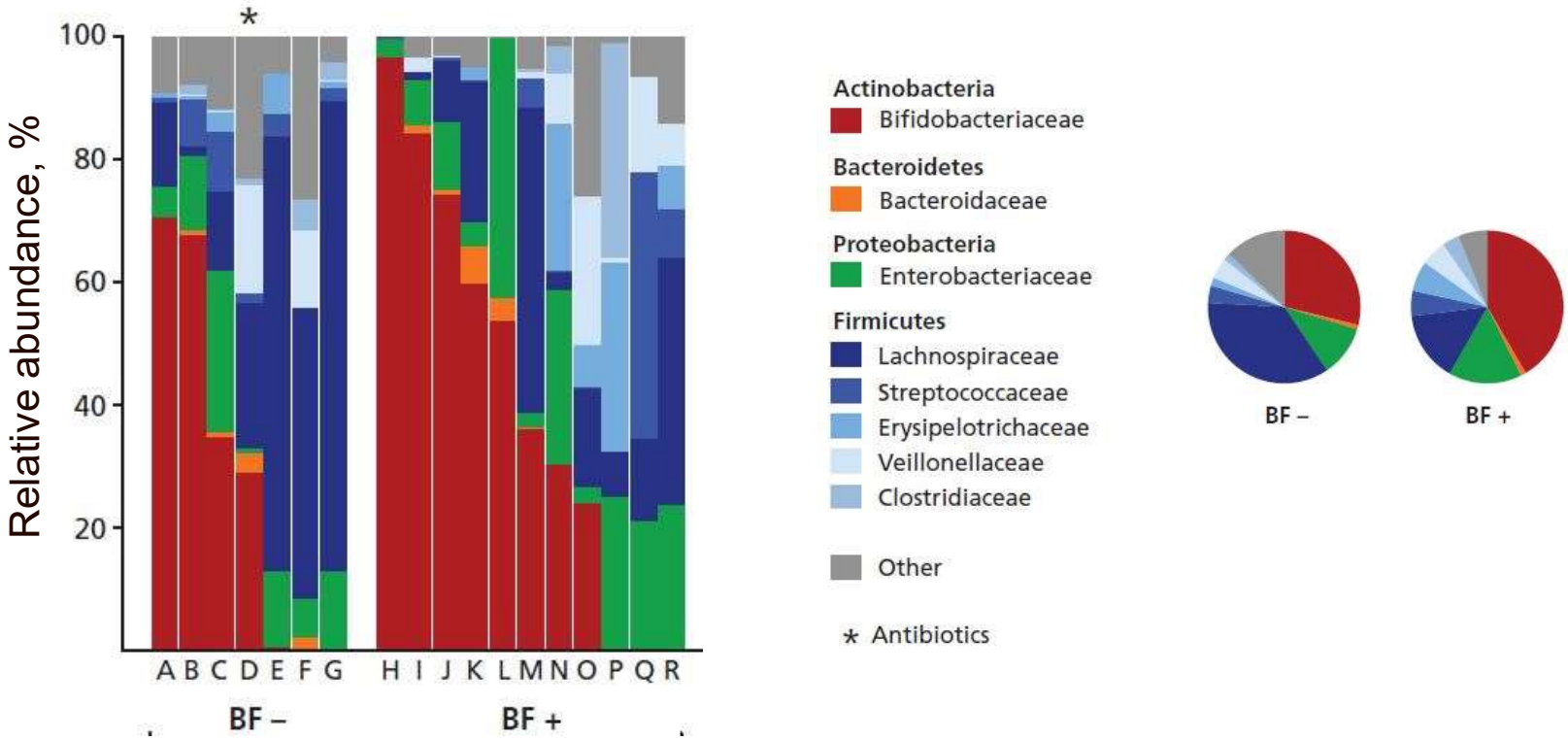
INFANT MICROBIOME BY FEEDING METHOD



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Azad M, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ*. 2013;185(5):385-94.

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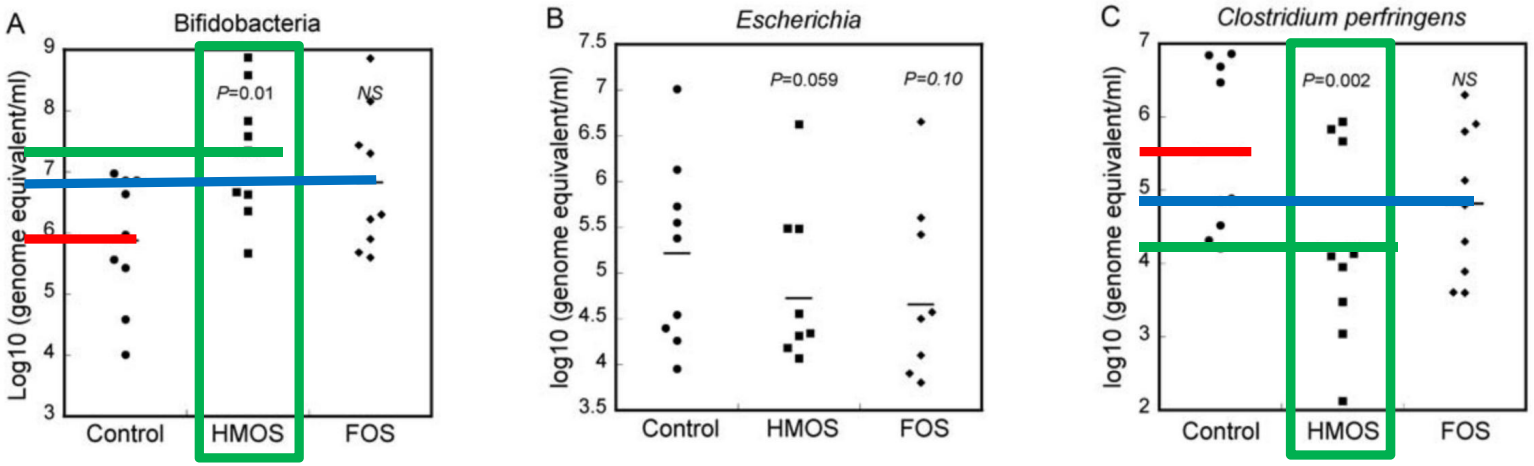
HMO IMPACT ON GUT MICROBIOTA IN INFANTS



Red = Control
Green = HMO
Blue = FOS

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Yu Z, et al. *Glycobiology.* 2013;23(2):169-177.

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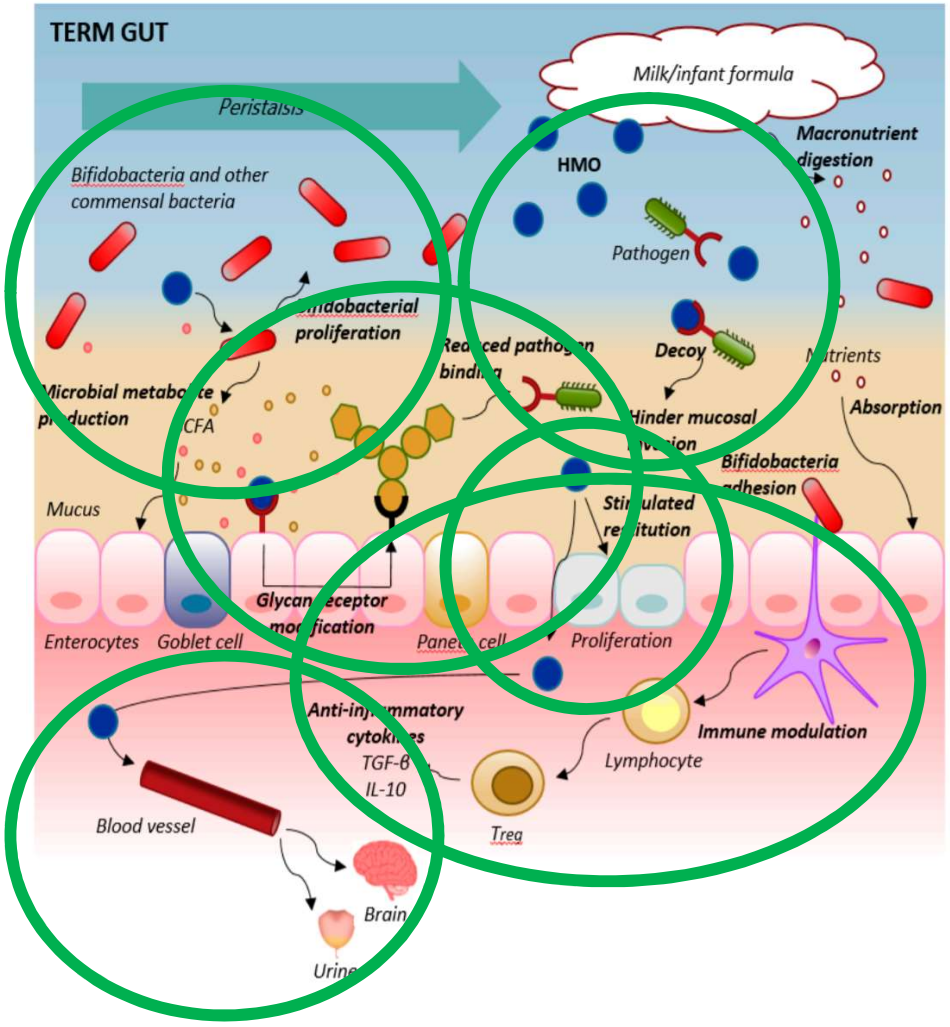
PREBIOTIC EFFECTS ON THE IMMUNE SYSTEM



Bering SB. *Nutrients*. 2018;10(10): pii:E1461.

INTRODUCTION OF SOLID FOODS AND BEYOND

- Mode of delivery has no lasting impact on infant's microbiome.
- Solid foods introduced →
  - Microorganisms that degrade complex sugars and starches flourish (*Prevotella*)
  - Microbiome dominated by *Bacteroides* and *Clostridium*, which indicates adaptation to a more complex diet
  - Diversity of microorganisms begin to resemble the adult microbiome

Penders, J. *Early diet and the infant gut microbiome: how breastfeeding and solid foods shape the microbiome*. In Microbiota in health and disease: from pregnancy to childhood (Chapter 5:1281-1292). Wageningen Academic Publishers; 2017.

7-12 YEARS

- Hollister, et al (2015)
- n= 22 healthy children aged 7-12, and 43 healthy adults
- Gene sequencing was performed on the stool samples
- Results:
  - *Bacteroides* account for 40% of the average healthy child's microbiome with *Faecalibacterium*, *Alistipes*, *Ruminococcus*, *Roseburia*, and other genera composing the balance
  - Richness, diversity, and community structure did not differ based on sex, ethnicity, or BMI. Richness and diversity didn't vary according to race variation
  - Microbiome of children contained a greater number of genera

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Hollister EB, et al. *Microbiome.* 2015;*3*(1):36.

11-18 YEARS

- Agans, et al (2011)
- n= 22 children aged 11-18, and 10 adults who followed a western diet, and had no GI symptoms + no antibiotics in the 3 months prior to stool sampling
- Gene sequencing was performed on the stool samples
- Results (when compared to adult stool samples):
  - Genus *Bifidobacterium* were higher in children than adults, and the level of *Bifidobacterium* gradually decreased with the child's age
  - *Ruminococcus* was the most prevalent genus in all stool samples examined (primary carbohydrate degraders in the gut)

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Agans R, et al. *FEMS microbiology ecology.* 2011;77(2):404-412.

PEDIATRIC OBESITY – 8-12 YEARS

- Borgo, et al (2017)
- n= 28 obese, and 33 age and sex matched normal weight children who were singletons born at term gestation, at ≥2,500gm. None of the children were on antibiotics or probiotics for 1 month prior to the start of the study. Obesity was defined using World Health Organization criteria.
- Results (when compared to normal weight children):
  - Obese children consumed significantly more calories, carbohydrates, protein, and total fat
  - No difference in *Bifidobacterium, Lactobacillus,* and enterobacteriaceae
  - *A. muciniphyla*, *F. prausnitzii*, and *Bacteriodes*/*Prevotella* were significantly less abundant

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Borgo F, et al. *Childhood Obesity.* 2017;*13*(1):78-84.

SO WHAT?

- *A. Muciniphyla*: Mucin degrading bacteria and dominant colonizer of the intestinal mucus layer. It increases the expression of acylglycerols, and reverses the thinning of the mucus layer caused by a high-fat diet
- *F. prausnitzii: A*ppears to have significant anti-inflammatory activity and increased production of anti inflammatory IL-10 in cell cultures. Low amounts could be caused by long-standing inflammation
  - Mouse model: Together with other commensal bacteria work to protect and preserve the intestinal mucosa
- *Bacteriodes:* Help degrade fat and protein
- *Prevotella*: Help degrade complex sugars and starches

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Borgo F, et al. *Childhood Obesity.* 2017;*13*(1):78-84.

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MICROBIOME IN NEONATAL/ PEDIATRIC DIAGNOSES

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DYSBIOSIS OF GUT MICROBIOTA ARE ASSOCIATED WITH…

- Celiac disease
- Irritable bowel disease
- Irritable bowel syndrome
- Necrotizing enterocolitis
- Anxiety
- Depressive behaviors
- Alzheimer's disease
- HIV/AIDS
- Autism spectrum disorders
- Multiple sclerosis
- Progression of chronic kidney disease
- Allergy
- Asthma
- Cardiovascular disease
- Parkinson's disease

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Carabotti M, et al. *Gastroenterology: quarterly publication of the Hellenic Society of Gastroenterology.* 2015;*28*(2):203.
Carding S, et al.. *Microbial ecology in health and disease.* 2015;*26*(1):26191
Ramezani A, Raj D. *Journal of the American Society of Nephrology.* 2013;ASN-2013080905
Davis BC, et al. In *Seminars in Liver Disease* (Vol. 37, No. 02, pp. 128-140). Thieme Medical Publishers.

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ANTIBIOTIC USE

- Antibiotics are most commonly prescribed medication for children
- There are associations between antibiotic usage in early infancy and occurrence of diseases such as obesity, diabetes, and asthma in later life
- There are short and long term effects of early life antibiotic use on the diversity and composition of gut microbes
- Broad spectrum antibiotics can cause:
    1. Unintended loss of microbes that are critical for maintaining homeostasis or proper host development (immune system)
    2. An overall loss of biodiversity (hygiene hypothesis)
    3. Blooms of pathogenic microbes
    4. Improper or incomplete recovery can cause a shift in functional capacity

Vangay P, et al. *Cell host & microbe.* 2015;*17*(5):553-564.

ANTIBIOTIC EXPOSURE

0-6 months

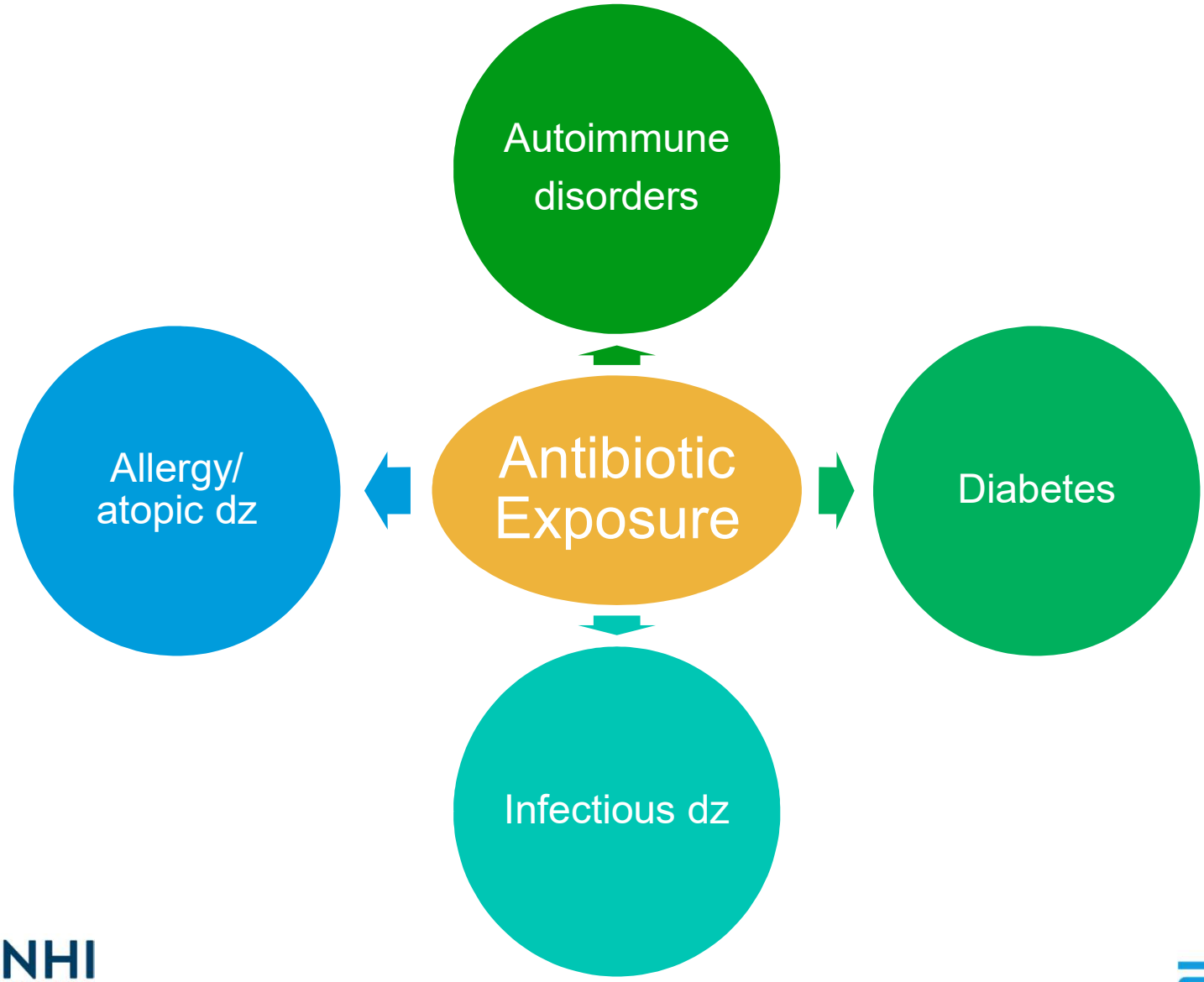
- Infants are most vulnerable to dysbiosis

0-24 months

- Exposure during the first 2 years of life predisposes the child to obesity during adulthood
- Changes in the microbiota result in functional changes that affect metabolism

Vangay P, et al. *Cell host & microbe.* 2015;*17*(5):553-564.
Mitre E, et al. *JAMA Pediatrics.* 2018;172(6)e180315-e180315.

ANTIBIOTIC EXPOSURE



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Vangay P, et al. *Cell host & microbe.* 2015;*17*(5):553-564.
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NEONATAL/PEDIATRIC DIAGNOSES – GI

Diagnosis	Microbiome
Necrotizing Enterocolitis (NEC)	Proteobacteria:Firmicute ratio altered in infants who subsequently develop NEC
Short Bowel Syndrome (SBS)	Proteobacteria:Firmicute ratio altered in infants who subsequently develop parenteral nutrition associated liver disease (PNALD) and central line associated blood stream infections (CLABSI) Children with SBS on TPN have overall decreased bacterial diversity, significantly more *Enterobacteriacae*

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PEDIATRIC DIAGNOSES – GI

Diagnosis	Microbiome
Irritable bowel syndrome	Proteobacteria bloom seen right before flare up
Crohn's Disease IBD	Strong dysbiosis. Increase in Enterobacteriaceae, Pasteurellacaea, Veillonellaceae, and Fusobacteriaceae. Decreased in Erysipelotrichales, Bacteroidales, and Clostridiales.
Ulcerative Colitis IBD	Decrease in microbial diversity however no strong dysbiosis
Celiac Disease	Classical GI manifestations: decreased microbial diversity, microbiota composition dominated by Proteobacteria

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PEDIATRIC DIAGNOSES – GI

Diagnosis	Microbiome
Eosinophilic Esophagitis (EoE)	Esophageal overall microbe count significantly higher, *Haemophilus, Neisseria*, and *Corynebacterium* increased in untreated EoE. Little is known about lower GI microbiome at this time
Cystic Fibrosis	Enterobacteriaceae over represented, *Bifidobacterium, Clostridium,* and *Parabacteroides* under represented. *Changes in diet seem to impact respiratory microbiome *repeated/chronic antibiotic use, low pH in intestinal lumen due to pancreatic insufficiency

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SUMMARY/CONCLUSIONS

- Scientific advancements have allowed researchers to explore the microbiome down to the species level of taxonomy
- Each day scientists are gaining a better understanding of the gut microbiome, but there is so much more to uncover
- Throughout infancy through adolescence the microbiome changes and develops with the child
- Shifts in microbes may be able to predict the onset of certain diseases. Different disease states can alter the microbiome

Thank You!

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QUESTIONS/COMMENTS?

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