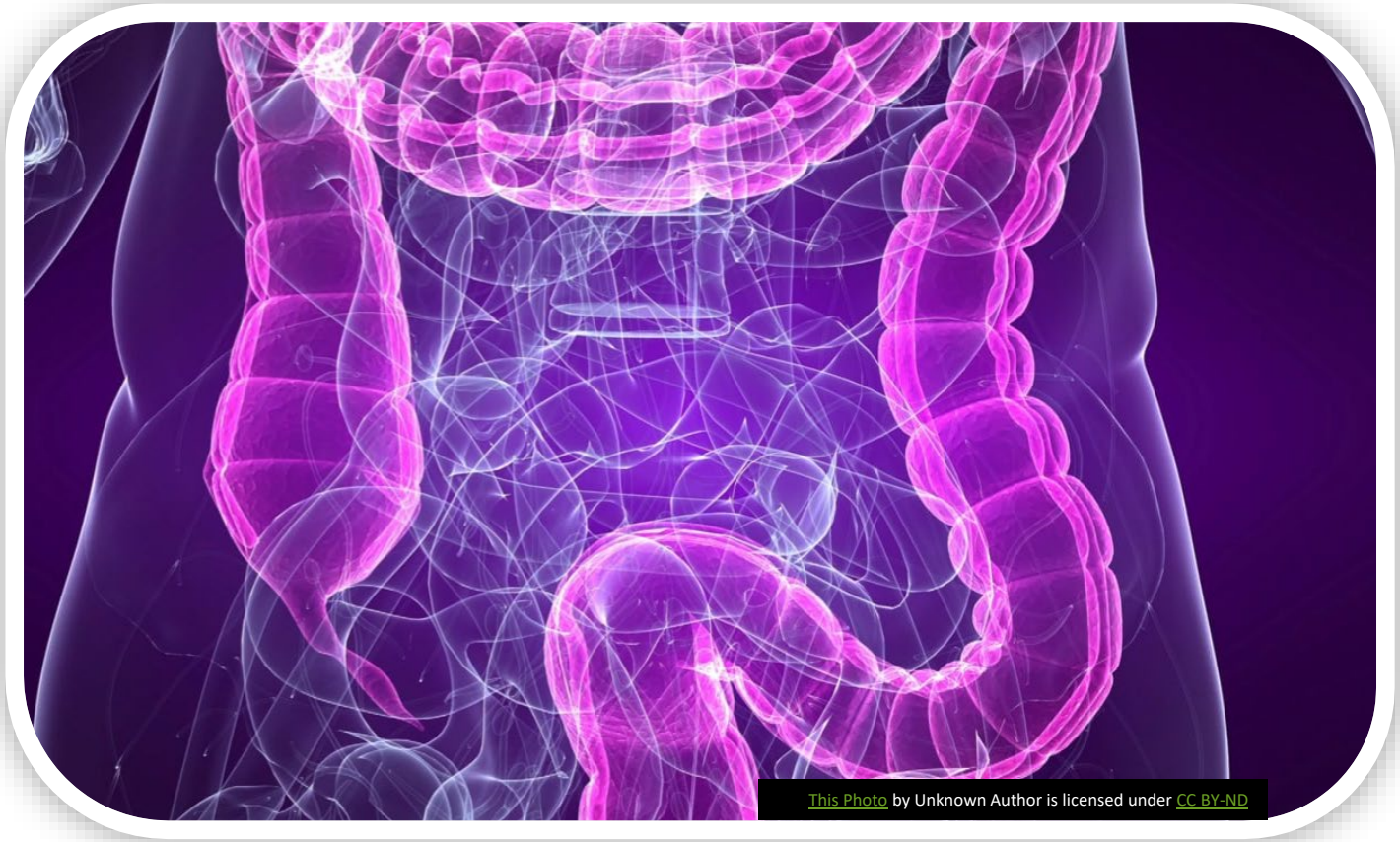
Gut Microbiota-Host Interactions: Assessment and Endocrine Implications

Julia Malkowski, ND, DC, BSc

LENS

Laboratory, Endocrine, & Neurotransmitter Symposium



"All disease begins in the gut,"
Hippocrates
3rd Century B.C.E

Gut microbiota

- First to colonize the infant gut; Clostridia
- Two of the major phyla, namely Bacteroidetes and Firmicutes
- Additional major pyla Actinobacteria, Verrucomicrobia, Tenericutes, Proteobacteria
- Bacterial abundance and diversity of phyla and species
- More than 10,000 microbial species occupy the human ecosystem
- The environment and diet during the first 3 years of life are crucial to the acquisition of an adult-like microbiota and to the establishment of bacterial–host symbiosis that influences the development of the immune and neurologic systems
- The human gut microbiota reaches the characteristics of an adult microbiota between the ages of 2 and 5 years

https://sciencing.com/types-spore-forming-bacteria-2504.html

Genome Med. 2016; 8:42.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4839080/

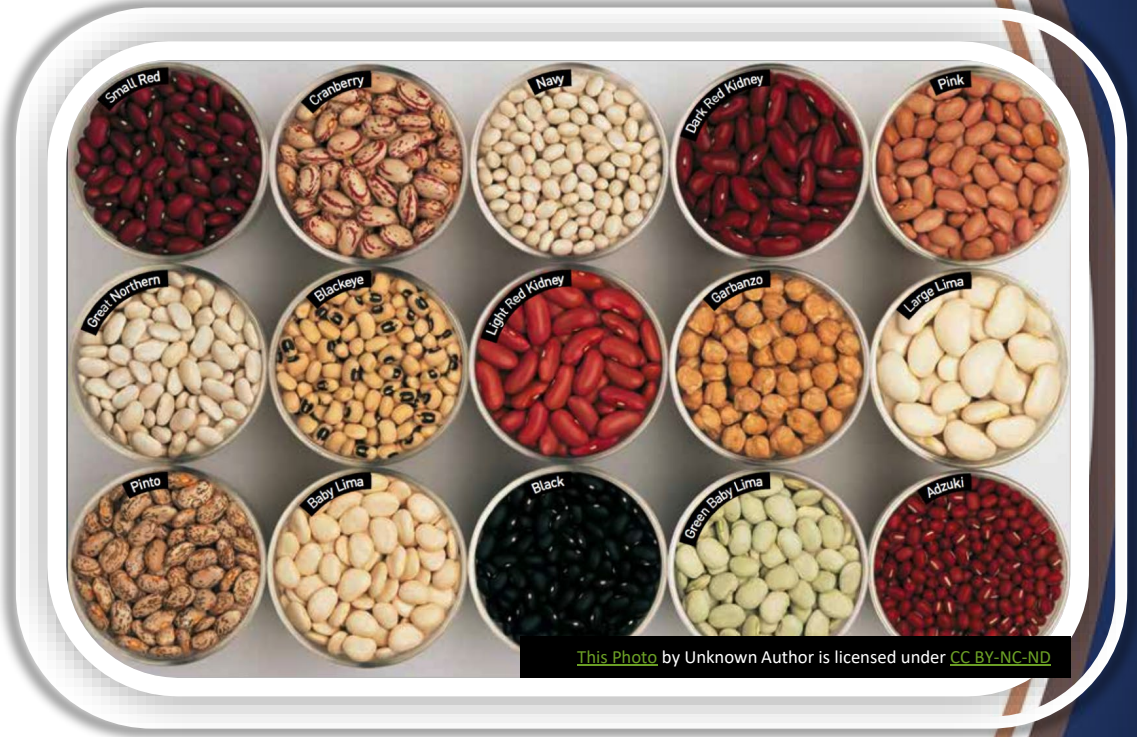
Microbiota

- Microorganisms within the colon. Includes bacteria, archaea, and viruses.
- Protection from invasive pathogens
- Processing of nutrients to bioactive molecules, including neurotransmitters, vitamins and fatty acids
- Influence levels of other neurotransmitters, including histamine, gasotransmitters, neuropeptides, steroids,, endocannabinoids, and many more
- Essential in priming the host's immune system
- It's alteration can trigger a wide range of physiological disorders, including low-grade inflammation, metabolic disorders, excess lipid accumulation, loss of insulin sensitivity, which increase the risk of developing metabolic diseases and autoimmunity

Genome Med. 2016; 8: 42. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4839080/

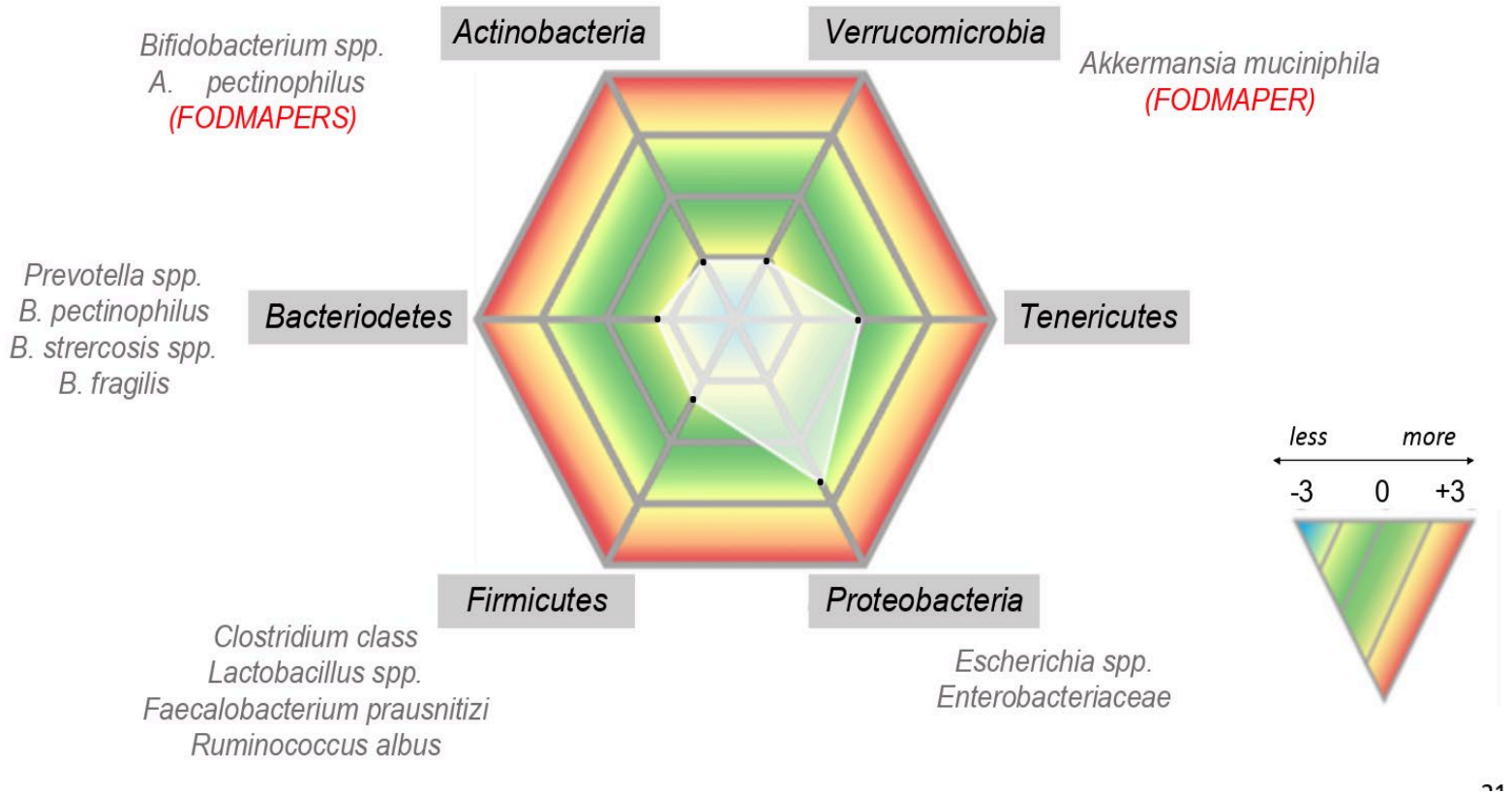
Gut microbiota

- The human body contains trillions of microorganisms — outnumbering human cells by 10 to 1
- Microorganisms make up only about 1 to 3 percent of the body's mass
- In a 200-pound adult, that's 2 to 6 pounds of bacteria
- Faecalibacterium prausnitzii (species)
- Akkermansia muciniphila (genus)
- Phascolarctobacterium (genus)



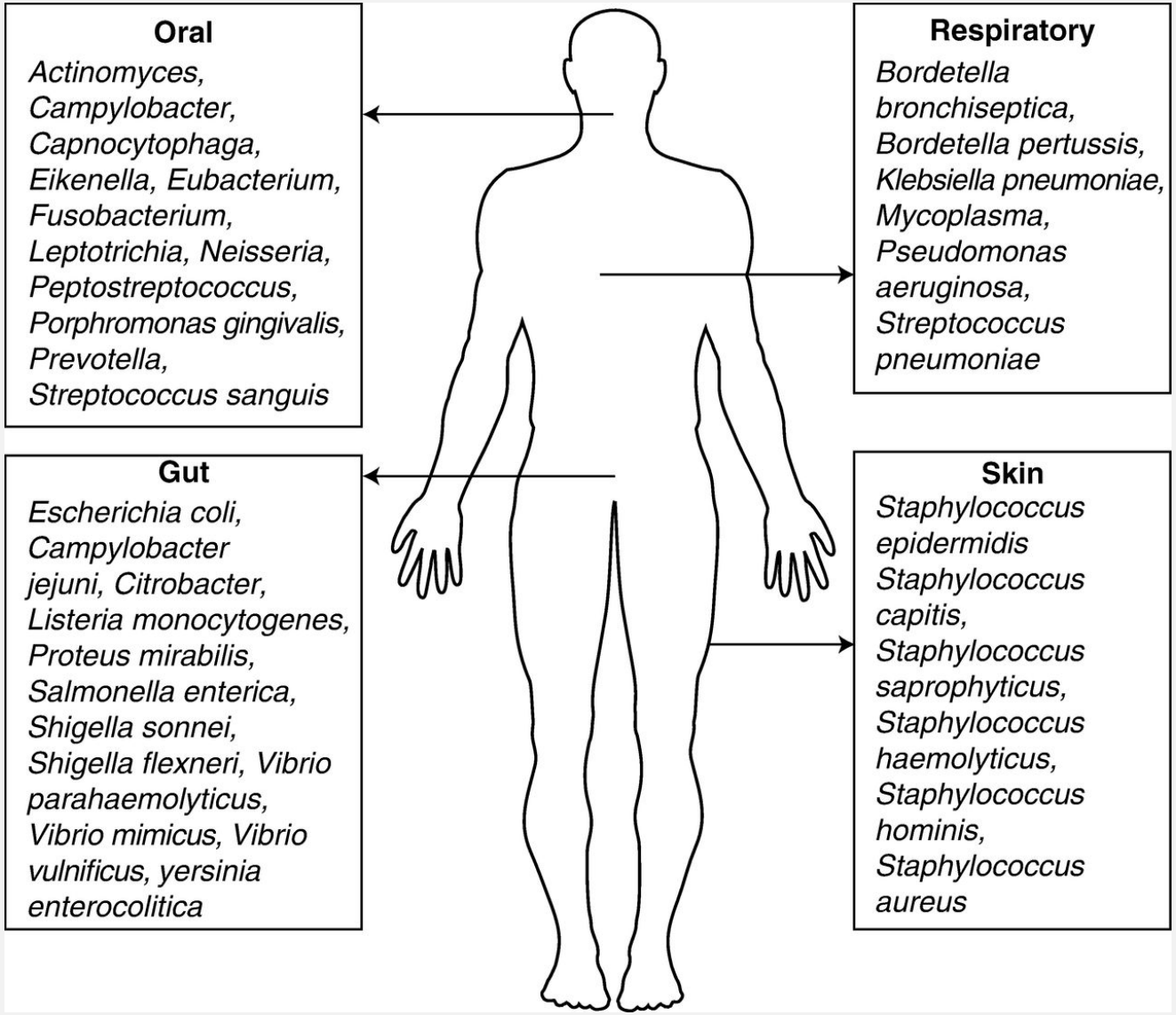
https://www.nih.gov/news-events/news-releases/nih-human-microbiome-project-defines-normal-bacterial-makeup-body

GUT MICROBIOTA



Microbiota & stress hormone-responsive bacteria

- Bacteria within the gut microbiome have evolved specificity in their recognition of host hormones
- Evidence has shown that bacteria have evolved sensory systems that are specific for the hormone they will encounter within their host niche
- *E. coli, Salmonella* and *Yersinia enterocolitica* found a significantly greater preference for NE and dopamine over adrenaline
- It has been reported that NE and adrenaline can bind to a kinase of *E. coli O157:H7,* leading to the proposal that this was the bacterial receptor for these catecholamines



Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

Gut Microbiome

- Gut bacteria and their genes
- A component of microbiome–gut–brain axis
- Microbiome formed via inoculation through the passage of the birth canal
- The vaginal microbiome undergoes a dramatic shift in bacterial species in preparation for birth
- 80 per cent of C-section-born babies had hospital-acquired bacteria in their guts when they were born, compared with 50 per cent of vaginally born babies
- Guts of c-section babies were dominated by opportunistic bacteria such as *Enterococcus* and *Klebsiella*
- *Bacteroides* were absent or present at very low levels in the microbiotas of nearly all c-section babies after birth
- Delayed colonization by the *Bacteroides fragilis* is associated with decreased T_H1 activation
- C-section babies more likely to develop obesity, asthma and eczema

https://www.nature.com/articles/nm.4039 https://www.nature.com/articles/d41586-019-02807-x https://www.nih.gov/news-events/news-releases/nih-human-microbiome-project-defines-normal-bacterial-makeup-body Cell Mol Gastroenterol Hepatol. 2015 Jan; 1(1): 28–40. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4339954/

Gut microbiome

- By the time babies are weaned at the age of around 6 to 9 months, the noted differences had largely disappeared
- Although about 60% of C-section babies still had few or no *Bacteroides* in their guts
- 2011 study on Vaginal Swabbing; Just before surgery, the researchers sampled the mother's vaginal microbes with sterile gauze. Within 2 minutes of birth, this gauze was swabbed all over the newborns' bodies
- All babies who received the swabs obtained skin, gut, anal and oral bacterial communities that were more like those of infants delivered naturally, compared to the C-section-delivered babies who did not go through the procedure
- In some countries, especially those in Latin America, C-sections account for more than half of all births
- US rate is about 30%

https://www.nature.com/articles/nm.4039 https://www.nature.com/articles/d41586-019-02807-x https://www.nih.gov/news-events/news-releases/nih-human-microbiome-project-defines-normal-bacterial-makeup-body

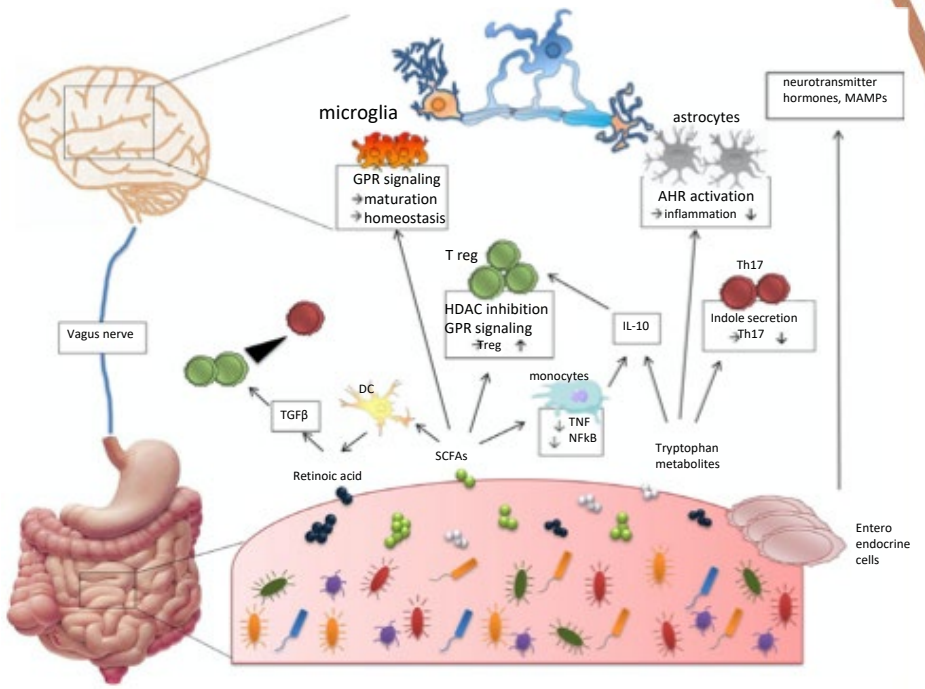
Klebsiella

Imbalance Bacteria	Result	NG	1+	2+	3+	4+	Reference Interval
Klebsiella aerogenes	4+						No Growth
Alpha hemolytic strep	4+						No Growth

Dysbiotic Bacteria	Result	NG	1+	2+	3+	4+	Reference Interval
Citrobacter freundii complex	3+						No Growth
Klebsiella oxytoca	4+						No Growth
Morganella morganii	4+						No Growth

Microbiome–Gut–Brain Axis

- Bi-directional communication system
- Includes the autonomic nervous system, the enteric nervous system, the vagus nerve and the hypothalamic pituitary adrenal axis
- Diet is an environmental factor influencing the complex crosstalk between the gut microbiota and the immune system



2018 John Wiley & Sons Ltd, Immunology

Ann Gastroenterol. 2015 Apr-Jun; 28(2): 203–209.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4367209/

Microbial effects on the host

Microbial effects on the host

- Gut bacteria influence host physiology
- Changes in the gastrointestinal microbiome are associated with inflammatory bowel disease, asthma, obesity, metabolic syndrome, cardiovascular disease, immune-mediated conditions, and neurodevelopmental conditions such as autism spectrum disorder
- Bacteria use sophisticated intercommunication systems to help maintain their niches; consequently, this microbial network is essential to host homeostasis
- Bacteria have developed cooperative mechanisms, such as horizontal gene transfer, biofilm formation, and quorum sensing to ensure the survival of their own community as a whole

Cell Mol Gastroenterol Hepatol. 2015 Jan; 1(1): 28–40.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4339954/

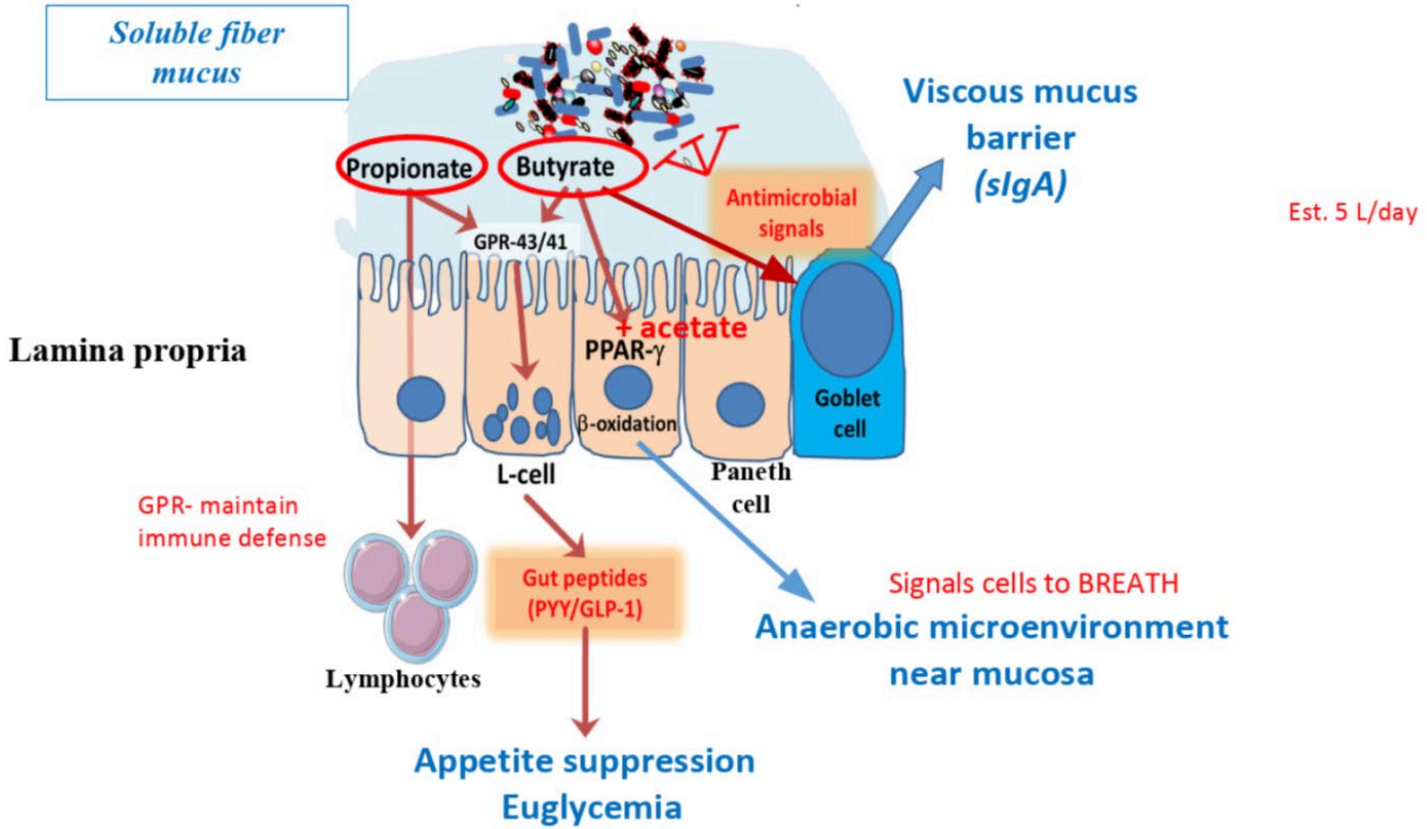
Microbial effects on the host

- *Bacteroides fragilis* express polysaccharide A, which profoundly influences the development of enteric tolerance by converting proinflammatory $CD4^+$ T cells into Tregs during colonization and mediates equilibrium between T_H1 and T_H2 responses
- The microbiota convert bile acids into secondary forms in the lumen by dehydroxylation, dehydrogenation, and deconjugation
- Microbial signaling via G protein-coupled and nuclear receptors can modulate synthesis and amounts of bile salts in the liver
- Gut microbiota influence intestinal permeability, and autoimmunity
- Gut microbiota directly influence catecholamines

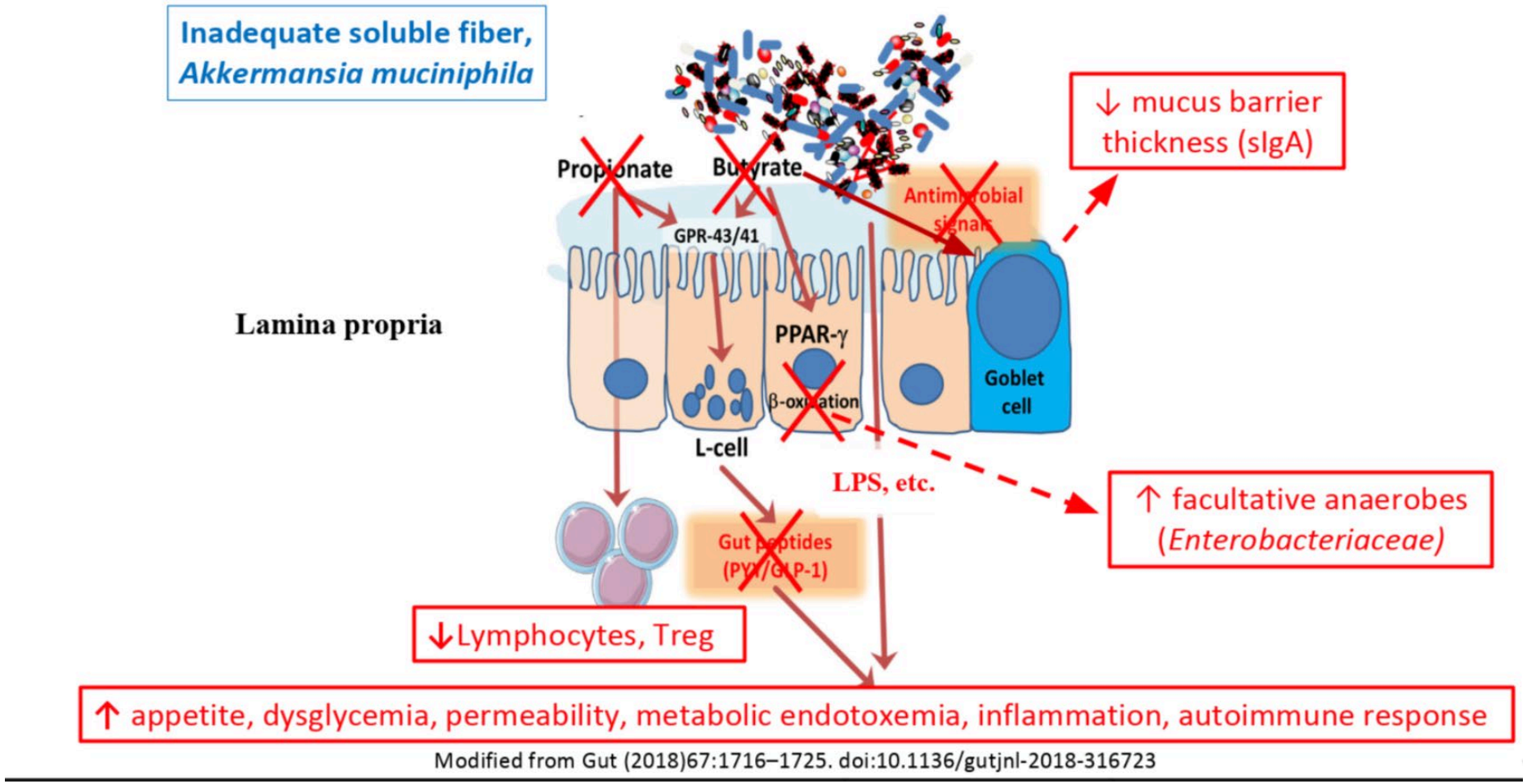
Cell Mol Gastroenterol Hepatol. 2015 Jan; 1(1): 28–40.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4339954/

Microbial-Host Communication

Healthy microbial host crosstalk



Modified from Gut (2018)67:1716–1725. doi:10.1136/gutjnl-2018-316723



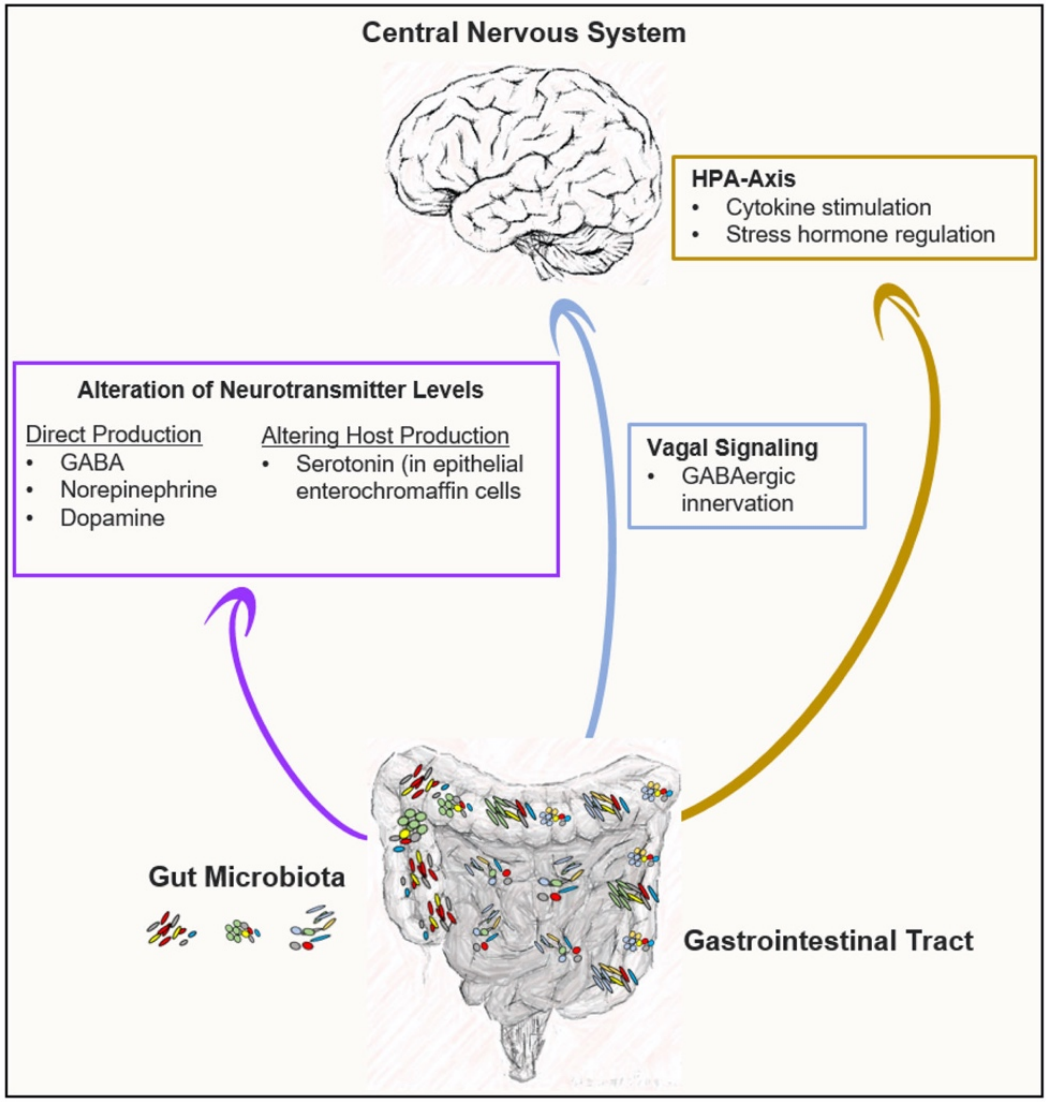
Dysbiosis induced breakdown

Akkermansia muciniphila

- 1-5% of microbiota in healthy microbiome, “specialized” mucus resident
- Decreased abundance with obesity type 2 diabetes and high fat diets
- **Prebiotic (oligofructose)**: normalized cecal A. muciniphlia abundance and insulin resistance, decreased adiposity and abolished metabolic endotoxemia (serum LPS)
- **Supplemental A. muciniphlia**: restored gut barrier functions (increased mucus thickness and colonic Reg3g mRNA, decreased serum LPS), improved body weight, adiposity and glucose homeostasis. Diminished GI and systemic inflammation (murine model)

PNAS (2013)110 doi: 10.1073/pnas.1219451110 Cell Metab(2014)205:779-86 doi: 10.1016/j.cmet.2014.07.003

Communication routes of the gut microbiota to the Communication routes of the gut microbiota to the brain



Brain Res. Author manuscript; available in PMC 2019 Aug 15 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005194/

GABA

- GABA is the major inhibitory neurotransmitter of the central nervous system, and it and its receptors are widely distributed throughout the mammalian host.
- Secretion of GABA serves as a mechanism to decrease intracellular pH via the glutamate acid resistance system
- E. coli can grow on GABA as a sole carbon and nitrogen source
- A broad diversity of bacteria have been reported to produce GABA ; such as several commensal organisms, including members of the Bifidobacterium and Lactobacillus genera
- Lactobacillus rhamnosus JB-1 was found its introduction into mice reduced depressive- and anxiety-like behavior in a vagus-dependent manner, with accompanying changes in cerebral GABAergic activity
- A ketogenic diet was shown to increase GABA levels in the CSF of children with refractory epilepsy, a response correlated with improvement of symptoms
- In a recent fecal transplant study, GABA was found to be the most altered metabolite in obese patients receiving allosteric fecal transplant from lean donors, a finding which was associated with improved insulin sensitivity.

Brain Res. Author manuscript; available in PMC 2019 Aug 15. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005194/

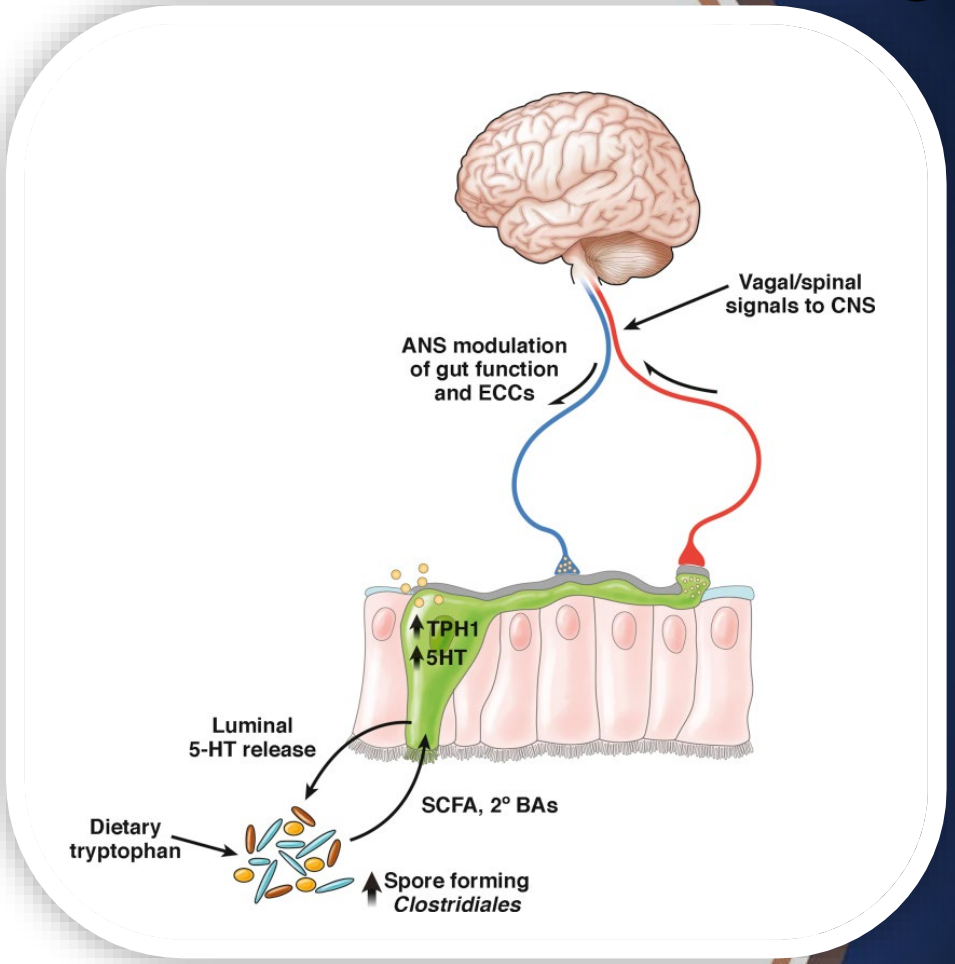
Serotonin

- Serotonin is involved in regulating numerous physiological processes, including gastrointestinal secretion and peristalsis, respiration, vasoconstriction, behavior, and neurological function
- 90–95% of serotonin resides in the gastrointestinal tract, mostly in epithelial enterochromaffin cell
- In germ free animals, there is a significant reduction of serotonin in the blood and colon of mice compared to controls (restored via re-colonization)
- Alteration of host serotonin levels appears to mediated via secretion of small molecules (like short chain fatty acids or secondary bile acids) that signal ECs to produce serotonin via expression of tryptophan hydroxylase
- The entrance of gut tryptophan into the immune-driven kynurenine pathway may play a major role serotonin dysregulation and the concomitant physiological consequences

Brain Res. Author manuscript; available in PMC 2019 Aug 15. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005194/

Enterochromaffin Cell Signaling

- Enterochromaffin cells (ECCs) contain more than 90% of the body's serotonin (5-HT)
- 5-HT/Serotonin synthesis in ECCs is modulated by SCFAs and 2BAs produced by spore-forming Clostridiales, increased in the presence of dietary tryptophan
- Enterochromaffin cells communicate with afferent nerve fibers through synapse-like connections between neuropod-like extensions and afferent nerve terminals
- The autonomic nervous system can activate ECCs to release 5-HT into the gut lumen, where it can interact with gut microbes



Cell Mol Gastroenterol Hepatol 2018;6:133–148; https://doi.org/10.1016/j.jcmgh.2018.04.003

Serotonin

- A balanced gut microbiota is necessary for the physiological function of the mucosa's immune defense.
- Increased levels of serotonin can enhance quorum sensing in *Pseudomonas aeruginosa* both in vitro and in vivo
- Quorum sensing is the process by which bacteria in close proximity are able to communicate with one another through chemical signaling
- In a mouse model, we observed that animals treated with exogenous serotonin and infected with P. aeruginosa exhibited an increase in intestinal bacterial load and mortality compared to untreated animals.
- The administration of exogenous serotonin enhanced the production of pro-inflammatory cytokines, increased biofilm formation on mouse intestines and worsened intestinal pathological manifestations

Brain Res. Author manuscript; available in PMC 2019 Aug 15. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005194/

Tummy aches & stress

- Serotonin receptors have been found to influence the severity of certain GI conditions
- Due to the presence of serotonin in both the brain and the gut, selective serotonin reuptake inhibitors (SSRIs) are not only able to treat depression and other neurological disorders/syndromes, but are also capable of decreasing pain and other symptoms associated with chronic GI disorders
- *E. coli* isolates respond to NE, dopamine and their metabolites, with growth increases of up to five logs over controls
- The host stress stimulated both attachment of the microflora to the gut wall and their translocation to the mesenteric lymph nodes, which are events preceding development of a gut-associated systemic infection
- The gut microbiome may return to normal, although this would take two weeks of living stress free
- *Salmonella enterica* increased plasma NE levels

J Cell Physiol. Author manuscript; available in PMC 2018 Jan 18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5772764/

CATECHOLAMINES

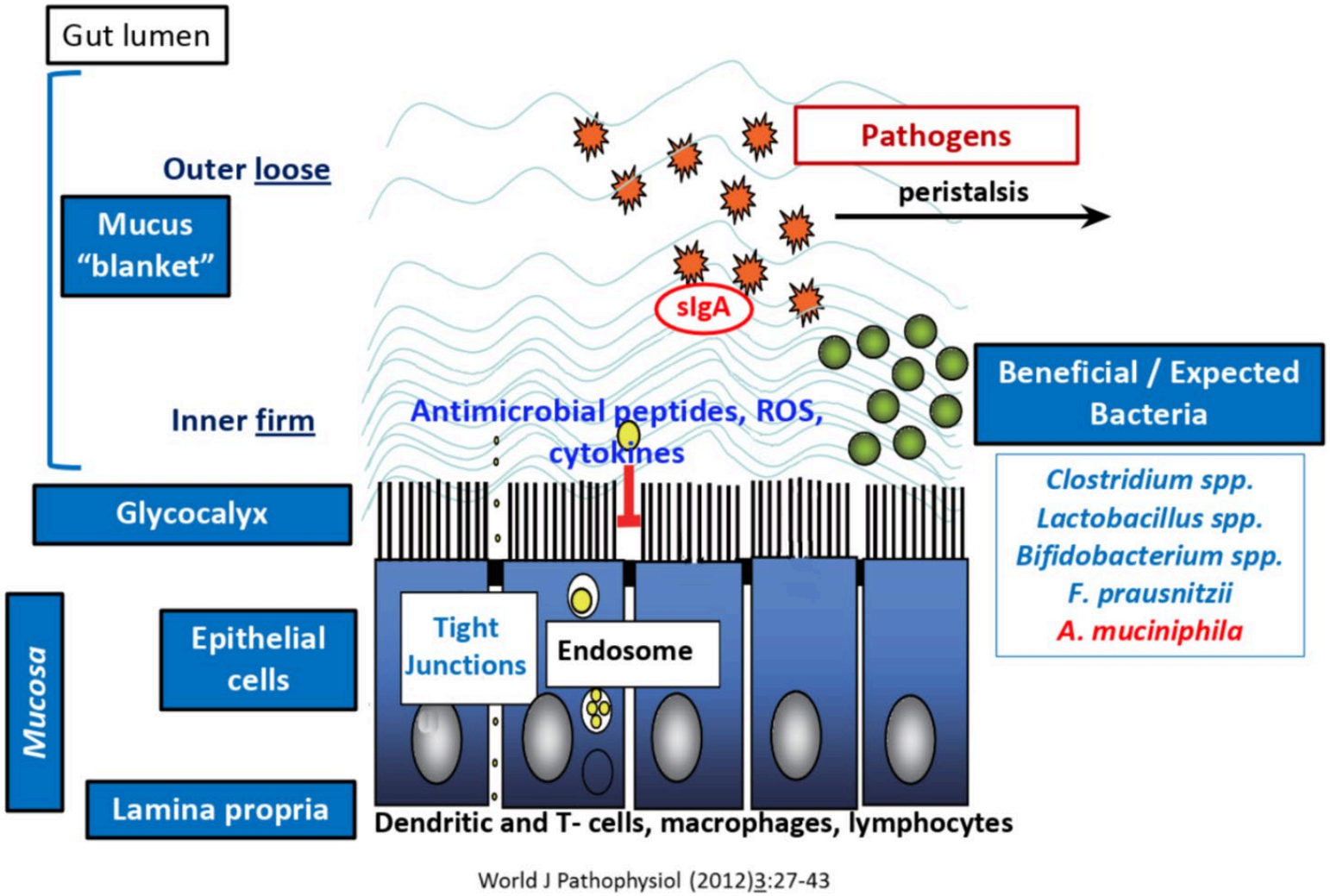
- Catecholamines are monoamines comprised of a catechol group and an amine side chain
- 3 main catecholamines: norepinephrine (noradrenaline), epinephrine (adrenaline), and dopamine; norepinephrine and epinephrine are also known as the “fight or flight” peripheral catecholamines while dopamine is a central acting catecholamine
- Epinephrine has the ability to potentiate the actions of other endogenous hormones.
- Besides glucose absorption, epinephrine also affects absorption of several other molecules and ion transporters.
- Dopamine plays a small role in regulating electrolyte absorption in the GI tract.
- Dopamine efflux in the reward pathway is produced by direct stimulation of the gastrointestinal tract.

Brain Res. Author manuscript; available in PMC 2019 Aug 15.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005194/

Clostridia species & norepinephrine

- Germ free animals were found to have substantially reduced levels of norepinephrine in the gut and the periphery
- *Clostridia* species added to the gut increased epinephrine to normal levels in the gut
- Germ free mice display an increased turnover rate of dopamine, norepinephrine, and serotonin in the brain which has lead to the reduction of decreased levels of these NT's in systemic circulation
- Decreased gut microbial abundance has been shown to increase activity of D1 Dopamine receptor Drd1 and the GluR2 AMPA receptor Gria2 in the nucleus accumbens. This leads to increased sensitivity to the behavioral effects of cocaine.
- The above was reversed with supplementation of short chained fatty acids, suggesting an indirect path for the microbiota to influence reward behavior.

Brain Res. 2018 August 15; 1693(Pt B): 128–133. doi:10.1016/j.brainres.2018.03.015.



World J Pathophysiol (2012)3:27-43

Intestinal barrier

Intestinal permeability

- Changes in the microbiota / metabolome
  ***DIET**- high fat, low soluble fiber, total parenteral nutrition*
- *Sustained* alterations in mucus production/degradation *(diet, starvation)*
- Direct adhesion of *any* bacteria to endothelial cells
- Gastrointestinal inflammation *(e.g. Crohn's disease)*
- Chronic stress
- Oxidative stress (*in vitro*)
- Life style- alcohol, drugs *(prednisone, NSAIDs, PPIs)*
- Gut-derived protein fragments (gliadin) and endotoxins
- Epithelial cell damage- environmental toxins *(e.g. glyphosate)*
- Cancer treatments/radiation
- Associated with common additives, including sugar, metal oxide nanoparticles, surfactants and sodium chloride
- Intestinal permeability can induce immune and inflammatory responses that exacerbate intestinal barrier damage and further increase IP
- Increased intestinal permeability has been correlated with aging and disease, including type 2 diabetes, Crohn'**s** disease, celiac disease, multiple sclerosis and irritable bowel syndrome.

BMC Gastroenterol (2014)14:189 Scientific Rep(2014)4:5551 DOI: 10.1038/srep05551 Clin Gastroenterol Hepatol(2012)10:1096-1100 World J Gastroenterol(2014)20:17107-14 Curr Nutr Food Sci (2013)9:99–107 Gastroenterol (2015)148 doi: 10.1016/S0016-5085(15)30192X

Stress

- Norwegian army undergoing training in the Arctic
- Stress & anxiety affect intestinal microbiota composition (e.g., decrease diversity, increase abundance of proinflammatory taxa, and decrease abundance of putatively beneficial taxa) and increase intestinal impermeability. Leads to GI distress
- A multiple-stressor environment can induce increases in intestinal permeability that are associated with inflammation, as well as intestinal microbiota composition and metabolism
- Intestinal microbiota responses were characterized by increased α-diversity and changes in the relative abundance of >50% of identified genera, including increased abundance of less dominant taxa at the expense of more dominant taxa such as *Bacteroides*
- Supplemental protein would exacerbate these decrements by promoting the generation of potentially harmful bacterially derived metabolites
- Carbohydrate supplementation would attenuate these decrements by reducing the magnitude of negative energy balance

https://doi.org/10.1152/ajpgi.00066.2017

Stress = intestinal permeability

Intestinal permeability

Zonulin; serum

	RESULT / UNIT	REFERENCE INTERVAL	LOW	MOD	HIGH
Zonulin*	**80.7 ng/mL**	< 45.0			

Restoration of the Gastrointestinal Mucosal Barrier

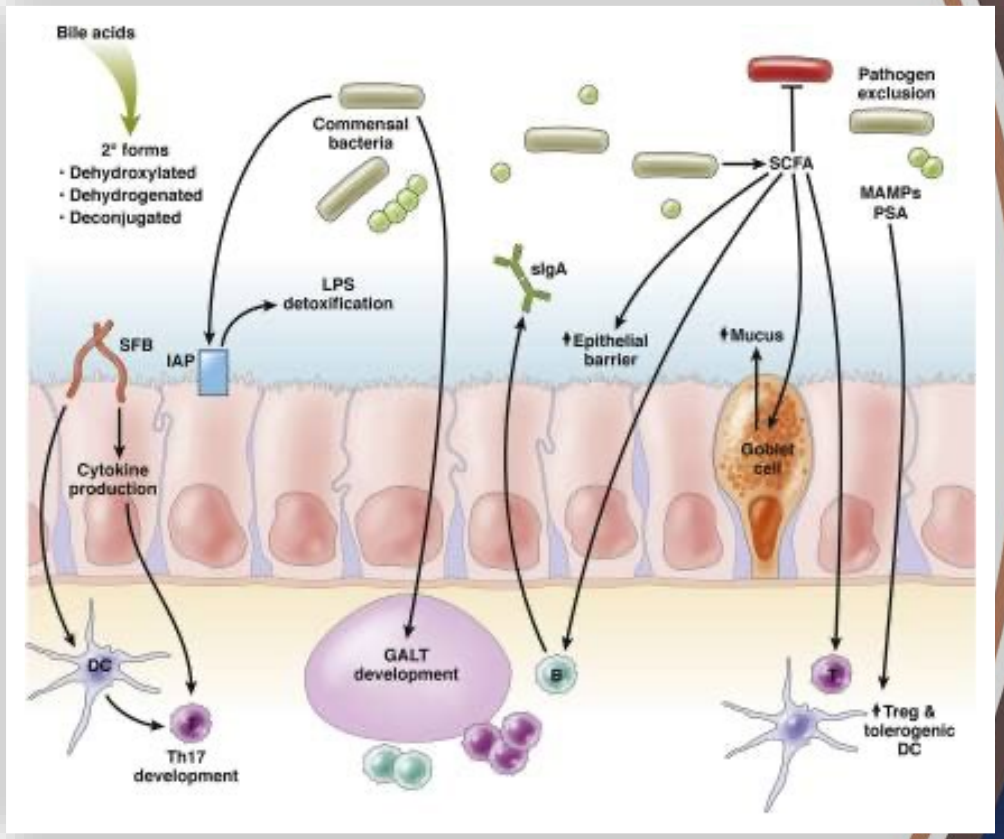
- Dietary changes, GF
- Treatment of dysbiosis
- Digestive supports and anti-inflammatory therapies
- Supplements such as quercetin, vitamin D, retinol, curcumin, gamma-linolenic acid, omega-3 fatty acids (EPA, DHA), and aloe vera
- Other nutrients such as zinc, beta-carotene, pantothenic acid, and L-glutamine may provide some support for rejuvenation of the GI mucosa

LOE: C

BMC Gastroenterol(2014)14:189 (www.biomedcentral.com/1471-230X/14/189)
Br J Nutr(2003)89:597-606 Frontiers Immunol(2015) doi: 10.3389/frimmu.2015.00612n

Microbial Induced Host Immune Tolerance

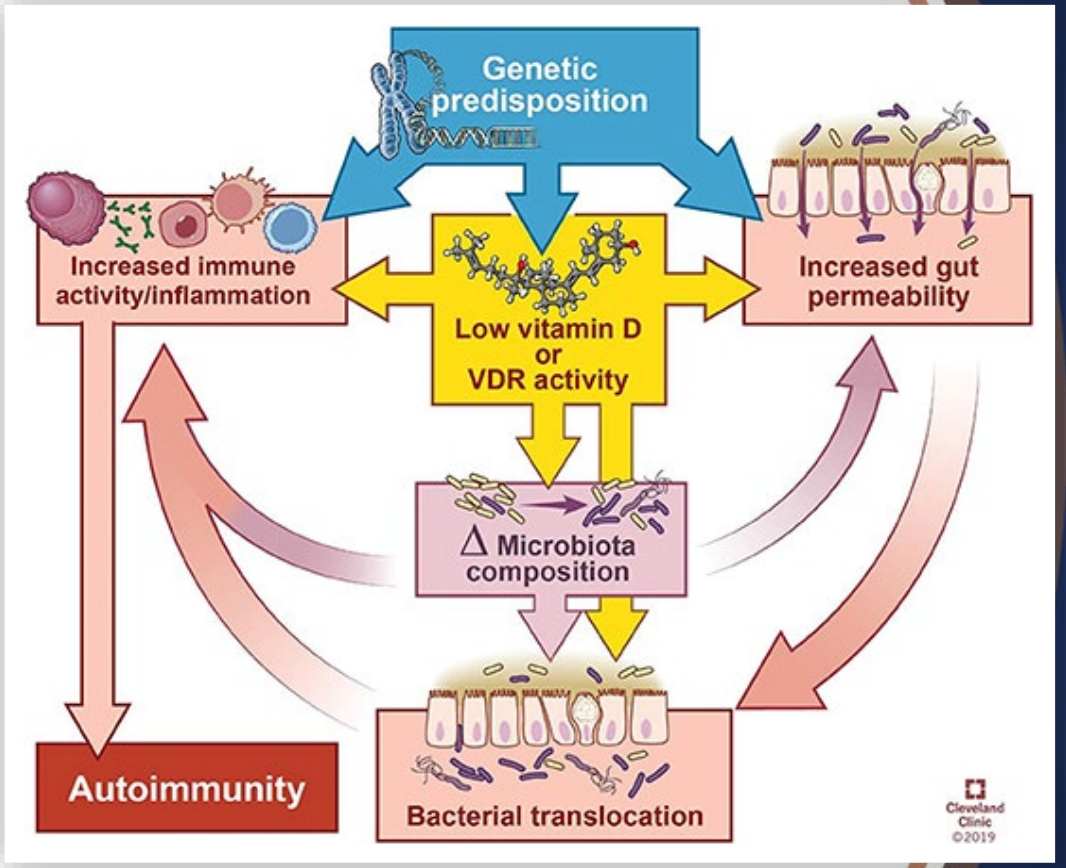
- The microbiota induces host immune tolerance to commensal bacteria directly via a microbe-associated molecular pattern (MAMP) and polysaccharide (PSA) signaling
- The microbiota induces host immune tolerance to commensal bacteria indirectly via the production of short-chain fatty acids (SCFA) and potentially through expression of epithelial intestinal alkaline phosphatase (IAP), which detoxifies luminal lipopolysaccharides (LPS)
- Segmented filamentous bacteria (SFB) promote immune development of Th17 cells
- The above contributes to proper gut-associated lymphoid tissue (GALT) development
- SCFA also induce IgA and mucus secretion into the lumen, promote epithelial barrier integrity, and prevent pathogen colonization
- The microbiota also participates in the formation of the active, secondary forms of bile acids.



Cell Mol Gastroenterol Hepatol. 2015 Jan; 1(1): 28–40.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4339954/

Microbiome & autoimmunity

- Interactions between genetics, gut integrity, microbiome, and vitamin D deficiency
- Low vitamin D increases the permeability of the gut barrier and heightens immune activity
- Low vitamin D and permeability of the gut alter microbial composition and the ability of microbes to translocate across the intestinal epithelium
- Leading to interaction with the host immune system
- Ultimately, immune system activation contributes to autoimmunity
- Microbiome studies in systemic autoimmune diseases demonstrate unique microbial patterns in Inflammatory Bowel Disease, Rheumatoid Arthritis, and Systemic Lupus Erythematosus



Front Immunol. 2019; 10: 3141.
doi: 10.3389/fimmu.2019.03141

Secretory IgA

- During cute conditions of stress, fecal sIgA may be upregulated as a protective mechanism
- Chronic stress will down regulate fecal sIgA, which increases the risk of GI infection
- HPA axis activation influences interleukins and sIgA production
- Luminal sampling by tolerogenic immune cells in the lamina propria, secretory IgA in the mucous layer, and the complement network operate together to maintain intestinal homeostasis
- SCFA also induce IgA and mucus secretion into the lumen, promote epithelial barrier integrity, and prevent pathogen colonization

Cell Mol Gastroenterol Hepatol. 2015 Jan; 1(1): 28–40./
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4339954/

Inflammation	Result	Unit	L WRI H	Reference Interval
Lactoferrin	1.4	µg/mL		< 7.3
Lysozyme*	102	ng/mL		≤ 500
Calprotectin	<10	µg/g		≤ 50

Immunology	Result	Unit	L WRI H	Reference Interval
Secretory IgA*	27.2	mg/dL		30 – 275

Short Chain Fatty Acids	Result	Unit	L WRI H	Reference Interval
% Acetate‡	64			50 – 72
% Propionate‡	16			11 – 25
% Butyrate‡	19			11 – 32
% Valerate‡	0.7			0.8 – 5.0
Butyrate‡	0.73	mg/mL		0.8 – 4.0
Total SCFA's‡	3.8	mg/mL		5.0 – 16.0

Stress

- Our modern lifestyles may induce chronic stress
- Increased sympathetic NS & Decreased PS
- HPA axis activation
- Decreased fecal sIgA
- Decreased GI microbial composition
- Decreased SCFAs
- Increased intestinal permeability

Intervention for Low sIgA

- Probiotics *(check strains)*
  Lactobacillus raminosus GG and *Bifidobacterium lactis* Bb-12
  S. boulardii- ~75% ↑ sIgA *(germ-free mouse model)*

- Prebiotics – *a cornucopia of unique oligosaccharides in breast milk (closest is camel milk)*
  Soluble fiber- vegetables, fruits, **chickpeas**, beans, etc.
  Fructooligosaccharides, galactooligosaccharides, inulin (up to 10 g/day)

- Glutamine
- Ω-3 fatty acids, olive oil, zinc, vitamins A and D_3
- **Chronically elevated cortisol (stress) and low DHEA diminish sIgA levels**

LOE: C

Clin Nutr(2015)34:1080-7 Amino Acids(2015)47:2143-54 J Steroid Biochem Mol Biol(2015)148:179-83 Biosci Biotech Biochem (2003)67:758-64 Scand J Food Nutr (2007)51:62-67 Eur J Nutr(2013)52:1089-98 Am J Surg(2010)199:35-42 Curr Nutr Food Sci(2013)9:99–107 Immunity(2007)26:812-26

Stress & Gut Microbiota

- GI tract is highly enervated by the ENS that has close connections to the CNS
- Approximately half of the NE made within the mammalian body is produced within the gut
- Within the ENS, NE is released from storage within sympathetic nerve fibers within the pre-vertebral ganglia innervating the gut mucosa
- Dopamine is synthesized in non-sympathetic enteric neurons located within the intestinal wall
- The endogenous microflora has the ability to also add to the levels of catecholamines
- Butyrate is synthesized by colonic bacteria and has been shown to enhance transcription of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis
- Bacillus spp., Proteus vulgaris, Serratia marcescens, and S. aureus can directly synthesize catecholamines that are exact analogues of the mammalian hormones
- Commensal gut bacteria express b-glucuronidase enzymes, which are able to generate free NE and dopamine via the cleavage of their pharmacologically inactive conjugated forms

Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

Stress & Gut Microbiota

- GI tract catecholamines can be isolated from the gut lumen, and that levels of NE and dopamine increased when there was a bacterial presence
- During periods of stress, excess catecholamines spill over into the gut from systemic circulation. The bacteria respond to these catecholamines and the gut microbiota changes
- Catecholamines within the gut may also shape the genetic composition of the microbiome bacteria
- NE increased the interspecies transfer efficiencies of a conjugative plasmid from *Salmonella typhimurium* to an *E. coli recipient*
- Enhancing the rate of local genetic exchange could speed adaptation of the gut microflora to the gut environment

Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

Stress Effects on the Gut Microbiome

- Under normal circumstances lactoferrin (Lf) and transferrin (Tf) act bacteriostatic as they iron limitation in mucosal secretions
- Under times of stress, excess gut-derived catecholamines, noradrenaline (NE) and dopamine, are released
- In the presence of NE, pathogens release virulence factor expressions such as host attachments and adhesions
- NE and dopamine interact with the lactoferrin, and transferrin, converting a normally bacteriostatic set of Fe-chelating proteins into a nutritional source of iron thereby providing support for increased bacterial growth in the gut
- Even several hours after a stressful event has resolved, the levels of catecholamines in the gut may have returned to normal; however, stress-related events are still occurring
- At 4 hours of catecholamine exposure Escherichia coli is stimulated to grow
- This moa allows E. coli O157:H7 also enhances shiga toxin expression

Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

Stress Effects on the Gut Microbiome

- Catecholamines can increase attachment of enteric pathogens to gut mucosa
- This increase in bacterial populations can lead to increased intestinal permeability
- Increased intestinal permeability can lead to to bacterial translocation either to the mesenteric lymphatic tissue or, in a worst-case scenario, directly into the systemic circulation
- Noradrenaline exposure of enteropathogenic and enterohaemorrhagic *Escherichia coli* can also lead to attaching and effacing lesions and destruction of gut tissue

Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

Microbiota-gut-brain Axis & Behavior

- A diverse gut microbiome has been associated with behaviors related to positive mood, curiosity, sociability, and impulsivity
- Extroverted behavior has been associated with bacteria of the Parabacteroides, Rikenellaceae, Ruminococcaceae families and Dialister genera
- Fear behavior has been associated with a less diverse microbiome
- A child's temperament, or how they handle stress, was influenced by their gut microbiome, especially for boys
- Bacterial metabolites which influence the pre-frontal cortex and gut derived amino acids
- Psychobiotics, bacteria administered for mental health benefit, may be utilized prophylactically and as potential adjunctive treatment.

LOE: C

Journal of Endocrinology (2015) 225, R21–R34 https://joe.bioscientifica.com/view/journals/joe/225/2/R21.xml

https://www.childwelfare.gov/topics/preventing/preventionmonth/resources/ace/ https://www.cdc.gov/violenceprevention/pdf/preventingACES.pdf

ACEs

- Adverse childhood experiences (ACEs) are traumatic events occurring before age 18
- ACEs include all types of abuse and neglect as well as parental mental illness, substance use, divorce, incarceration, and domestic violence
- ACES linked to obesity, diabetes, depression, suicide attempts, STDs, heart disease, cancer, stroke, COPD (in adulthood)
- ACEs have been found to have a graded dose-response relationship with 40+ outcomes to date
- A significant relationship between the number of ACEs a person experienced and a variety of negative outcomes in adulthood, including poor physical and mental health, substance abuse, and risky behaviors
- The more ACEs experienced, the greater the risk for these outcomes
- Public health policies aimed at prevention & intervention, yet not at microbiome

ACES Survey

Adverse Childhood Experiences (ACE) 10-Question Survey

PRIOR TO YOUR 18th BIRTHDAY:

1. Did a parent or other adult in the household **often or very often**…
   Swear at you, insult you, put you down, or humiliate you? OR
   Act in a way that made you afraid that you might be physically hurt? **If YES, enter 1_____**

2. Did a parent or other adult in the household **often or very often**…
   Push, grab, slap, or throw something at you? OR
   Ever hit you so hard that you had marks or were injured? **If YES, enter 1_____**

3. Did an adult or person at least 5 years older than you **ever**…
   Touch or fondle you or have you touch their body in a sexual way? OR
   Attempt or actually have oral, anal, or vaginal intercourse with you? **If YES, enter 1_____**

4. Did you **often or very often** feel that…
   No one in your family loved you or thought you were important or special? OR
   Your family didn't look out for each other, feel close to each other,
   or support each other? **If YES, enter 1_____**

5. Did you **often or very often** feel that…
   You didn't have enough to eat, had to wear dirty clothes, and had no one to protect you? OR
   Your parents were too drunk or high to take care of you or
   take you to the doctor if you needed it? **If YES, enter 1_____**

6. Was a biological parent **ever** lost to you through divorce, abandonment,
   or other reason? **If YES, enter 1_____**

7. Was your mother or stepmother:
   Often or very often pushed, grabbed, slapped, or had something thrown at her? OR
   Sometimes, often, or very often kicked, bitten, hit with a fist, or hit with something hard?
   OR
   Ever repeatedly hit over at least a few minutes or threatened
   with a gun or knife? **If YES, enter 1_____**

8. Did you live with anyone who was a problem drinker or alcoholic or
   who used street drugs? **If YES, enter 1_____**

9. Was a household member depressed or mentally ill or did a household
   member attempt suicide? **If YES, enter 1_____**

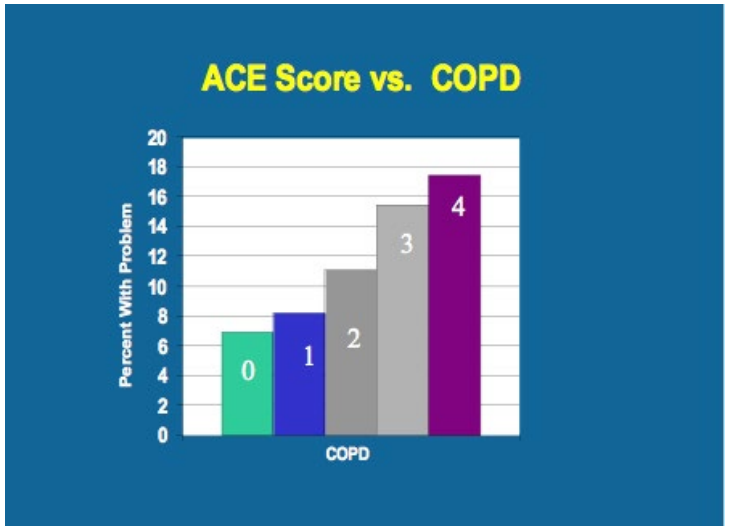
10. Did a household member go to prison? **If YES, enter 1_____**

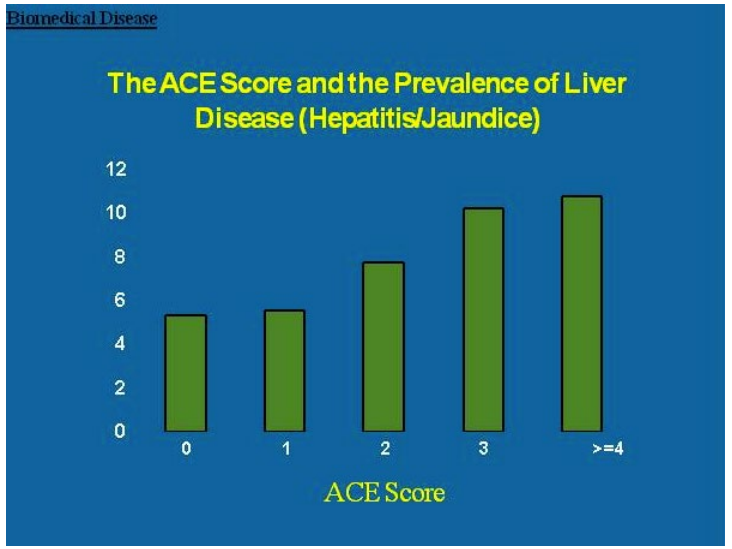
Now add up your YES answers – **TOTAL "Yes": _____**

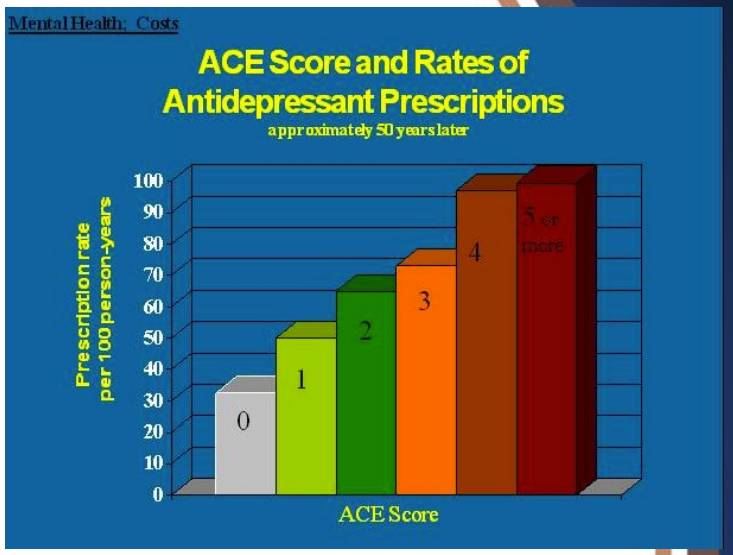
This is your ACE score

MENTOR BOOKS
MentorBooks.com

HeidiFrancine.com • MentorBooks.com • heidi@mentorbooks.com







With an ACE score of 4 or more

- The likelihood of chronic pulmonary lung disease increases 390 %
- The likelihood of hepatitis, 240 %
- The likelihood of depression 460 %
- The likelihood of attempted suicide, 1,220 %

http://acestoohigh.com/got-your-ace-score/

Microbiota: the missing link

- HPA Axis
- NT dysfunction (microbial influence)
- Sympathetic dominant NS
- Microbial abundance and diversity (*Lactobalili, Clostridia, Bifidobacterium)*
- Intestinal permeability
- Fecal sIgA
- What are we treating in adults with COPD, liver disease and depression, with ACES?
- Psychobiotics
- In patients, with stress; is adrenal support sufficient? Stool testing warranted.

Psychobiotics

- Lactobacillis has been shown to lower corticosterone
- Supplementation of Lactobacillus helveticus and Bifidobacterium longum for two weeks lowered scores in anxiety tests in an animal model
- Lactobacillis helveticus and rhamnosus reduced anxiety and depression like behaviors in murine models
- Probiotic treatment reduced depression-like behaviors and increased levels of a neuroprotective acid via microbial metabolism of tryptophan
- Children consuming prebiotics had a significant decrease in plasma IL-6, and a significant increase in Bifidobacterium spp
- B. infantis in adulthood improved immune system abnormalities and depressive like behaviors, which were a result of early maternal separation
- The microbial stress response to ACTH, was reversed by administration of B. infantis, and there was a greater response in subjects whom had B. infantis colonization early in life
- Proper microbial colonization early in life serves to influence the HPA axis

LOE: C

Nutrients. 2018 Nov; 10(11): 1783. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6267105/

Stress & Bifidobacterium and Lactobacillus

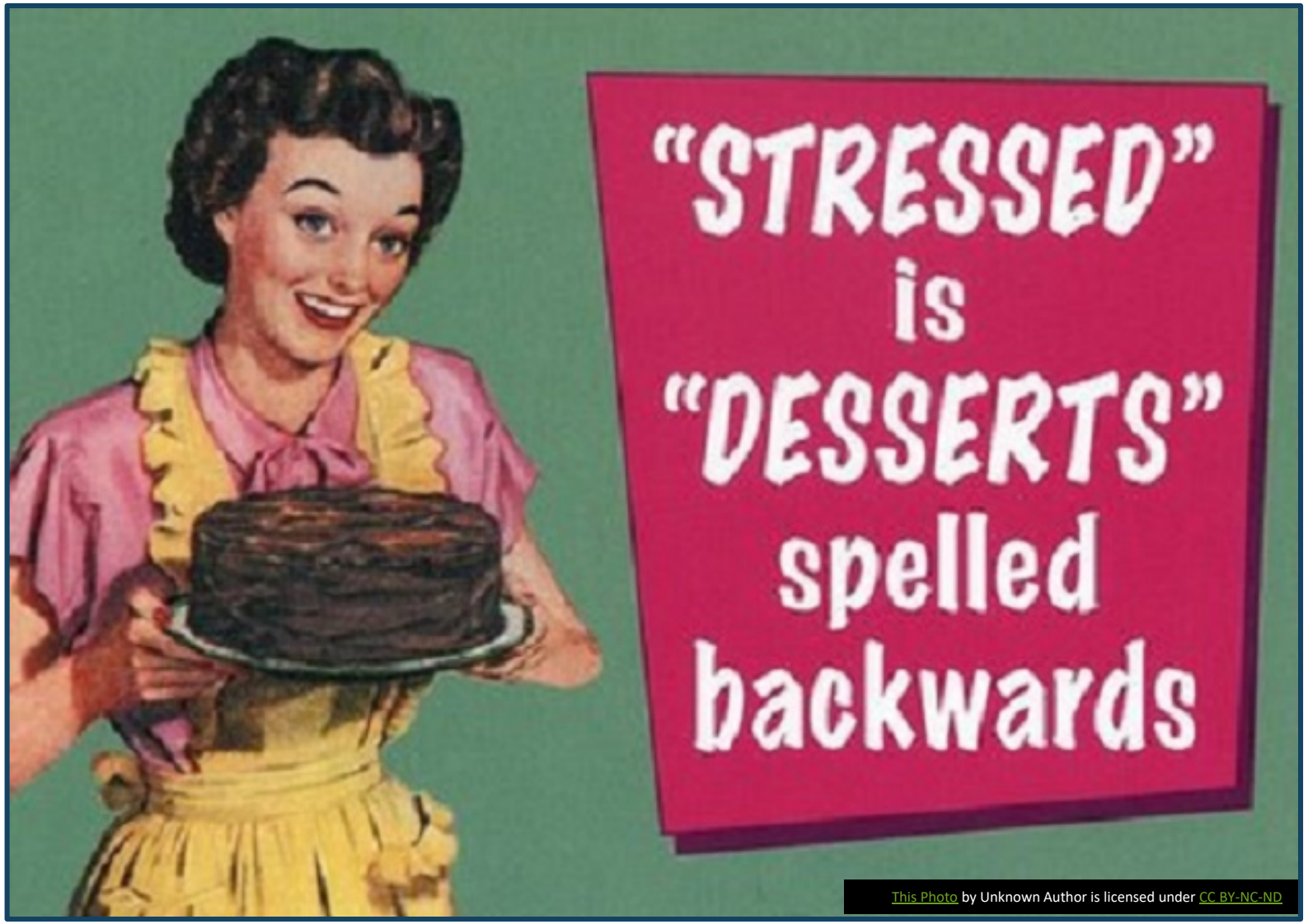
- Stress caused by maternal separation of rhesus monkeys changed their microbiome via decreased *Bifidobacterium* and *Lactobacillus*
- Rat pups separated from their mother exhibited decreased *Lactobacillus*
- *Lactobacillus* remained decreased into adulthood
- Intestinal dysbiosis and subsequent chronic low-grade inflammation follow
- Gastrointestinal dysbiosis and chronic low-grade inflammation have been implicated in IBS and depression
- *Lactobacillis* has been shown to lower corticosterone
- Supplementation of *Lactobacillus helveticus* and *Bifidobacterium longum* for two weeks lowered scores in anxiety tests
- *Lactobacillis helveticus* and *rhamnosus* reduced anxiety and depression like behaviors in murine models

Alper E., & Ceylan M., (2015) The Gut-Brain Axis: The Missing Link in Depression. Clin Psychopharmacol Neurosci. doi: 10.9758/cpn.2015.13.3.239
Campos-Rodriguez, R., et all. Stress modulates intestinal secretory immunoglobulin A. Frnters Intgrtv Nurosci. 02 December 2013.
Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology. 2009;136:2003–2014. DOI: 10.1053/j.gastro.2009.01.075
Dinan TG, et al., Collective unconscious: How gut microbes shape human behavior, Journal of Psychiatric Research (2015), http://dx.doi.org/10.1016/j.jpsychires.2015.02.021
Farzi, A., Frohlich, E., Holzer, P., Gut Microbiota and the Neuroendocrine System. Neurotherapeutics, 27 January 2018
Moloney, R., et all. The microbiome: stress, health and disease. Mamm Genome. 27 November 2013. DOI: 10.1007/s00335-013-9488-5.0

BACTERIOLOGY CULTURE		
Expected/Beneficial flora	**Commensal (Imbalanced) flora**	**Dysbiotic flora**
3+ Bacteroides fragilis group	2+ Alpha hemolytic strep	
1+ Bifidobacterium spp.	2+ Citrobacter freundii complex	
3+ Escherichia coli	1+ Gamma hemolytic strep	
2+ Lactobacillus spp.	1+ Pseudomonas aeruginosa	
NG Enterococcus spp.	3+ Pseudomonas chlororaphis group	
	2+ Staphylococcus aureus	
3+ Clostridium spp.		
NG = No Growth		

Parasympathetic dominance

- Low *Bifidobacterium spp.*
- Lower *Lactobacillus spp.*
- No growth *Enterococcus spp*
- Low beneficial bacteria & many commensals



Stress Eating

- Consuming food in response to your feelings, especially when you are not hungry
- Stress eating is also sometimes called emotional eating
- Emotional eating means that your emotions — not your body — dictate when and how much you eat
- High-carbohydrate, high-calorie foods with low nutritional value
- Using food as a substance, rather than sustenance
- Opioid response to gluteomorphins (gluten) & caseomorphins (milk)
- Sugar is more addictive than cocaine (dopamine influence, microbiome abundance)
- Frankenfoods & SAD

https://www.apa.org/news/press/releases/stress/2013/eating
https://www.sciencedirect.com/science/article/abs/pii/S0195666317309625?via%3Dihub

Stress Eating

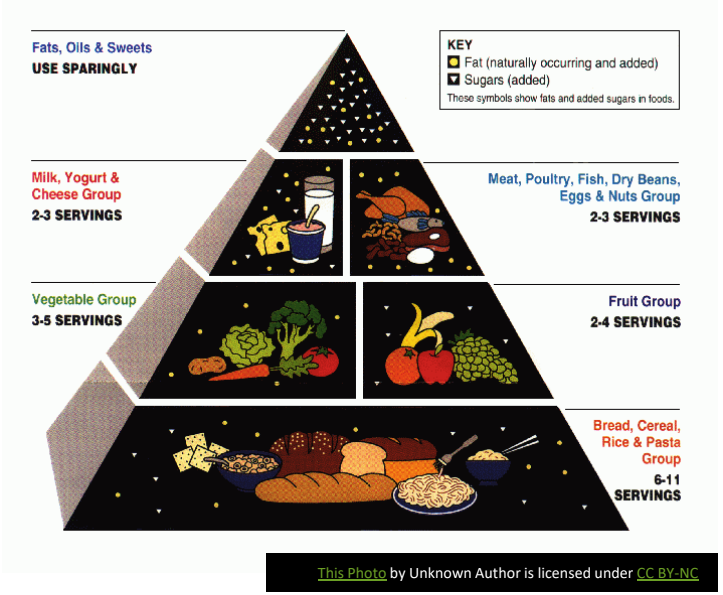
- Impact of stress on unhealthy eating may begin as early as 8 or 9 years old
- Foods eaten during stress are high fat and/or sugar content
- Thirty-eight percent of adults say they have overeaten or eaten unhealthy foods in the past month because of stress. Half of these adults report engaging in these behaviors weekly or more.
- Millennials are more likely than other generations to say they stress eat — 50 percent say they have done so in the past month, compared to 36 percent of Gen Xers, 36 percent of Boomers and 19 percent of Matures

https://www.apa.org/news/press/releases/stress/2013/eating
https://www.sciencedirect.com/science/article/abs/pii/S0195666317309625?via%3Dihub

Inflammation

- A low-fiber, high-protein, and high-fat diet have been reported to increase intestinal inflammation
- Dysbiosis is associated with inflammation, increased metabolic and immune disorders
- An impaired intestinal barrier can increase the translocation of the Gram-negative bacteria cell membrane component LPS into the circulation, which results in metabolic endotoxemia and low-grade inflammation

Nutrients. 2018 Nov; 10(11): 1783. . doi: 10.3390/nu10111783



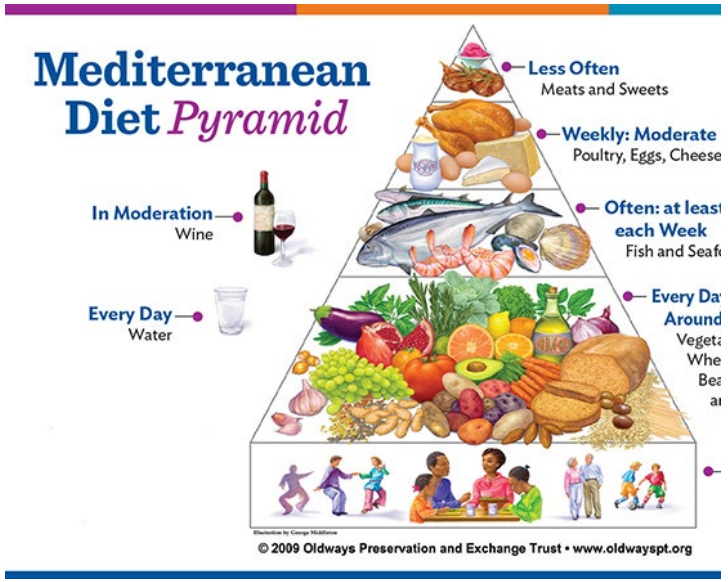
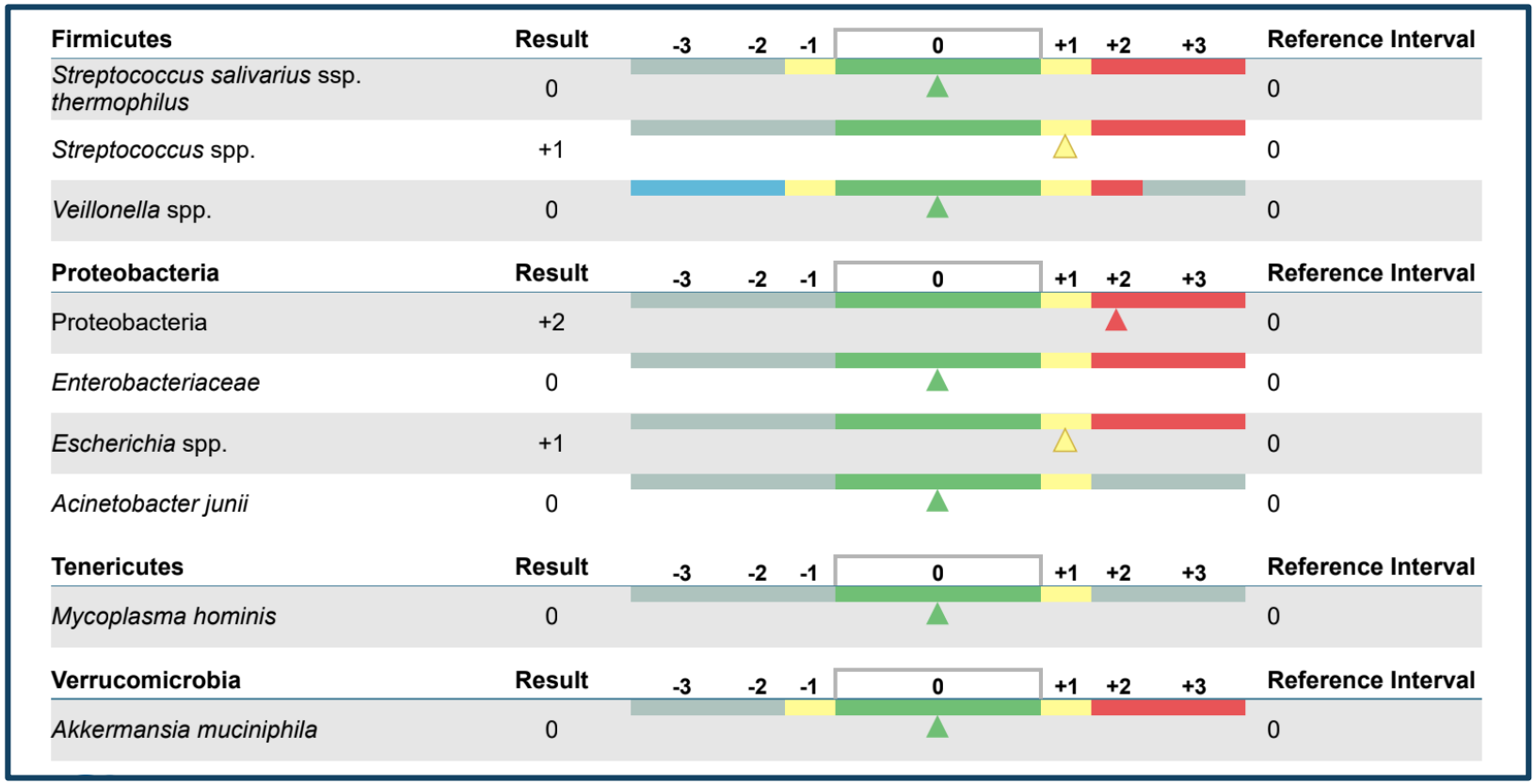


IMAGE COURTESY OF HEALTH CANADA.

Inflammatory diets…?



Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Streptococcus salivarius ssp. *thermophilus*	0		0
Streptococcus spp.	+1		0
Veillonella spp.	0		0
Proteobacteria	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Proteobacteria	+2		0
Enterobacteriaceae	0		0
Escherichia spp.	+1		0
Acinetobacter junii	0		0
Tenericutes	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Mycoplasma hominis	0		0
Verrucomicrobia	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Akkermansia muciniphila	0		0

Sad

Firmicutes

10–15 g of prebiotics per day increased fecal Bifidobacteria and

Faecalibcterium prausnitzii

Firmicutes	Result	Reference Interval
Clostridia Class	0	0
Clostridium methylpentosum	0	0
Clostridium L2-50	0	0
Coprobacillus cateniformis	0	0
Dialister invisus	0	0
Dialister invisus & Megasphaera micronuciformis	0	0
Dorea spp.	0	0
Eubacterium biforme	0	0
Eubacterium hallii	-1	0
Eubacterium rectale	0	0
Eubacterium siraeum	0	0
Faecalibacterium prausnitzii	-1	0
Lachnospiraceae	-1	0
Lactobacillus ruminis & Pediococcus acidilactici	0	0
Lactobacillus spp.	0	0
Phascolarctobacterium spp.	+1	0
Ruminococcus albus & R. bromii	0	0
Ruminococcus gnavus	0	0
Streptococcus agalactiae & Eubacterium rectale	0	0
Streptococcus salivarius ssp. *thermophilus & S. sanguinis*	0	0

LOE: C

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6267105/

Putting it all together

- Dysbiotic gut bacteria can influence neurotransmitters and HPA axis, fecal sIgA, SCFA's, increased intestinal permeability, inflammation, disease and autoimminuity
- Gut bacteria and catecholamines interact regularly
- Addressing the gut microbiota can benefit stress related adverse health outcomes such as further dysbiosis and pathogen advantages, and increased intestinal permeability
- ACES; stress in childhood profoundly influences adult health, consider the underlying mechanism of action may very well lie in the gut microbiota
- Stress can lead to stress eating which further disrupts the gut microbiota and vicious cycle
- Gut, catecholamines, stress, intestinal permeability are all closely interconnected and should be addressed as such.

LOE: C

QUESTIONS?