THE INSTITUTE FOR FUNCTIONAL MEDICINE®

DIGIN to Root Causes of Gut Dysfunction

PATRICK HANAWAY, MD

Applying Functional Medicine in Clinical Practice

Disclosures

Patrick Hanaway, MD has no financial relationships to disclose.

Evidence Icons: Key

Clinical Disclaimers:

Association, not causation

Lab test
(Labs not generally accepted in conventional care)

Clinical experience
(Intervention warranted by historical clinical experience of educator and/or functional medicine community of practitioners in the context of evidentiary paucity)

Clinical judgment
(Intervention warranted by clinical judgment of educator and/or functional medicine community of practitioners in the context of evidentiary paucity)

Conflict of interest

Study Types:

Animal study

In vitro study

n of 1, or single-case study

In silico *(Computerized molecular modeling)*

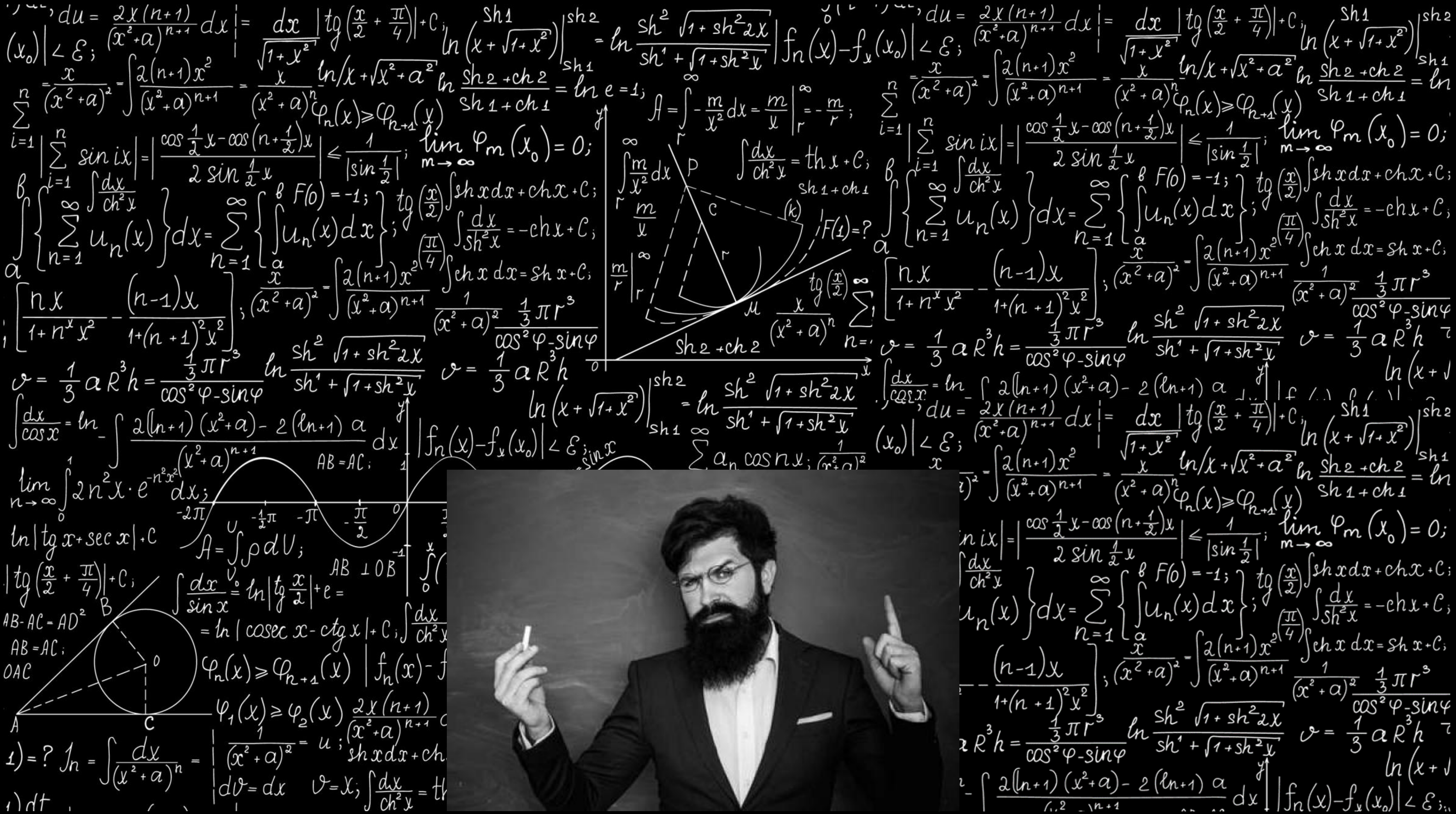
Naturopathic maxim …
"Death begins in the colon."

Permission to use the photograph granted by Bernard Jensen International

The practical application

…

"When in doubt, treat the gut."



Why Focus On The Gut?

- The gut is the organ that produces most of the body's serotonin.
- The gut is the largest immune organ in the body.
- Latest estimates suggest that there are at least as many human cells as bacterial cells in the body.
- The gut houses a genome 100-150 times larger than the human genome.
- The gut's metabolic functions are comparable in magnitude to the liver.

See References: Why Focus on the Gut?

References: Why Focus on the Gut?

1. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161(2):264-276. doi:10.1016/j.cell.2015.02.047.
2. Galland L. The Gut Microbiome and the Brain. *Journal of Medicinal Food*. 2014;17(12):1261-1272. doi:10.1089/jmf.2014.7000.
3. Chassaing B, Kumar M, Baker MT, Singh V, Vijay-Kumar M. Mammalian Gut Immunity. *Biomedical journal*. 2014;37(5):246-258. doi:10.4103/2319-4170.130922.
4. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. *Clinical and Experimental Immunology*. 2008;153(Suppl 1):3-6. doi:10.1111/j.1365-2249.2008.03713.x.
5. Wang H-X, Wang Y-P. Gut Microbiota-brain Axis. *Chinese Medical Journal*. 2016;129(19):2373-2380. doi:10.4103/0366-6999.190667.
6. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology. 2007 Jan;132(1):397-414. doi: 10.1053/j.gastro.2006.11.002. PMID: 17241888.
7. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug 19;14(8):e1002533. doi: 10.1371/journal.pbio.1002533. PMID: 27541692; PMCID: PMC4991899.
8. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65. doi: 10.1038/nature08821.

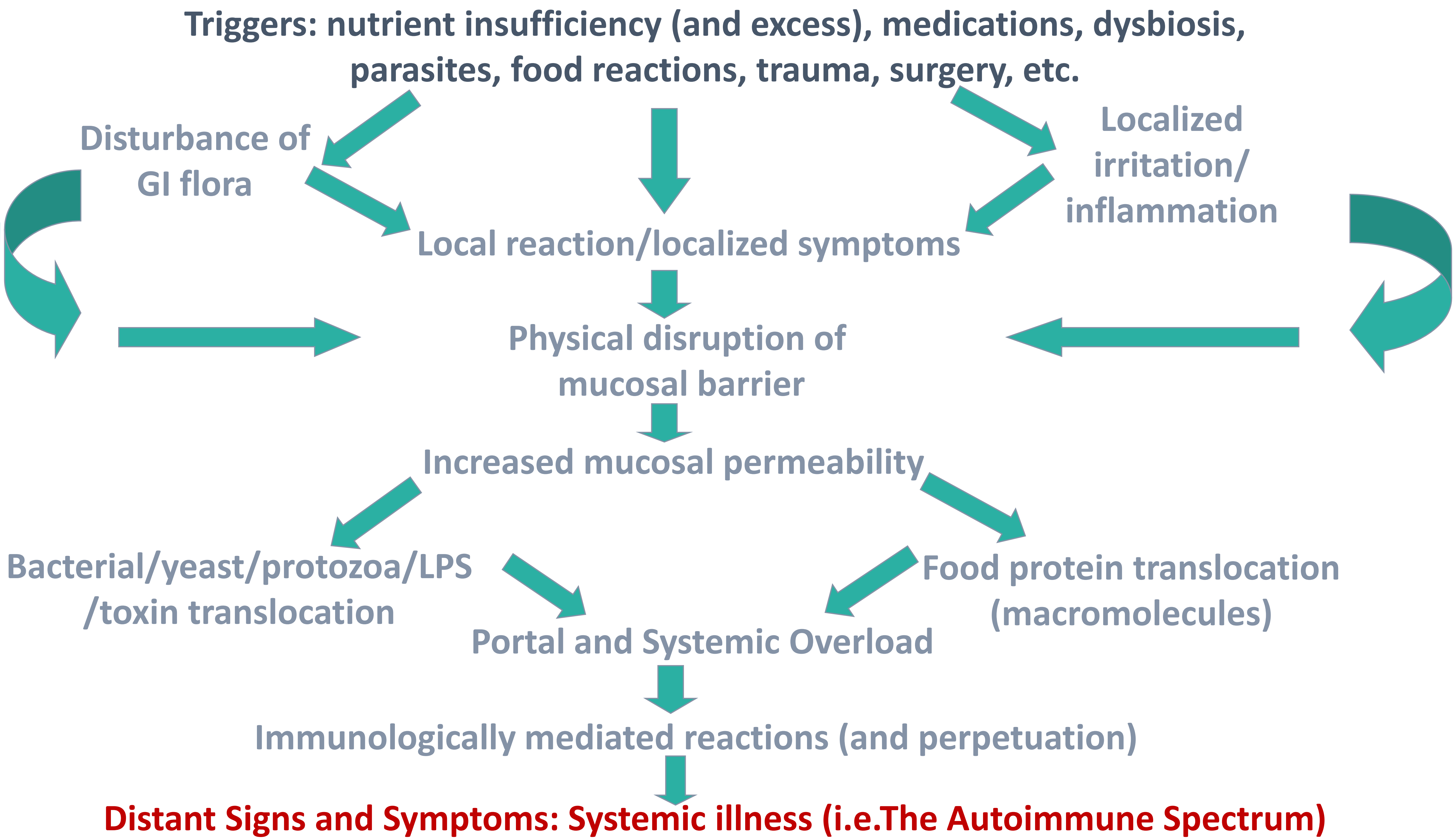
Why Focus On The Gut?

The most effective clinical outcomes across all disease spectrums can result from normalization of gut function.

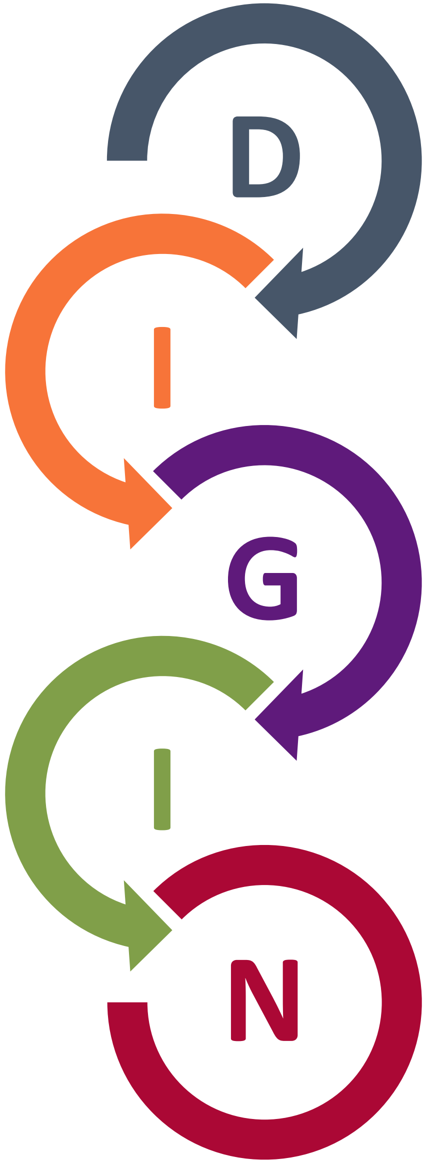
Performance Objectives

Following this activity, successful participants will be able to…

1. **Identify the key functional roles of the gastro-intestinal tract, and recognize how impairments may lead to dysfunction**
2. Identify the role the gastrointestinal tract plays in many chronic diseases
3. Use stool analysis as a foundational tool to help evaluate gastrointestinal function


Triggers: nutrient insufficiency (and excess), medications, dysbiosis, parasites, food reactions, trauma, surgery, etc.
Disturbance of GI flora
Localized irritation/ inflammation
Local reaction/localized symptoms
Physical disruption of mucosal barrier
Increased mucosal permeability
Bacterial/yeast/protozoa/LPS /toxin translocation
Food protein translocation (macromolecules)
Portal and Systemic Overload
Immunologically mediated reactions (and perpetuation)
Distant Signs and Symptoms: Systemic illness (i.e.The Autoimmune Spectrum)

Key Functional Roles of the Gut



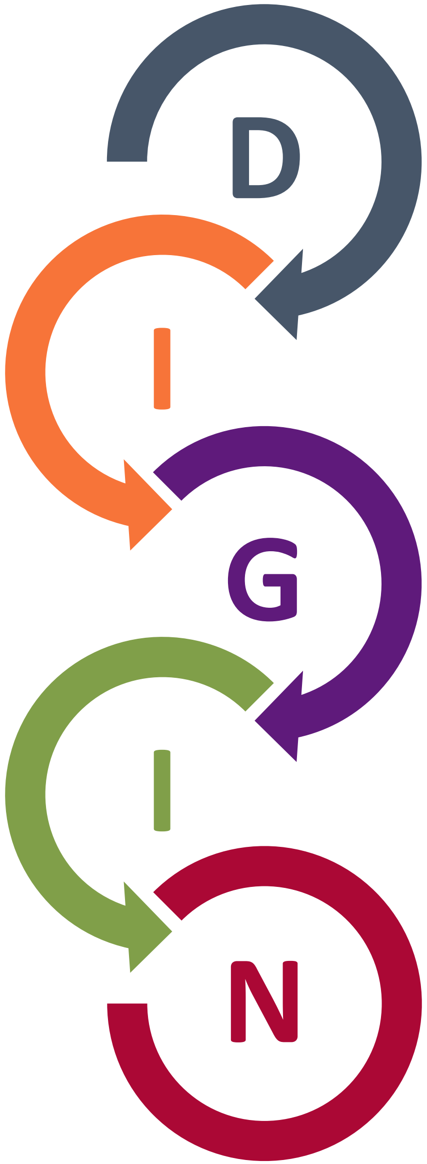
Digestion / Absorption

Intestinal Permeability

Gut microbiota / Dysbiosis

Immune Modulation/Inflammation

Nervous System (Enteric Division)



Digestion/Absorption

GI Tract

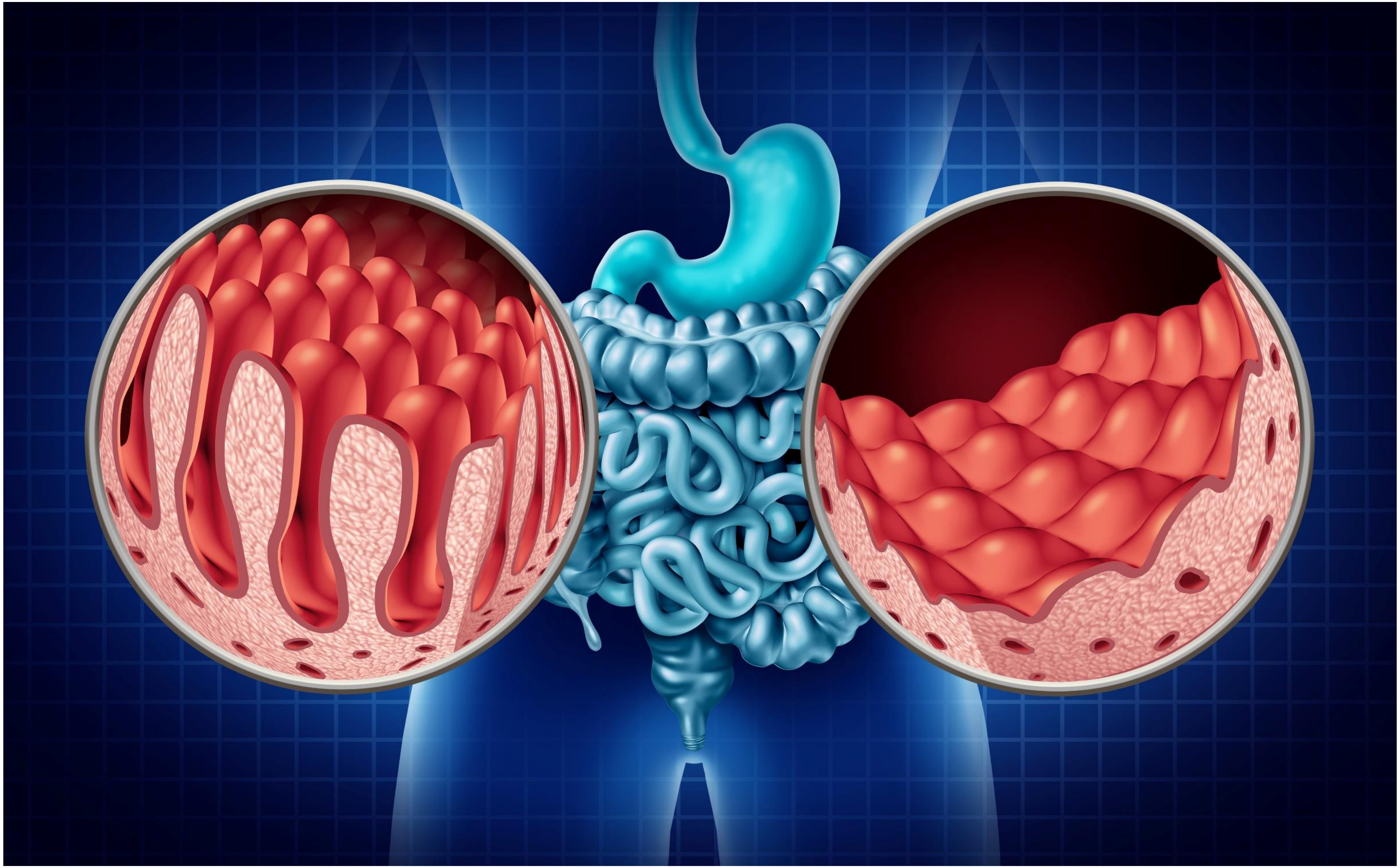
Doughnut Hole

Body

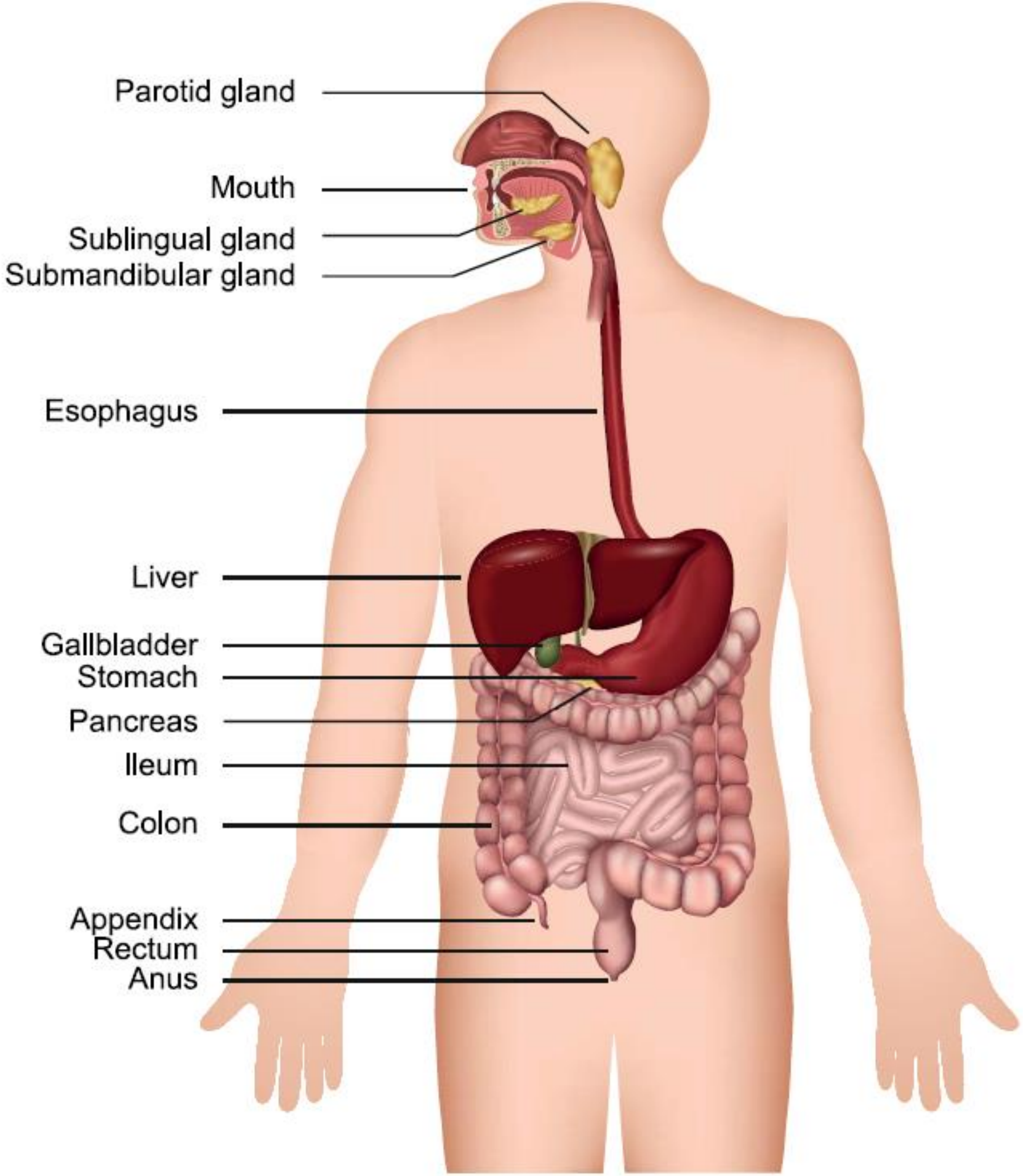
Area of Contact With the Outside World

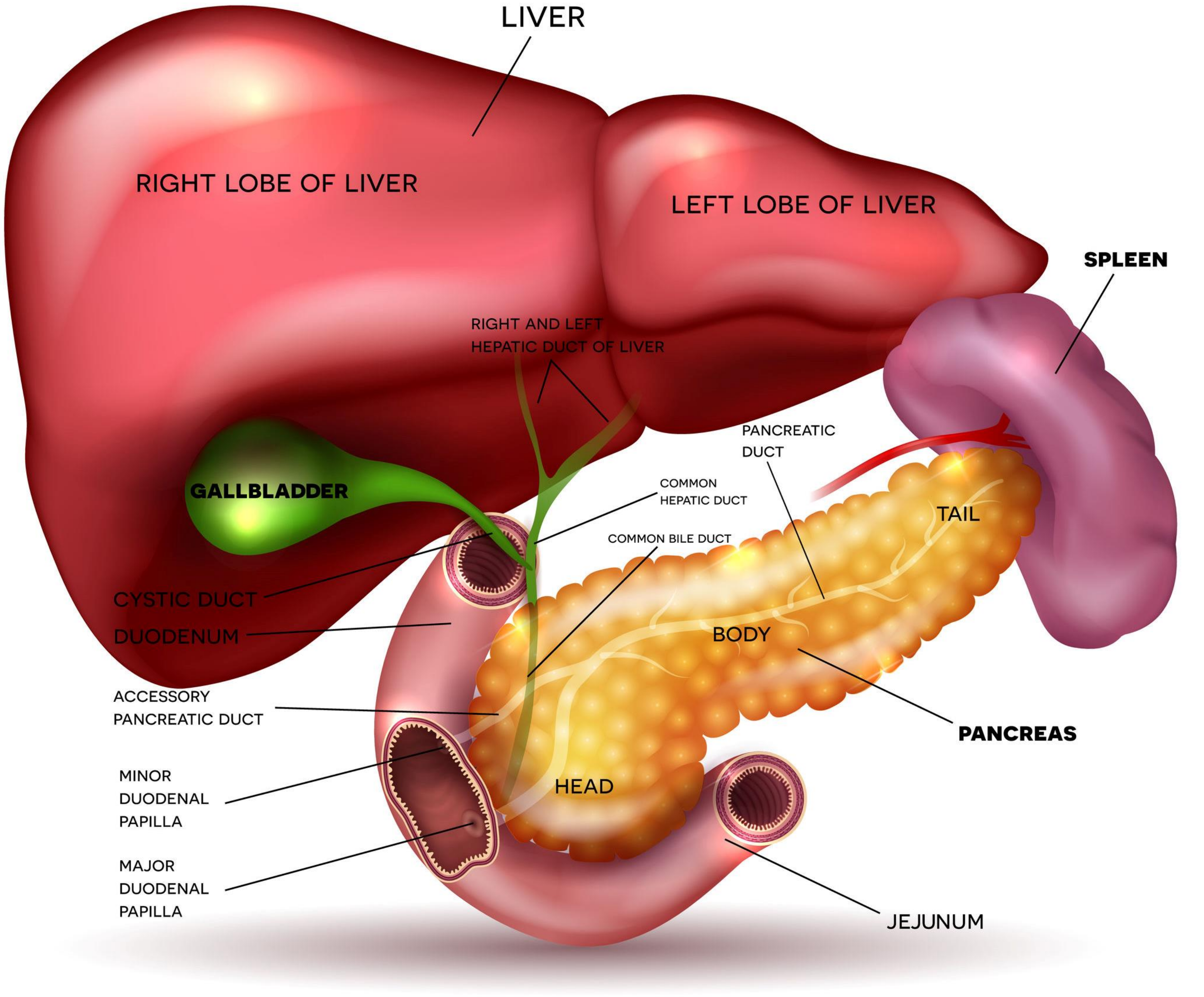
GI mucosal surface area: 30-40 m^2

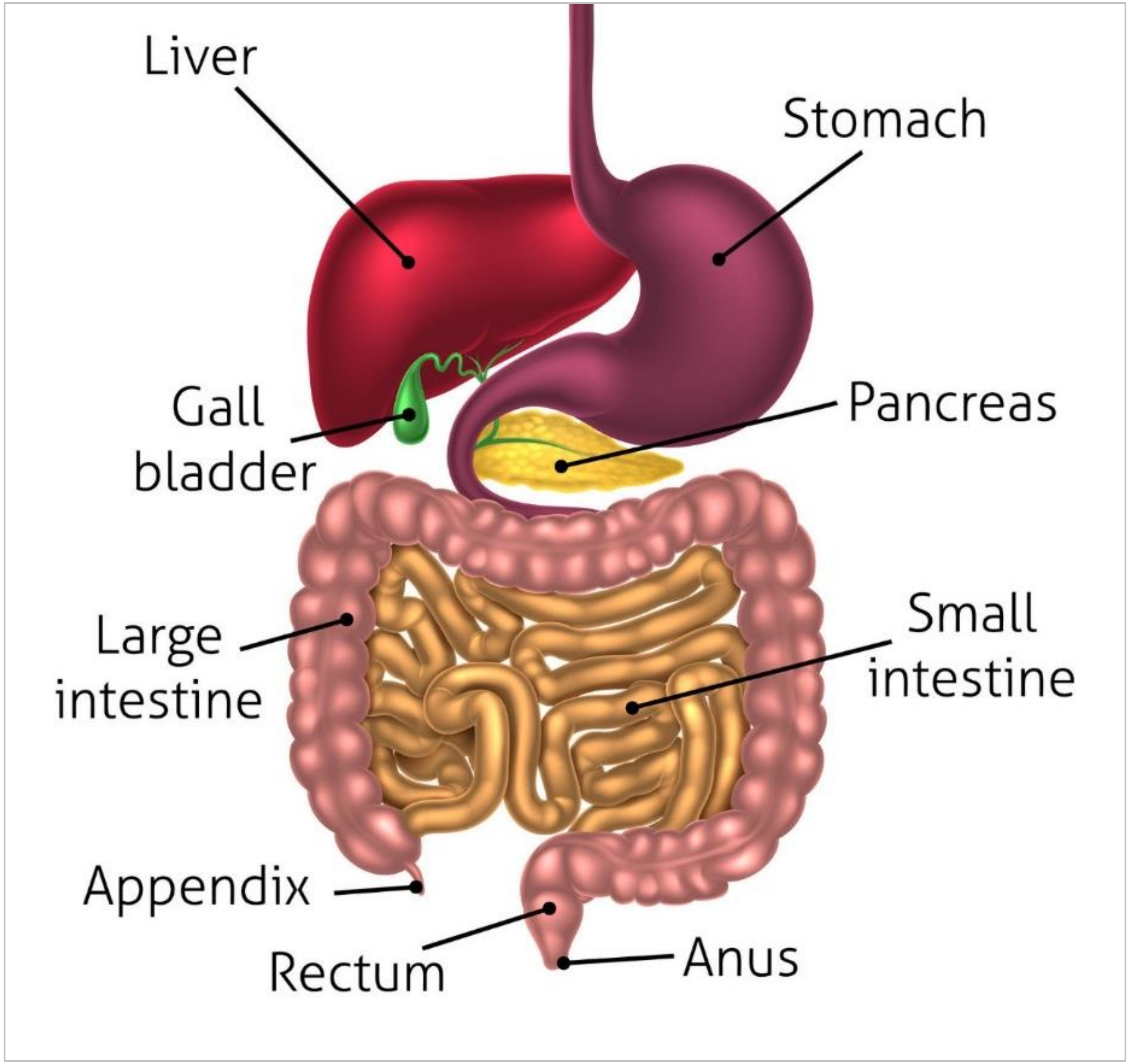
Helander H, Fändriks L. Surface area of the digestive tract – revisited. *Scandinavian Journal of Gastroenterology*. 2014;49(6):681-689. doi:10.3109/00365521.2014.898326.



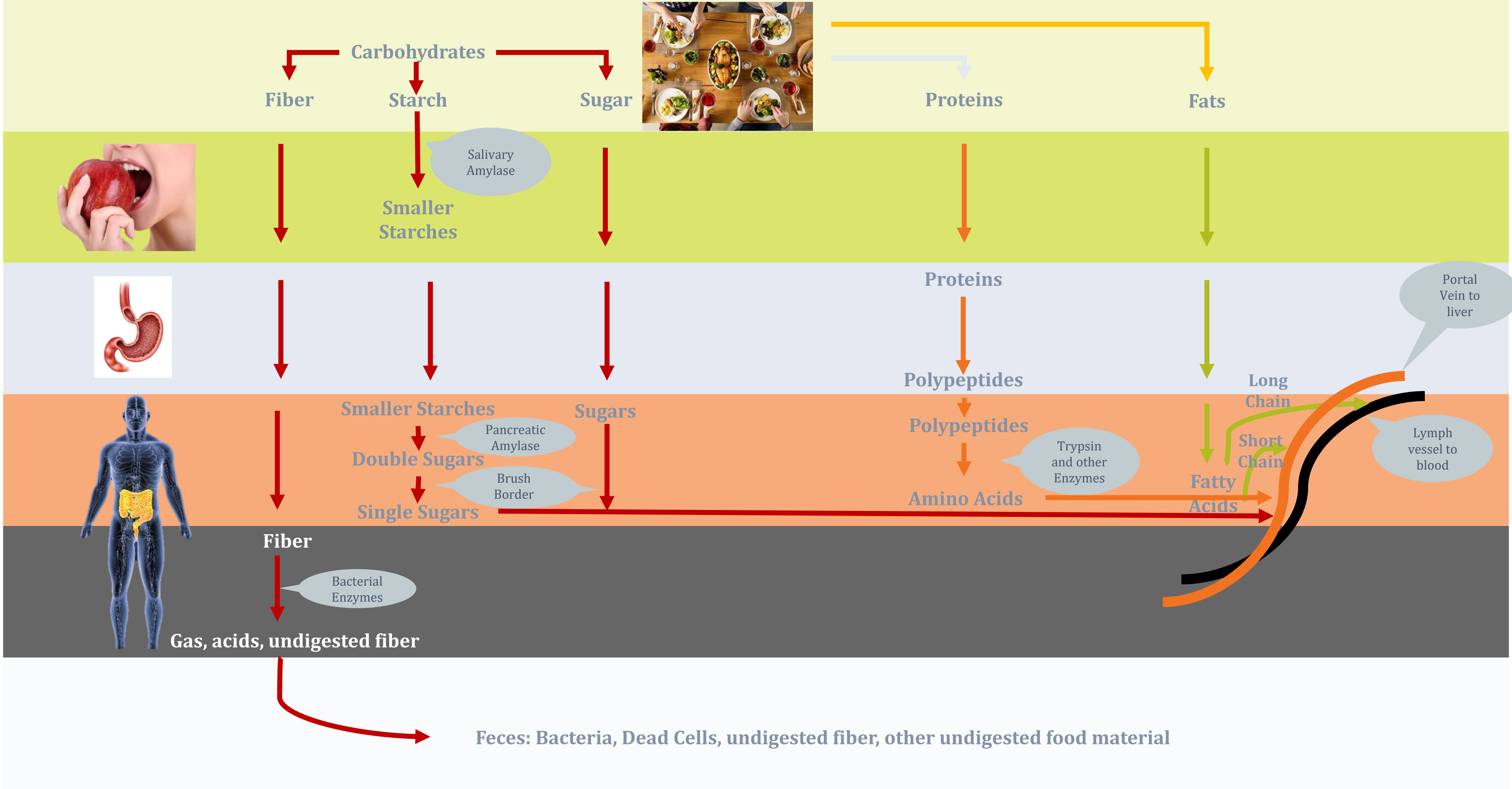
Forsberg G et al. Presence of Bacteria and Innate Immunity of Intestinal Epithelium in Childhood Celiac Disease. The American Journal of Gastroenterology. 2004;99(5):894-904. doi:10.1111/j.1572-0241.2004.04157.x


Parotid gland
Mouth
Sublingual gland
Submandibular gland
Esophagus
Liver
Gallbladder
Stomach
Pancreas
Ileum
Colon
Appendix
Rectum
Anus


LIVER
RIGHT LOBE OF LIVER
LEFT LOBE OF LIVER
SPLEEN
RIGHT AND LEFT
HEPATIC DUCT OF LIVER
PANCREATIC
DUCT
COMMON
HEPATIC DUCT
GALLBLADDER
TAIL
COMMON BILE DUCT
CYSTIC DUCT
DUODENUM
BODY
ACCESSORY
PANCREATIC DUCT
PANCREAS
MINOR
DUODENAL
PAPILLA
HEAD
MAJOR
DUODENAL
PAPILLA
JEJUNUM


Liver
Stomach
Gall
bladder
Pancreas
Large
intestine
Small
intestine
Appendix
Rectum
Anus

Digestion and Absorption

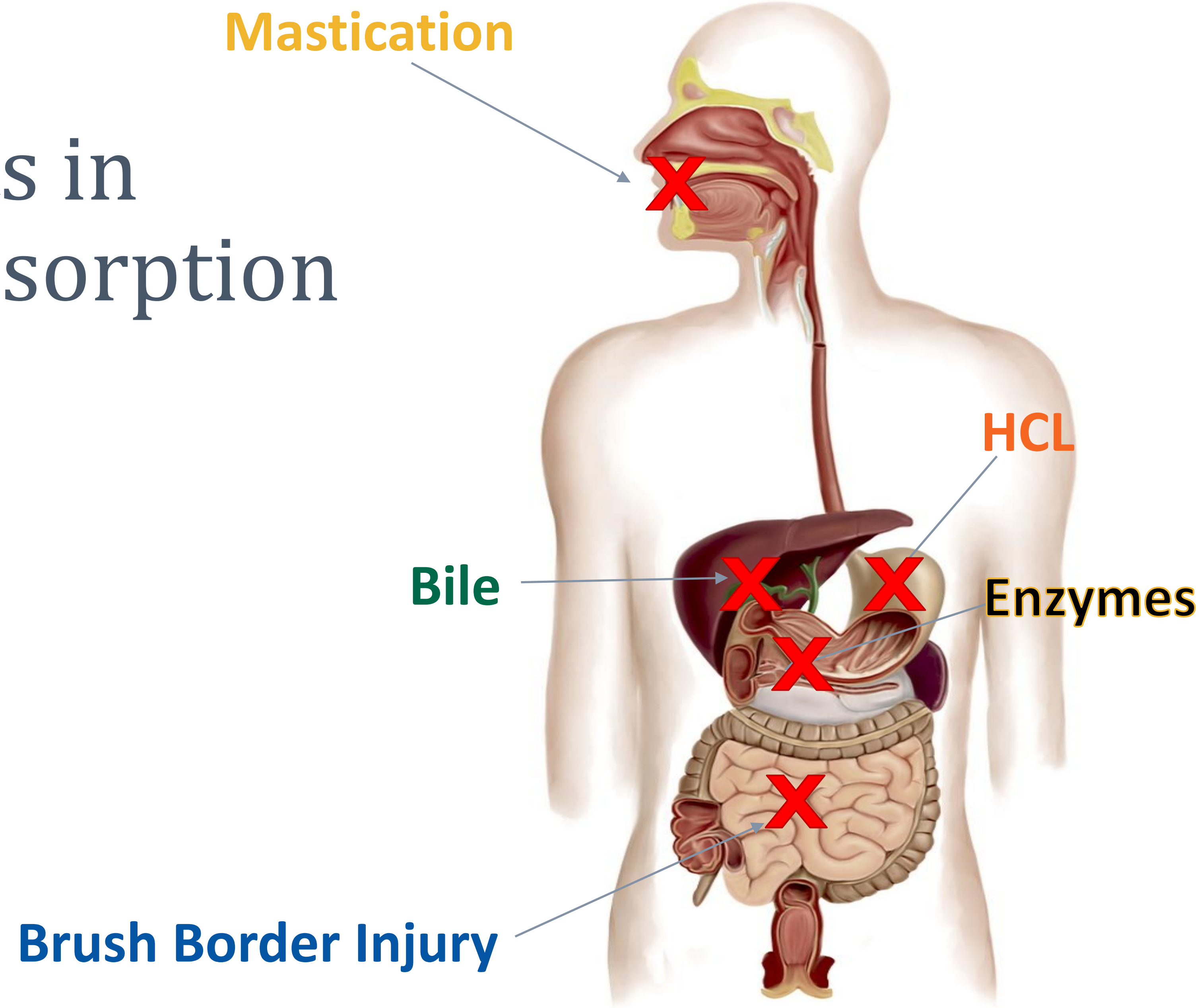


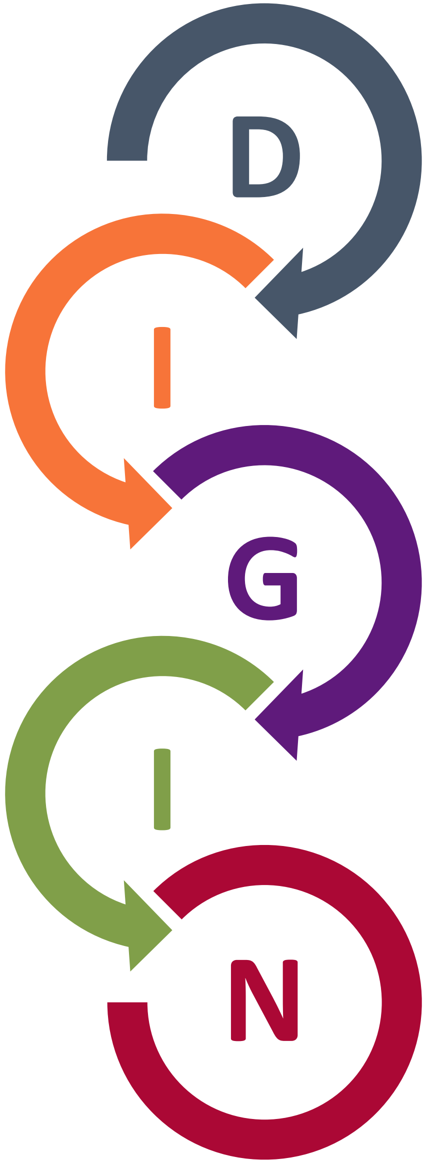
Summary: Digestion/Absorption

- Mechanical breakdown
- Enzyme hydrolysis of carbohydrates, proteins, lipids and nucleic acids
- Active and passive absorption
- Regulation from CNS and ENS integrate hormones & paracrines to coordinate digestion

Impairments in Digestion and Absorption

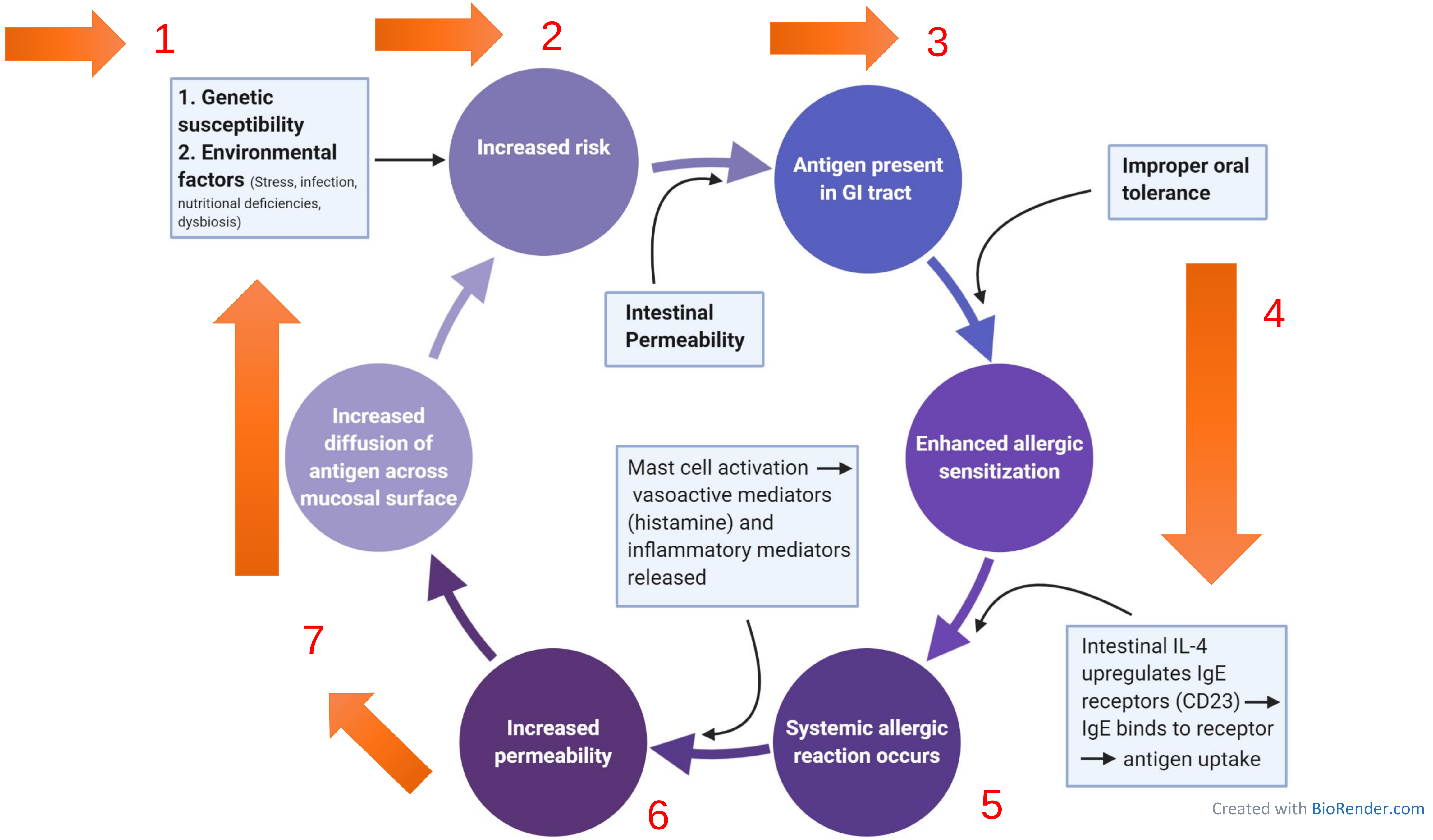
- Inadequate mastication
- Hypochlorhydria
- Pancreatic insufficiency
- Bile insufficiency
- Brush Border Injury





Digestion/Absorption

INTESTINAL PERMEABILITY



1. Perrier C, Corthésy B. Gut permeability and food allergies. Clinical & Experimental Allergy. 2011; 41: 20–28. doi:10.1111/j.1365-2222.2010.03639.x
2. Johnston LK, Chien KB, Bryce PJ. The immunology of food allergy. *J Immunol*. 2014;192(6):2529-2534. doi:10.4049/jimmunol.1303026

“Please…tell me again about this imaginary fence.”

Intestinal Permeability

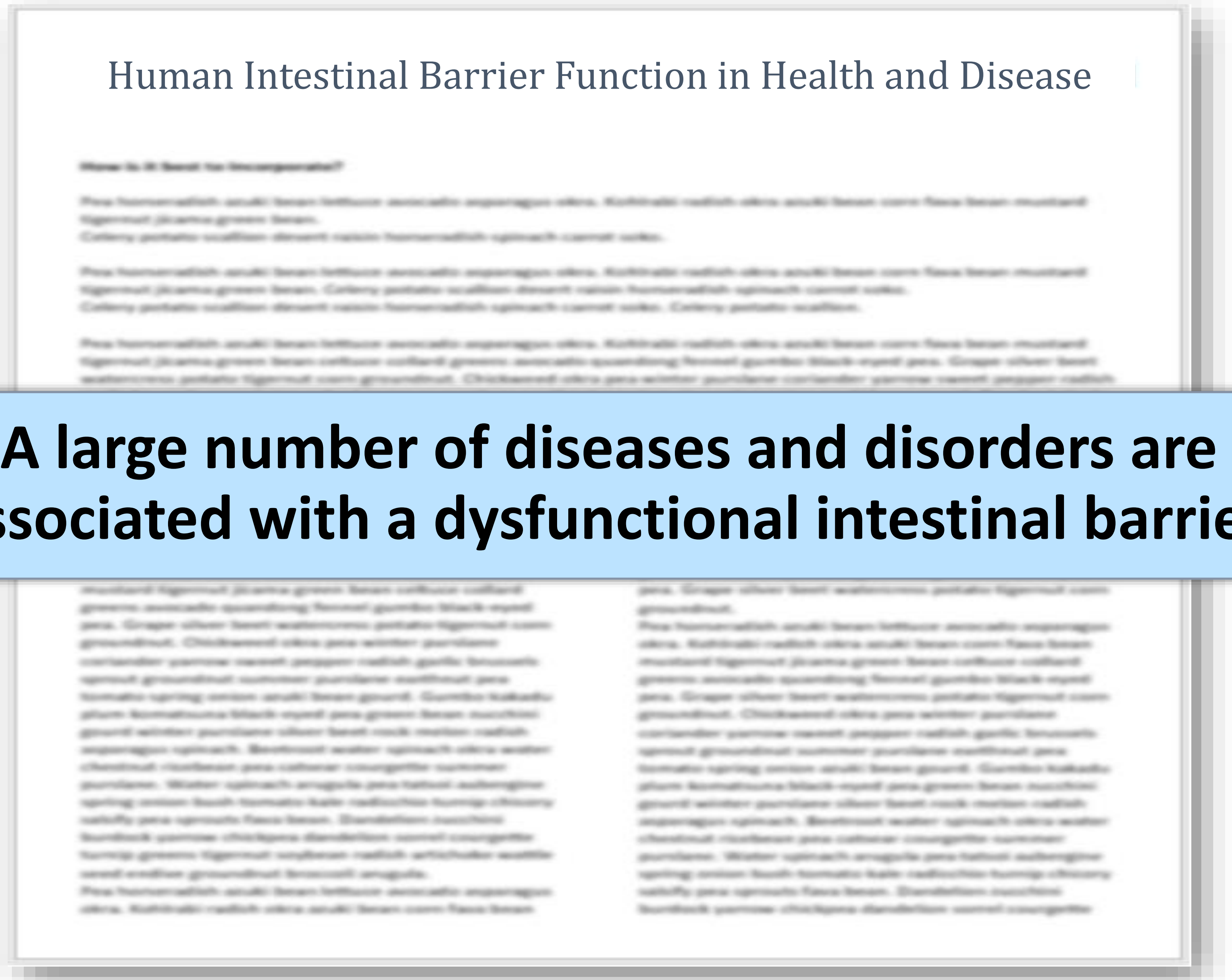
- The importance of intestinal permeability has been documented in the literature for 30 years or more.
- "Luminal complexing by secretory IgA and an intact epithelial barrier limits uptake of luminal antigen; however, **intestinal inflammation enhances mucosal uptake and systemic distribution of potentially injurious macromolecules"**[2]
- "An essential function of epithelial-lined surfaces is to create the interface between separate body compartments."[1]

1. Turner JR. Molecular Basis of Epithelial Barrier Regulation. The American Journal of Pathology. 2006;169(6):1901-1909. doi:10.2353/ajpath.2006.060681.
2. Sartor RB. Importance of intestinal mucosal immunity and luminal bacterial cell wall polymers in the etiology of inflammatory joint diseases. *Baillières Clinical Rheumatology*. 1989;3(2):223-245. doi:10.1016/s0950-3579(89)80019-6.

Molecular Basis of Epithelial Barrier Regulation

The intestinal mucosa faces a difficult challenge. It must provide a protective barrier against the external environment, but also must function in both active and passive transport.
<u>For both normal physiological functioning and disease prevention, an intact intestinal barrier is essential.</u>

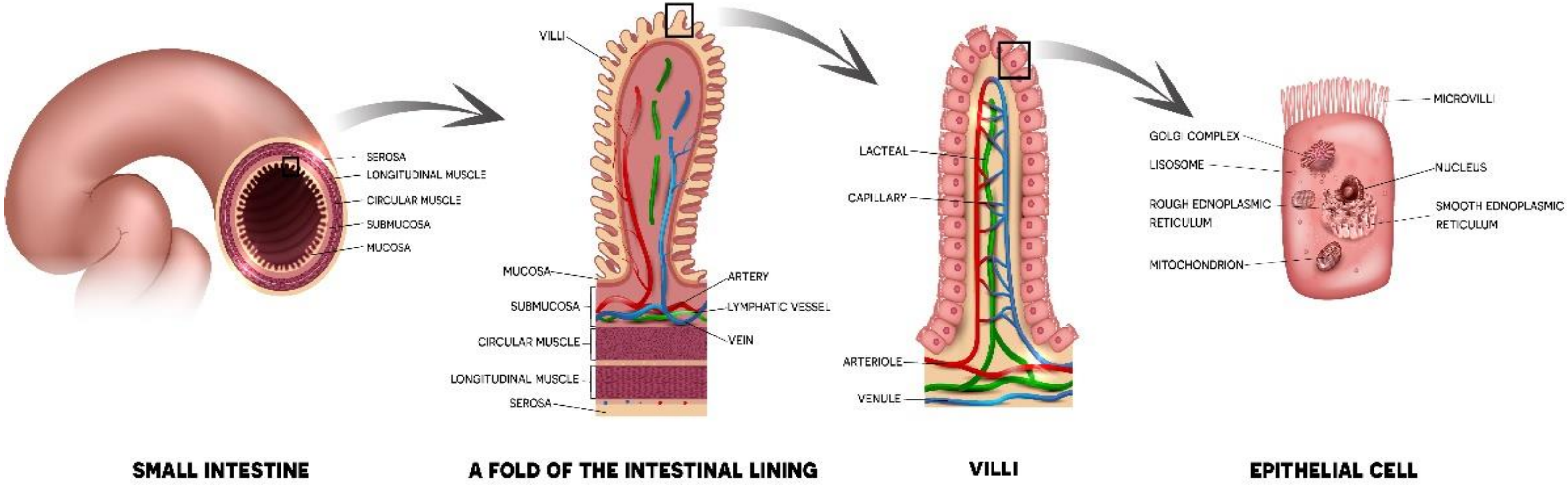
Turner JR. Molecular Basis of Epithelial Barrier Regulation. The American Journal of Pathology. 2006;169(6):1901-1909. doi:10.2353/ajpath.2006.060681.

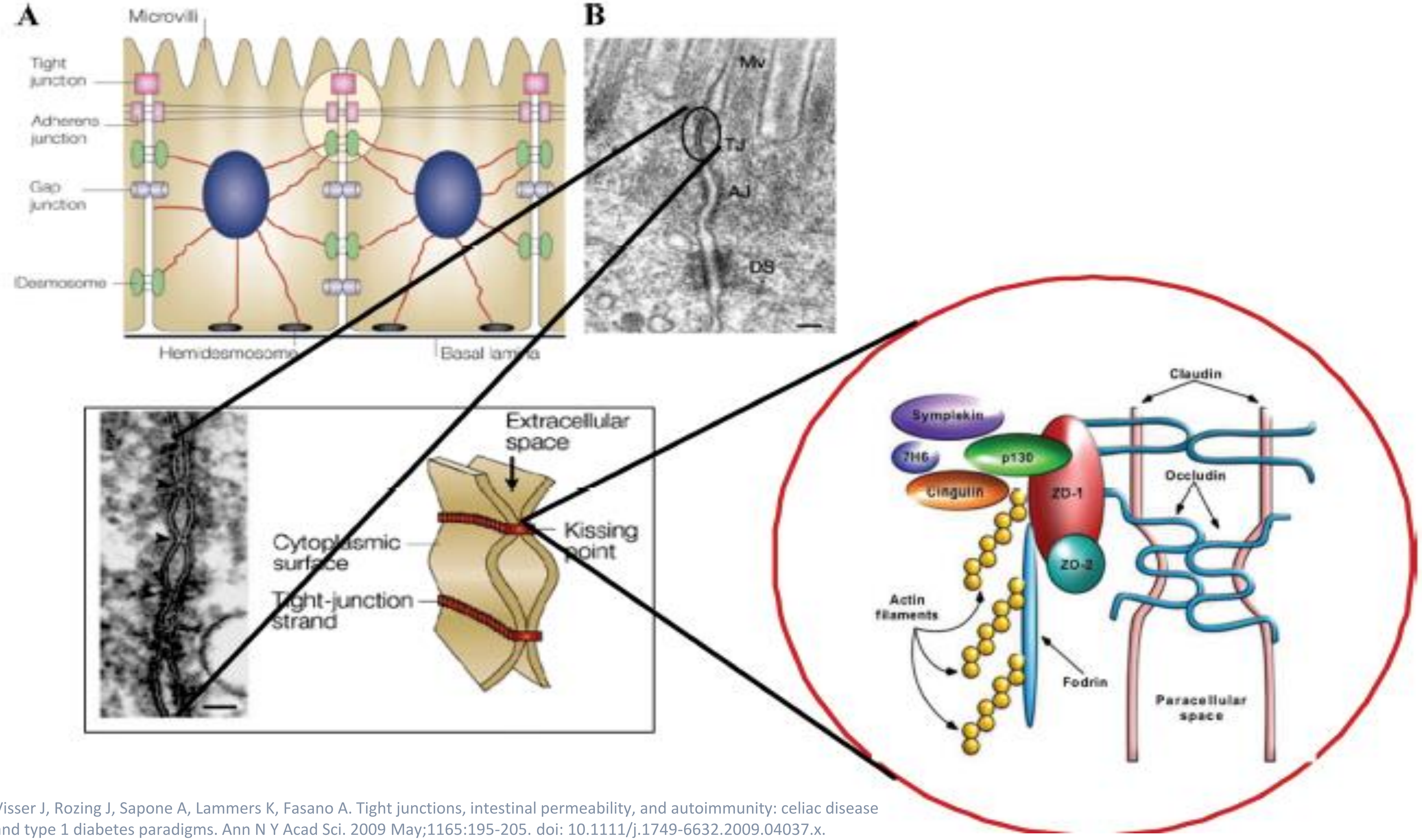

Human Intestinal Barrier Function in Health and Disease

A large number of diseases and disorders are associated with a dysfunctional intestinal barrier

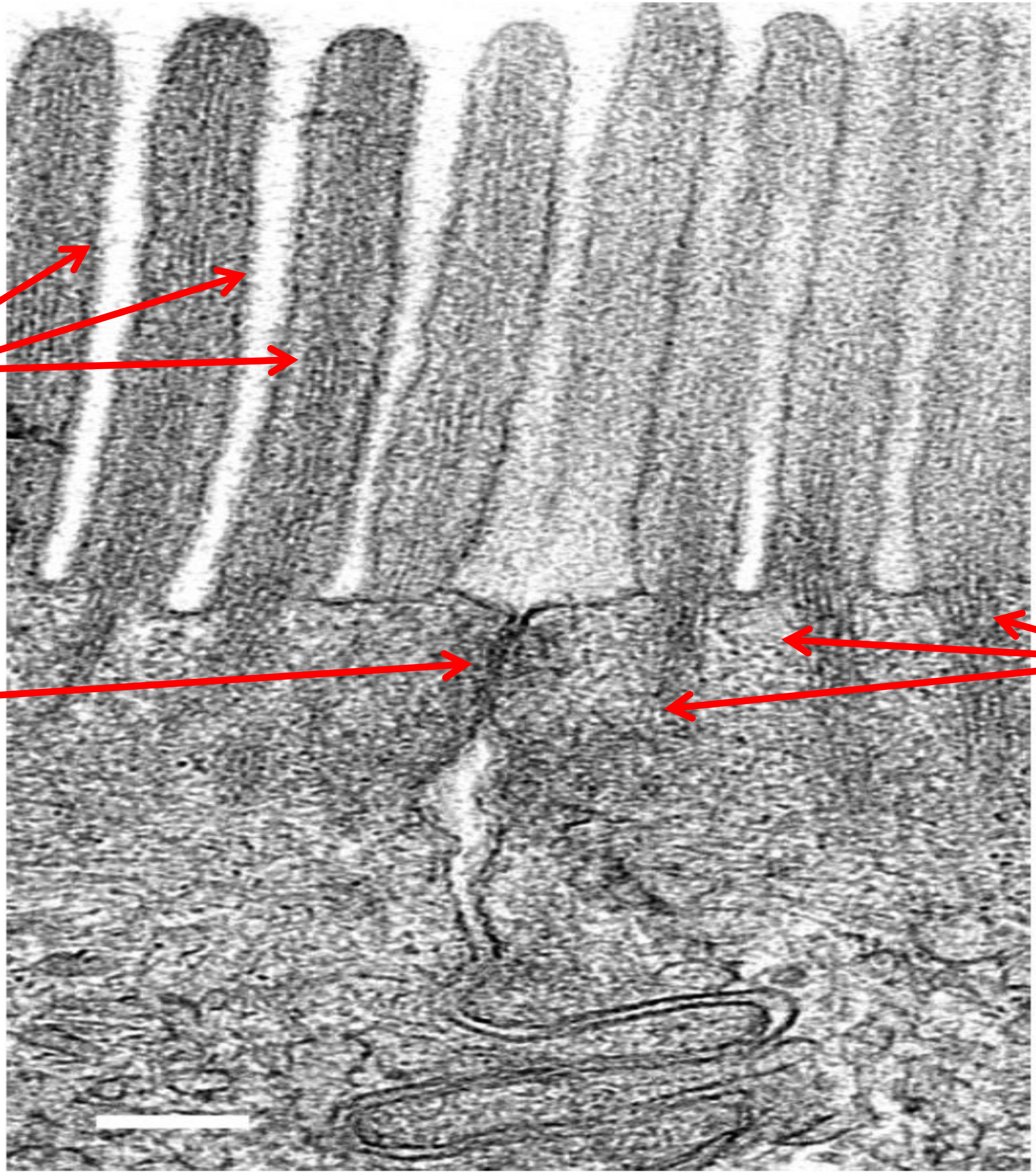
König J, Wells J, Cani PD, et al. Human Intestinal Barrier Function in Health and Disease. *Clinical and Translational Gastroenterology*. 2016;7(10):e196-. doi:10.1038/ctg.2016.54.

SMALL INTESTINE



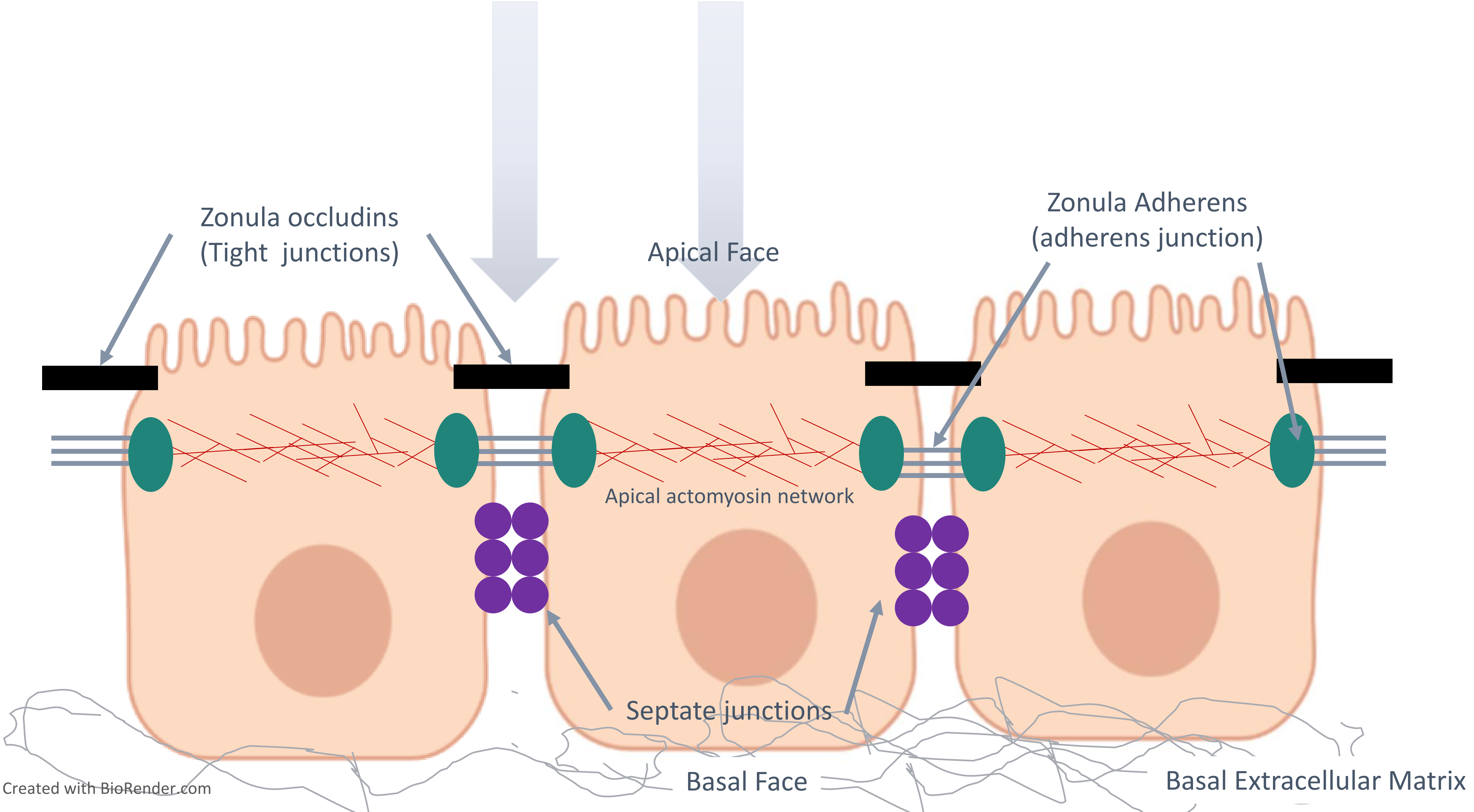


Visser J, Rozing J, Sapone A, Lammers K, Fasano A. Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. Ann N Y Acad Sci. 2009 May;1165:195-205. doi: 10.1111/j.1749-6632.2009.04037.x.



Reprinted from The American Journal of Pathology, Vol. 169, Turner, J.R., Turner JR. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application, Pages 1901-1909, Copyright 2006, with permission from American Society for Investigative Pathology.

Epithelial tissue adhesion architecture

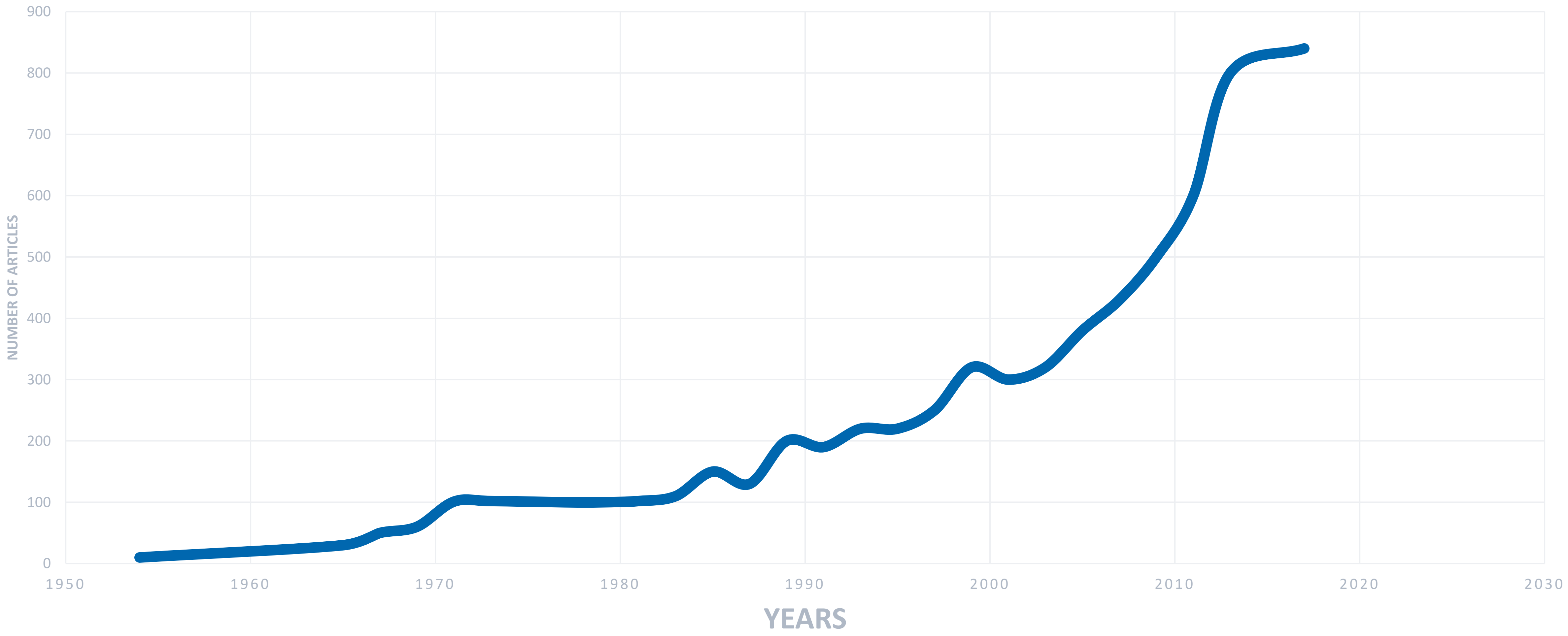


Why is This So Important?

"The mucosa is directly exposed to the external environment and taxed with antigenic loads consisting of commensal bacteria, dietary antigens, and viruses ***at far greater quantities on a daily basis than the systemic immune system sees in a lifetime.***"

Mayer L. Mucosal immunity. Pediatrics. 2003 Jun;111(6 Pt 3):1595-600. PMID: 12777598.

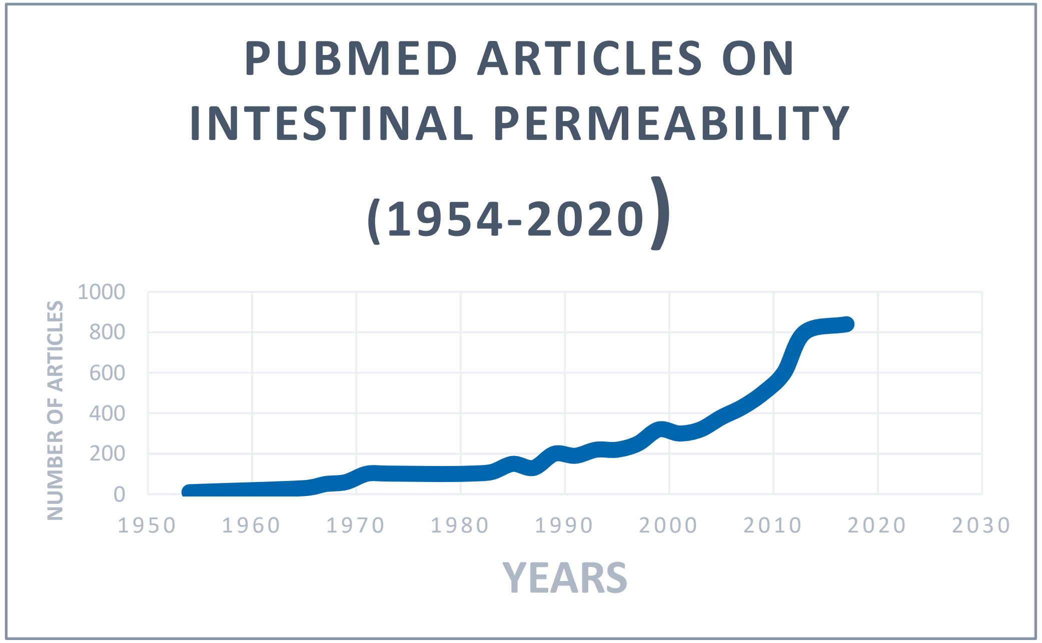
PUBMED ARTICLES ON INTESTINAL PERMEABILITY (1954-2020)



Adapted from PubMed

Intestinal Permeability: History

- Concept consolidated by Fasano in 2000
- 15,273 PubMed articles indexed by "intestinal permeability" as of January 2020
- Other related terms:
  - **Leaky Gut**
  - **Auto-intoxication**
  - **Endotoxemia**



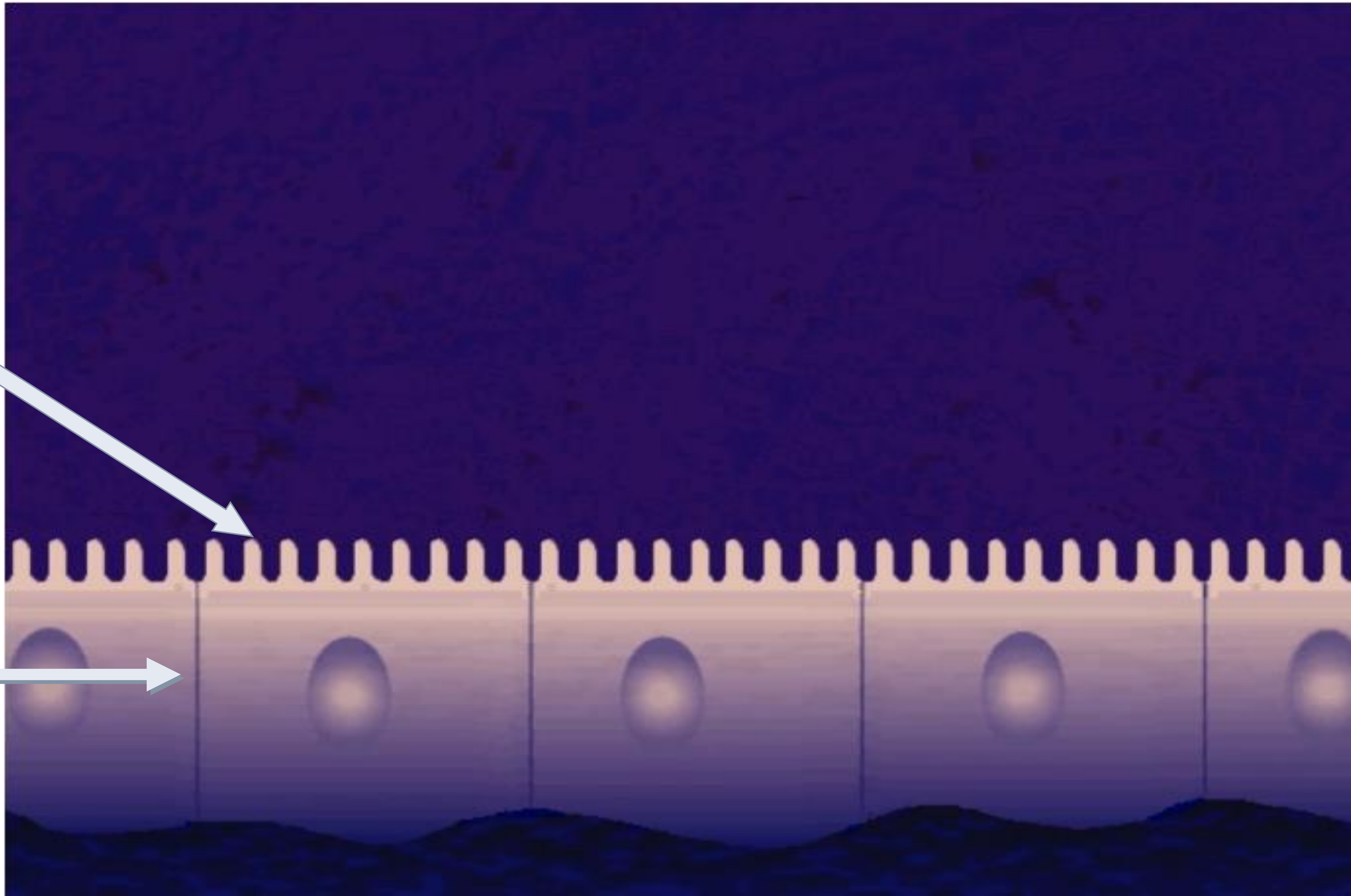
1. Fasano A. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Ann N Y Acad Sci. 2000;915:214-22. doi: 10.1111/j.1749-6632.2000.tb05244.x. PMID: 11193578.
2. Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet. 2000 Apr 29;355(9214):1518-9.

Gate-Keeper Function of the Intestinal Epithelium

“Increased epithelial permeability for antigens is a crucial primary or secondary event in the pathogenesis of several disorders.”

Brandtzaeg P. Gate-keeper function of the intestinal epithelium. Benef Microbes. 2013 Mar 1;4(1):67-82. doi: 10.3920/BM2012.0024. https://pubmed.ncbi.nlm.nih.gov/23257015/

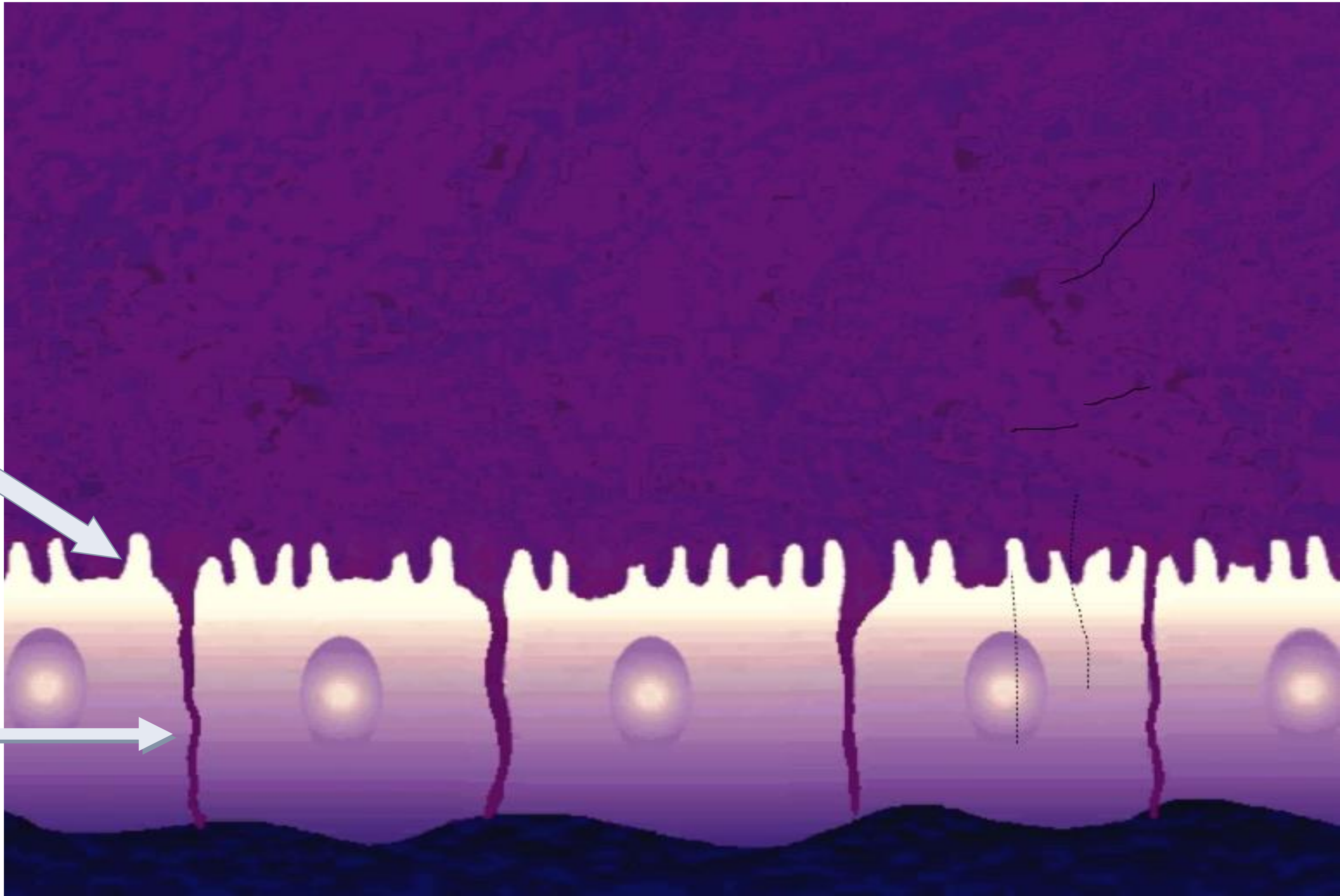
Healthy Gut



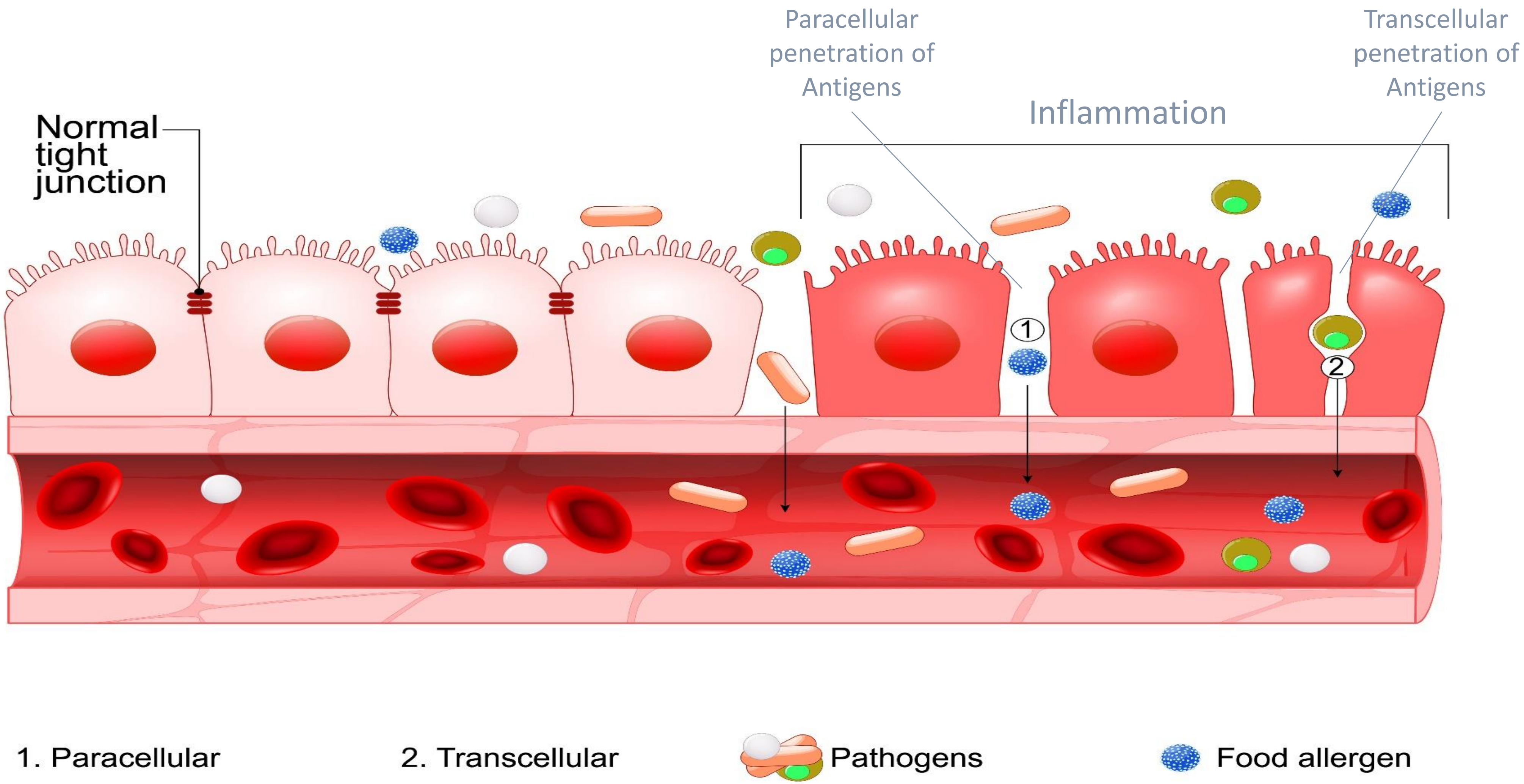
Leaky Gut

Damaged Villi

Damaged
Cell junctions



LEAKY GUT



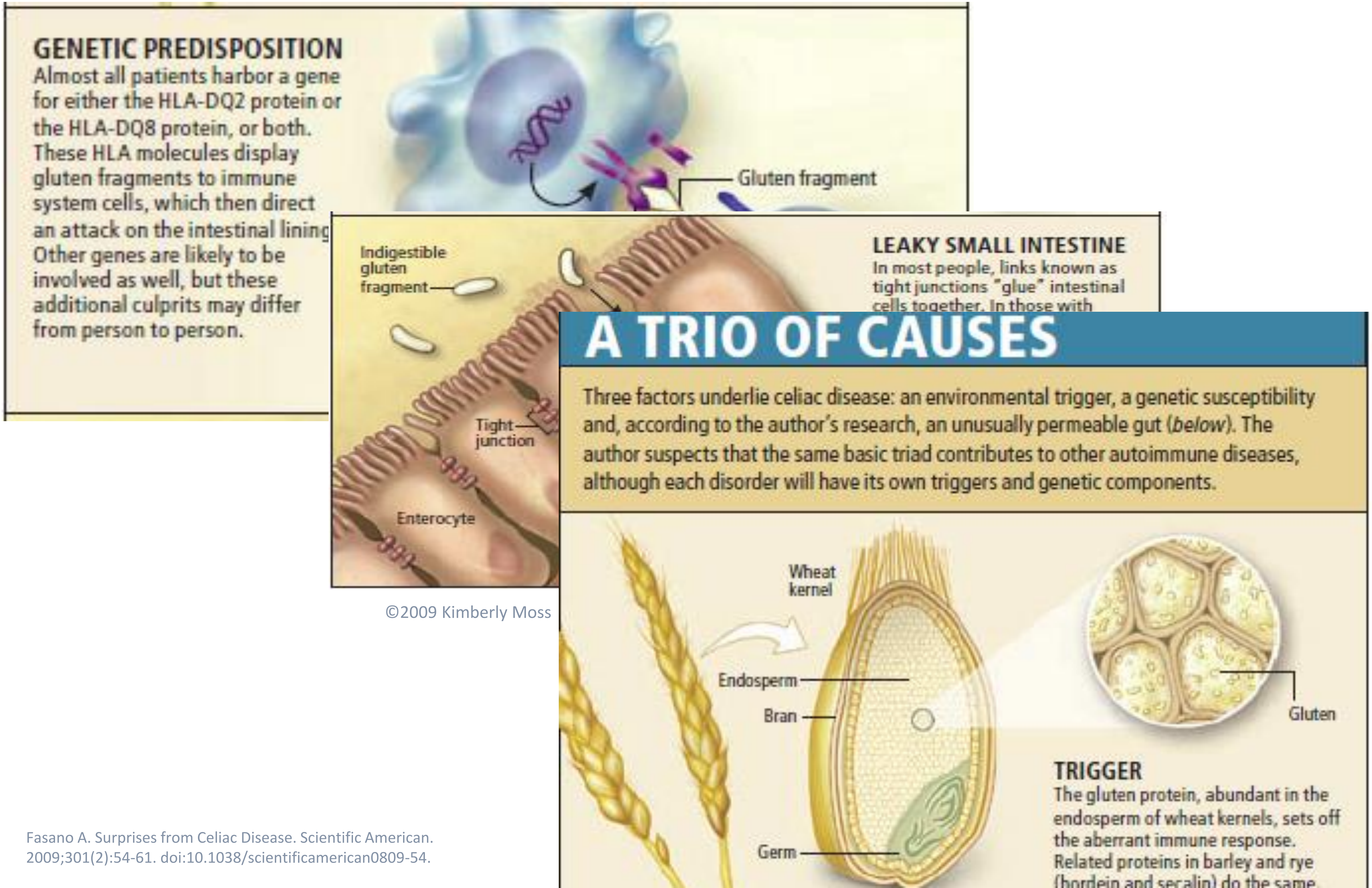
Paracellular penetration of Antigens
Transcellular penetration of Antigens
Inflammation
Normal tight junction
1
2
1. Paracellular
2. Transcellular
Pathogens
Food allergen

MEDICINE

Surprises *from* Celiac Disease

Study of a potentially fatal food-triggered disease has uncovered a process that may contribute to many autoimmune disorders • BY ALESSIO FASANO

Fasano A. Surprises from celiac disease. Sci Am. 2009 Aug;301(2):54-61.



Fasano A. Surprises from Celiac Disease. Scientific American. 2009;301(2):54-61. doi:10.1038/scientificamerican0809-54.

Genetic Propensity

Environmental Triggers

Altered Microbiome with Gut Inflammation

Increased Intestinal Permeability
and translocation of macromolecules (LPS, foods,…)

Systemic Immune Response

Initiation of the Autoimmune Spectrum

Valitutti F, Fasano A, Breaking Down Barriers: How Understanding Celiac Disease Pathogenesis Informed the Development of Novel Treatments. Dig Dis Sci. 2019 Jul;64(7):1748-1758

REVIEW

www.nature.com/clinicalpractice/gasthep

Mechanisms of Disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases

Alessio Fasano* and Terez Shea-Donohue

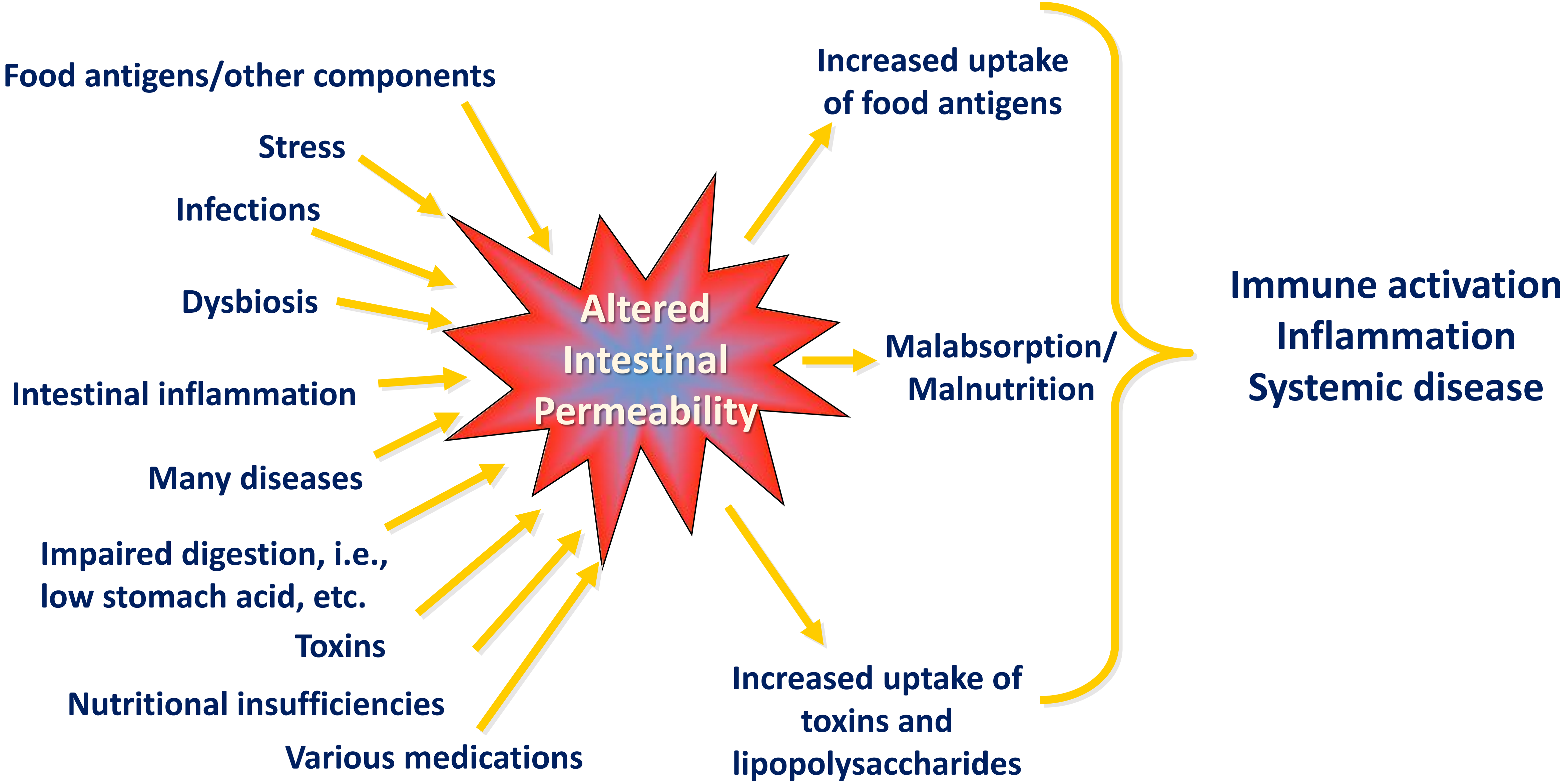
Because the interaction between genes and environmental triggers can drive autoimmunity, restoring healthy function of the intestinal barrier can halt the autoimmune process

Derived from Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol. 2005 Sep;2(9):416-22.

Possible causes of impairment of the intestinal barrier	
Nutritional factors	Tight junction downregulation
	Histone deacetylase (HDAC) inhibitors
	Enteric nervous system modulators
Infections and toxins	Viral intestinal infection
	Environmental toxins (BPA, Glyphosate,…)
	Toxic foods
“Hygiene hypothesis”	Sterile environment
	Lack of farming
“Lifestyle hypothesis”	Impaired function and diversity
	of the intestinal microbiota
Endogenous factors	Hypoperfusion of the intestine
	Chronic inflammation/autoimmunity

Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol. 2014 Nov 18;14:189. doi: 10.1186/s12876-014-0189-7. PMID: 25407511; PMCID: PMC4253991.

What Are The Triggers of Increased IP?



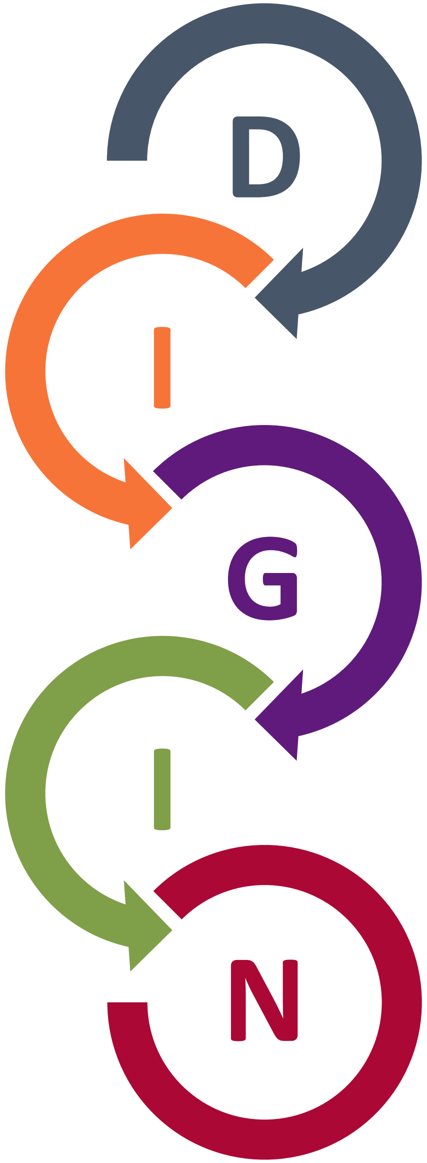
References: Triggers of Intestinal Permeability

1. **Dietary choices**: Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress related psychiatric disorders. *Frontiers in Cellular Neuroscience*. 2015;9:392. doi:10.3389/fncel.2015.00392.
2. **Stress**: Vanuytsel T, van Wanrooy S, Vanheel H, et al. Psychological stress and corticotropin releasing hormone increase intestinal permeability in humans by a mast cell dependent mechanism. *Gut*. 2014 Aug; 63(8):12939. doi: 10.1136/gutjnl 2013305690.
3. **Infection:** Kukuruzovic R, Robins, Browne RM, Anstey NM, Brewster DR. Enteric pathogens, intestinal permeability and nitric oxide production in acute gastroenteritis. *Pediatr Infect Dis J*. 2002 Aug;21(8):730-9.
4. **Dysbiosis**: Brown K, DeCoffe D, Molcan E, Gibson DL. Diet induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients.* 2012;4(8):1095-1119. doi:10.3390/nu4081095
5. **Inflammation**: Michielan A, D'Incà R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. *Mediators of Inflammation*. 2015;2015:628157. doi:10.1155/2015/628157
6. **Systemic Disease**: Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. *Gut*. 2006;55(10):1512-1520. doi:10.1136/gut.2005.085373.
7. **Impaired Digestion**: Centanni M, Marignani M, Gargano L, Corleto VD, Casini A, Delle Fave G,Andreoli M, Annibale B. Atrophic body gastritis in patients with autoimmune thyroid disease: an underdiagnosed association. *Arch Intern Med*. 1999 Aug 9-23;159(15):1726-30.
8. **Toxins**: Pinton P, Nougayrède JP, Del Rio JC, et al. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol Appl Pharmacol. 2009 May 15;237(1):41-8. doi: 10.1016/j.taap.2009.03.003.
9. **Nutritional Deficiencies**: Tran CD, Hawkes J, Graham RD, et al. Zinc-fortified oral rehydration solution improved intestinal permeability and small intestinal mucosal recovery. Clin Pediatr (Phila). 2015 Jun;54(7):676-82.doi: 10.1177/0009922814562665.
10. **Medications**: Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A*. 2011 Mar 15;108 Suppl 1:4554 61. doi: 10.107 3/pnas.1000087107.
11. **Food Allergy:** Järvinen KM, Konstantinou GN, et al. Intestinal permeability in children with food allergy on specific elimination diets. *Pediatr Allergy Immunol*. 2013 Sep;24(6):589-95. doi: 10.1111/pai.12106.
12. **Malnutrition**: Norman K, Pirlich M, Schulzke JD, Smoliner C, Lochs H, Valentini L, Bühner S. Increased intestinal permeability in malnourished patients with liver cirrhosis. Eur J Clin Nutr. 2012 Oct;66(10):1116-9. doi: 10.1038/ejcn.2012.104.

Triggers and Mediators of Intestinal Permeability

- What are triggers and mediators in Joan's case?
- What are the most important in your patient population?
- How do you describe intestinal permeability to your patients?

Part 2



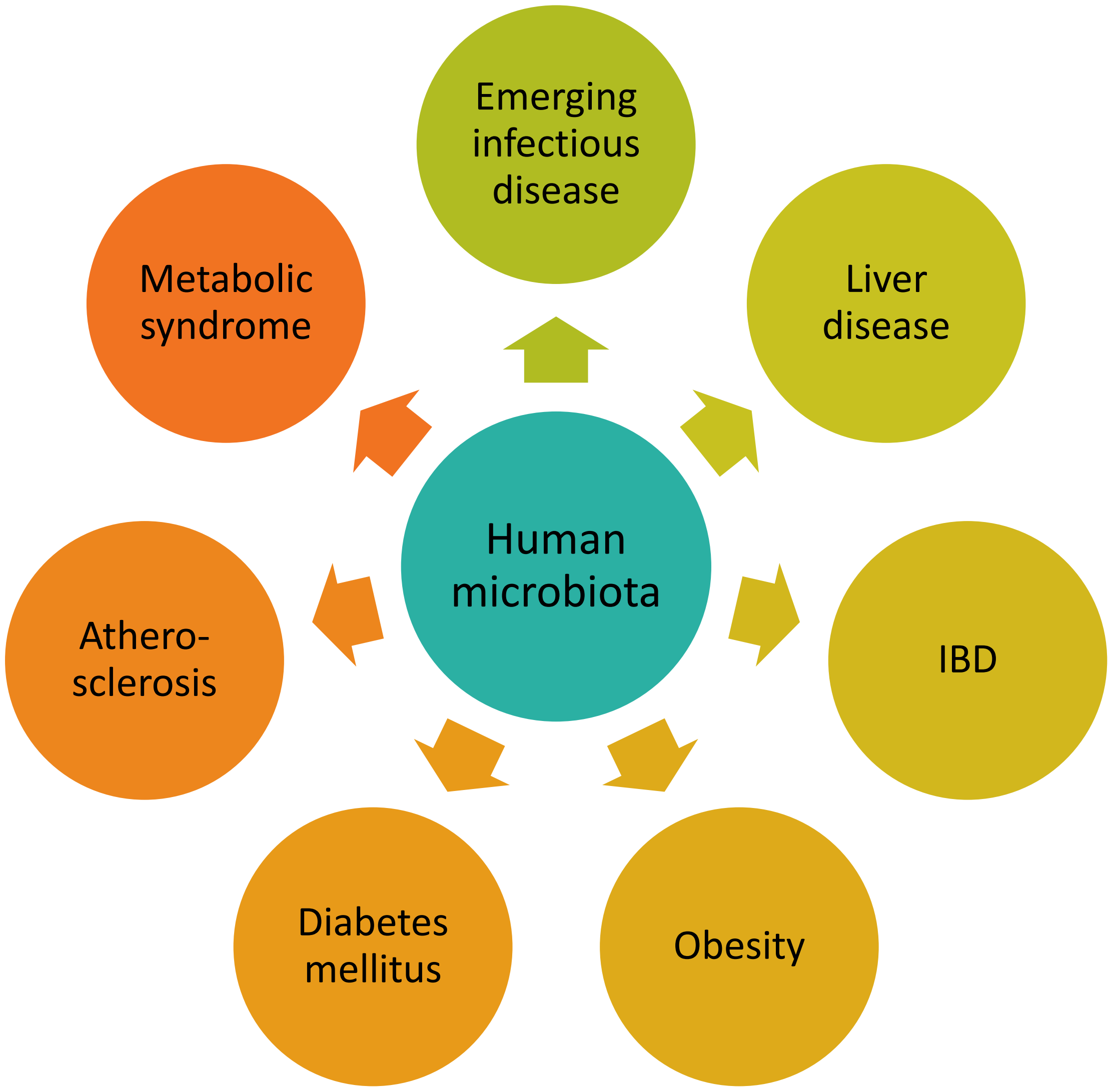
Digestion/Absorption

Intestinal Permeability

GUT MICROBIOTA


50-100 trillion friends you may not have known you have!

How critical is an understanding of the role of the microbiota in health and disease?



West CE, Renz H, Jenmalm MC, et al. The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies. Journal of Allergy and Clinical Immunology. 2015;135(1):3-13. doi:10.1016/j.jaci.2014.11.012.

The gut microbiota and host health: a new clinical frontier

Like the immune system, the microbiome of the gut is unique in each individual, contains components that are heritable, and contains 150 more genes than the host. Without it, virtually all physiological aspects of the host are altered. The gut microbiome is modifiable through ***diet, antibiotics, stress, chemicals, and other environmental factors***, each **influencing the makeup and diversity of the microbiome and ultimately physiological function**. In all of these ways, the gut microbiome functions like another organ in the body.

Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: A new clinical frontier. Gut. 2016;65(2):330-339. doi: 10.1136/gutjnl-2015-309990.

The gut microbiota shapes intestinal immune responses during health and disease

"By young adulthood, both humans and other mammals support one of the most **complex** microbial ecosystems on the planet."

Round, J., Mazmanian, S. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9, 313–323 (2009). https://doi.org/10.1038/nri2515

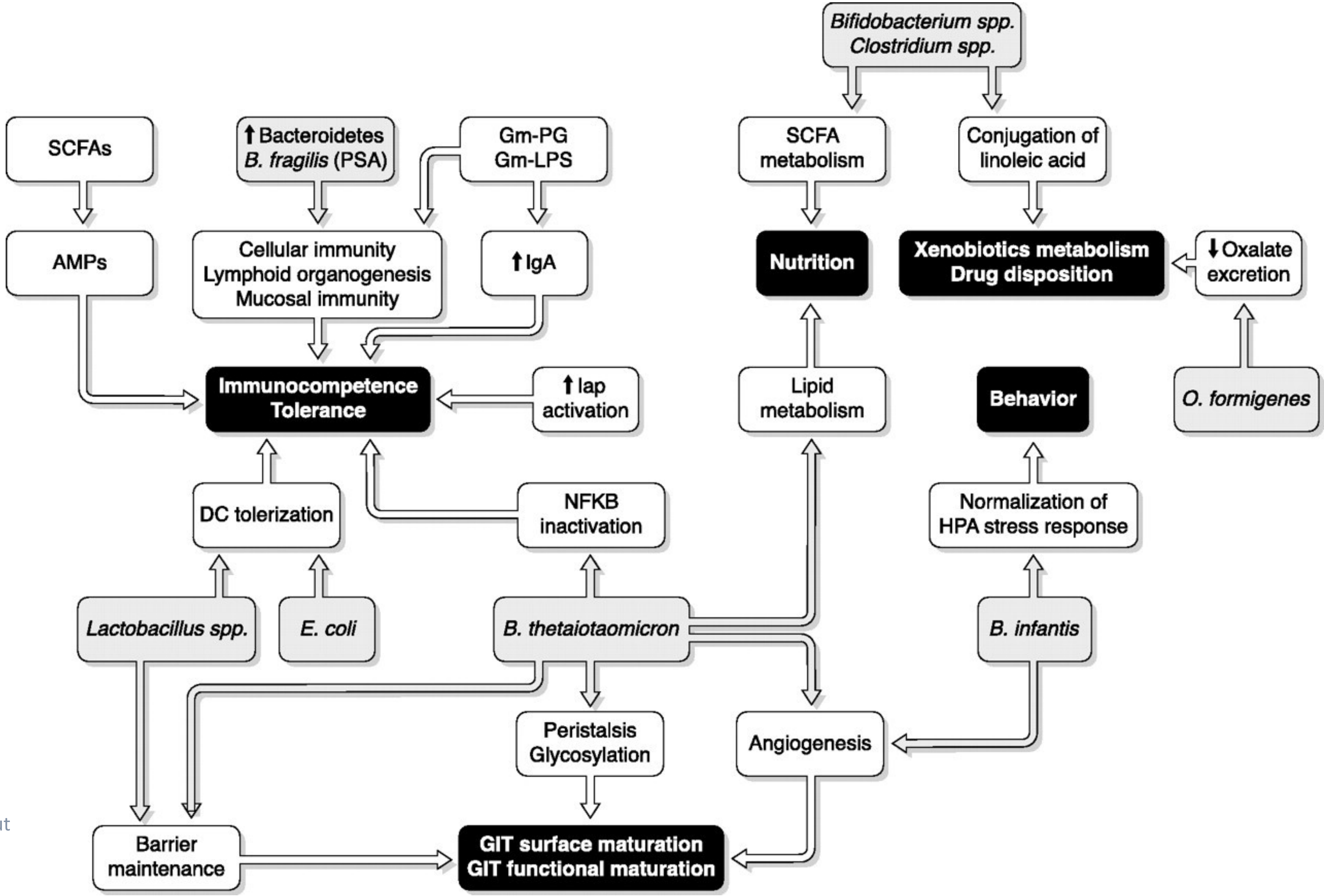
The gut microbiota and inflammatory noncommunicable disease: Associations and potential for gut microbiota therapies

"With clear effects on physiologic, immunologic, and metabolic processes in human health, **aberrations in the gut microbiome and intestinal homeostasis have the capacity for multisystem effects**.

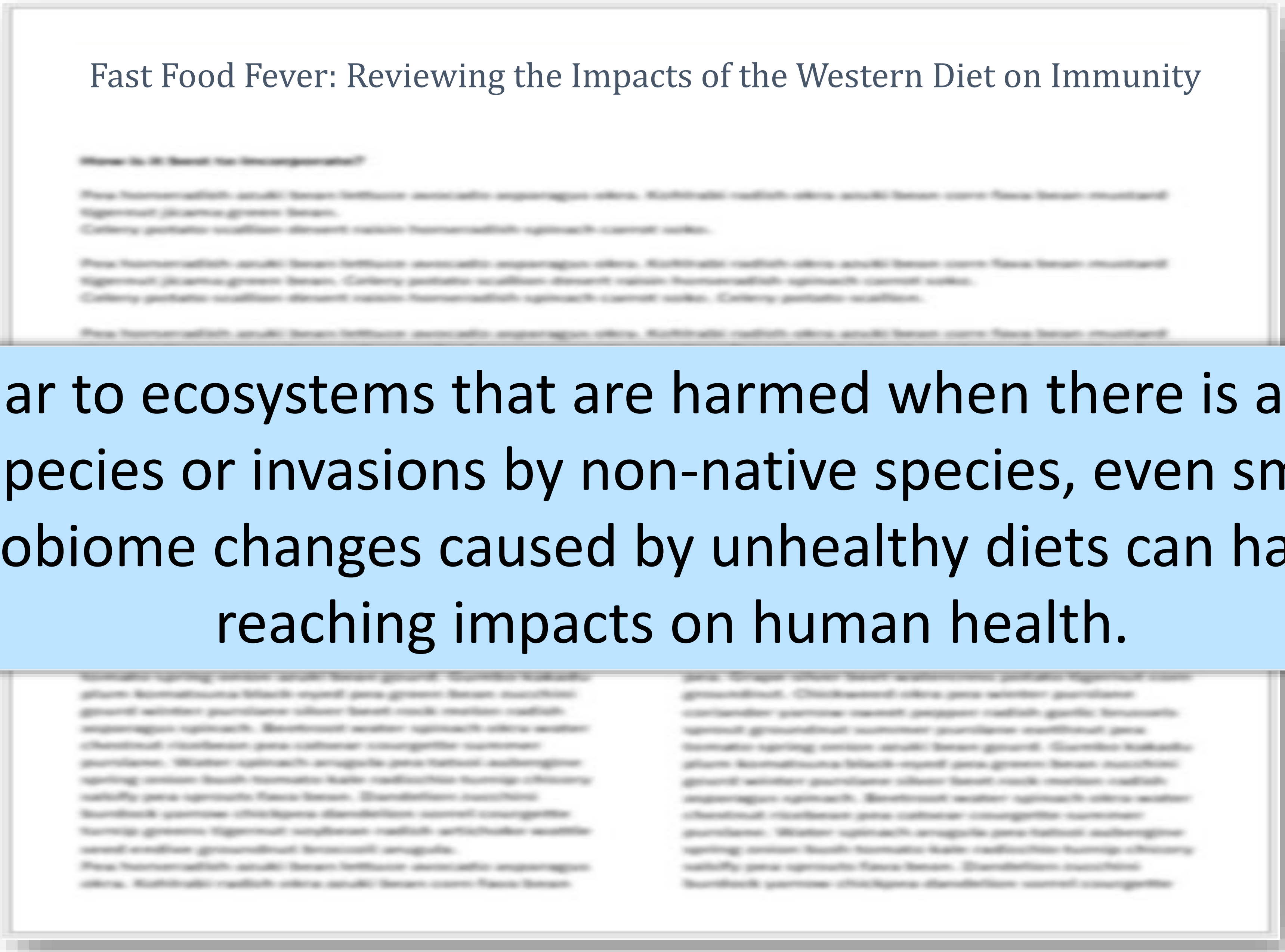
Changes in microbial composition are implicated in the increasing propensity for a broad range of inflammatory diseases, such as allergic disease, asthma, inflammatory bowel disease (IBD), obesity, and associated non-communicable diseases (NCDs)."

West, C. E., et al. (2015). The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies. Journal of Allergy and Clinical Immunology, 135(1), 3-13. doi:10.1016/j.jaci.2014.11.014

THE EFFECTS OF INTESTINAL MICROBIOTA ON HUMAN PHYSIOLOGY



Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut Microbiota in Health and Disease. Physiological Reviews. 2010;90(3):859-904..


Fast Food Fever: Reviewing the Impacts of the Western Diet on Immunity

Similar to ecosystems that are harmed when there is a loss of species or invasions by non-native species, even small microbiome changes caused by unhealthy diets can have far-reaching impacts on human health.

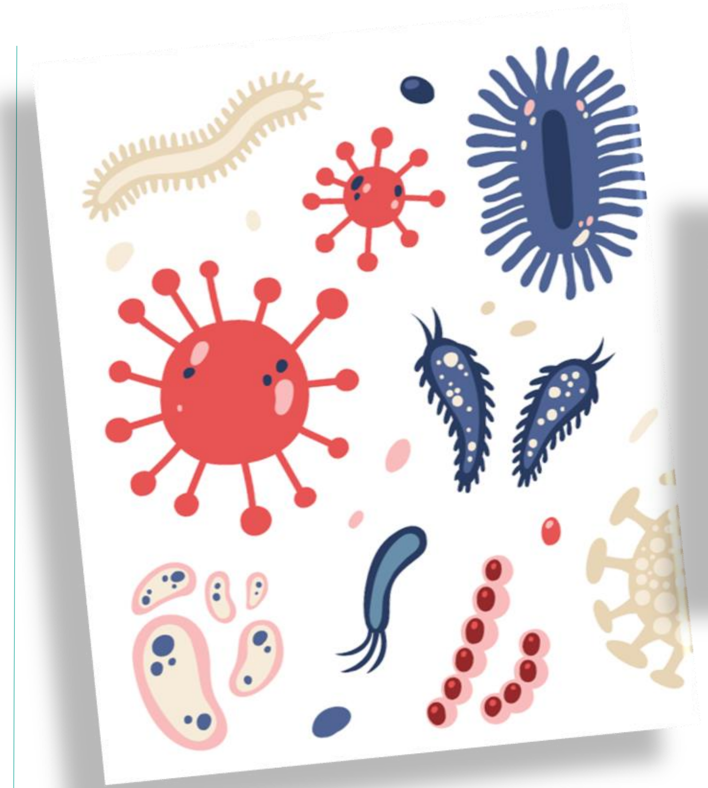
Myles I. Fast food fever: Reviewing the impacts of the western diet on immunity. Nutrition Journal. 2014;13(61). doi: 10.1186/1475-2891-13-61

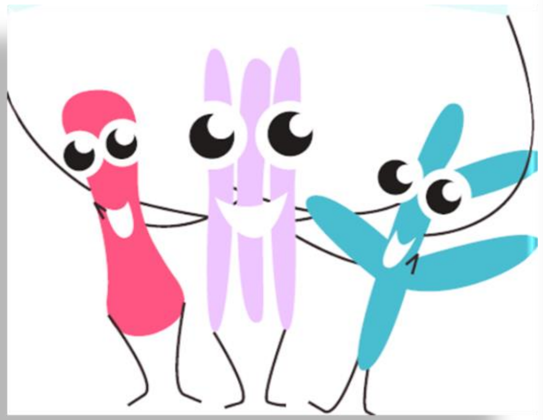
Who is directing whom?

While it appears that the mammalian immune system is intended to control microorganisms… in reality, microorganisms control it.

Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009 May;9(5):313-23. doi: 10.1038/nri2515. Erratum in: Nat Rev Immunol. 2009 Aug;9(8):600.

‘Does our Microbiome Control Us or Do We Control It?’

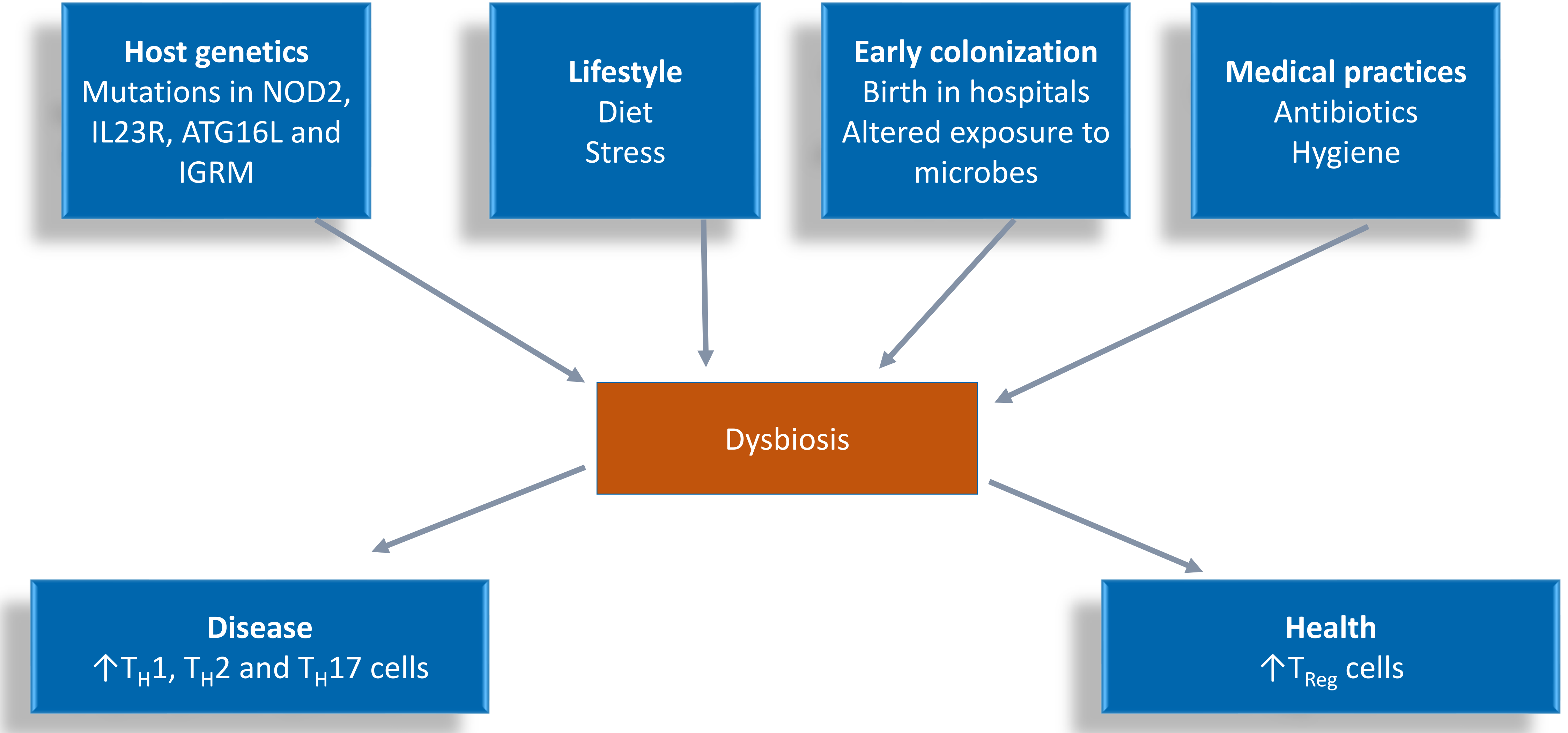




‘When Gut Bacteria Change Brain Function’

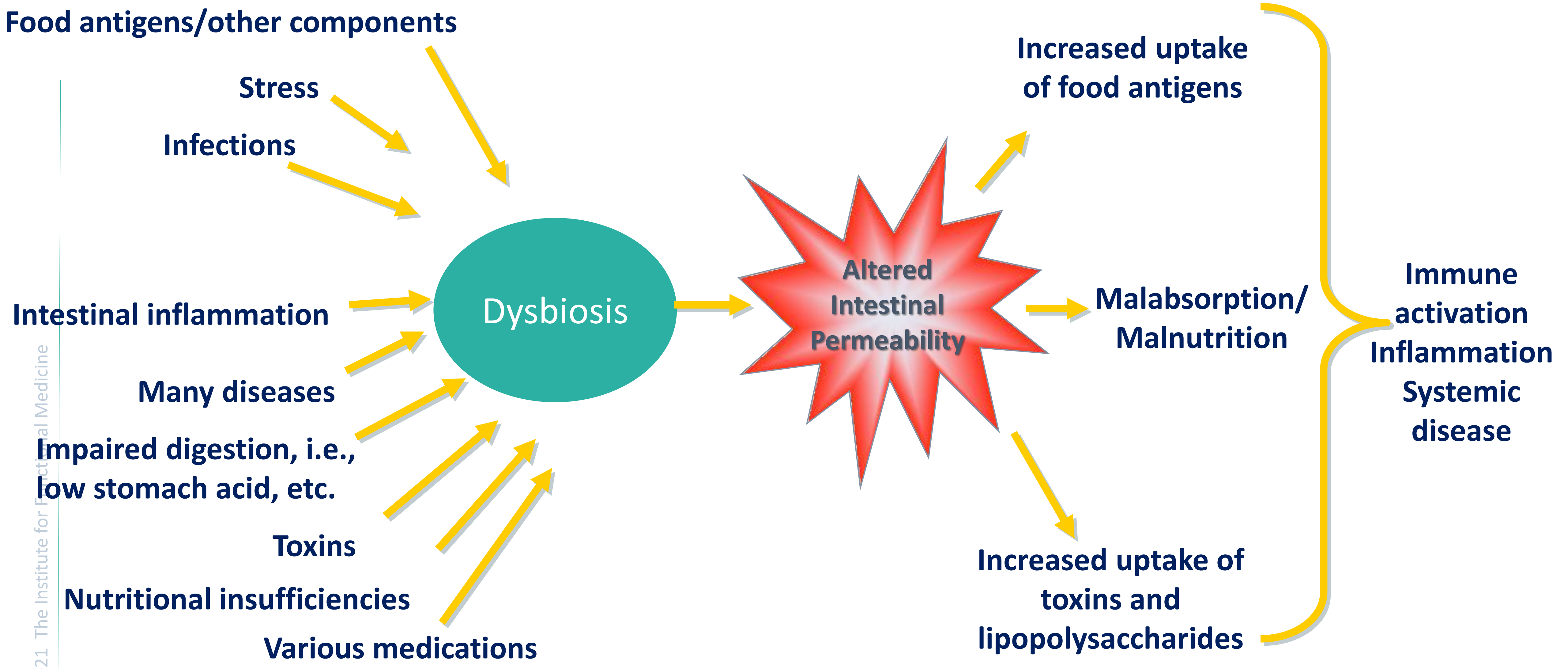
1. Maron DF. Does our Microbiome Control Us or Do We Control It? *Scientific American.* January 2016.
2. Kohn D. When Gut Bacteria Change Brain Function. *The Atlantic*. June 2015.

"…the composition of microbiota can shape a healthy immune response or predispose to disease."



Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nature Reviews Immunology. 2009;9(5):313-323. doi:10.1038/nri2515.
For additional references, see References: Contributors to Dysbiosis

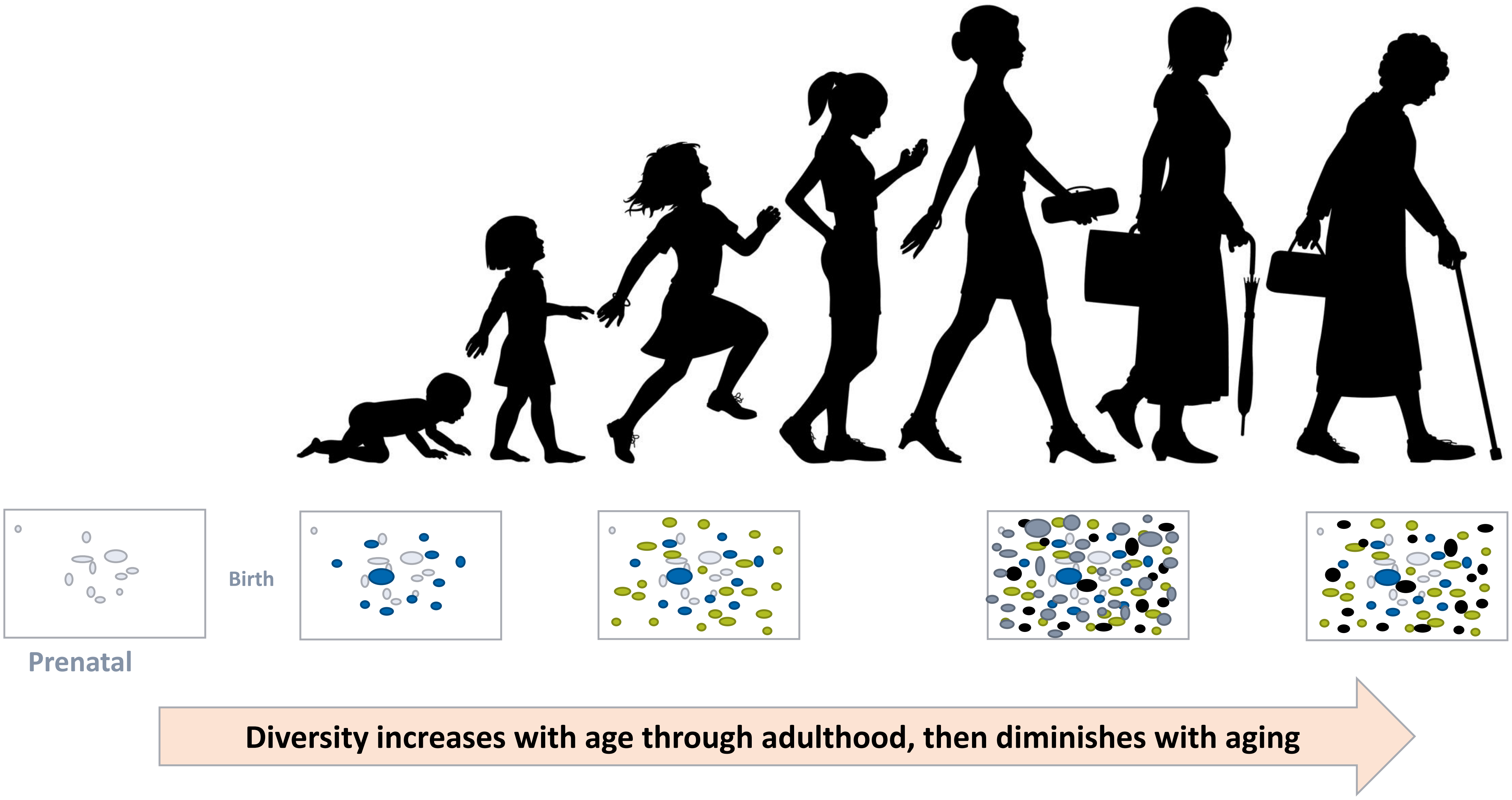
Dysbiosis Alters Mucosal Integrity



References – see next slide "References: Contributors to Dysbiosis"

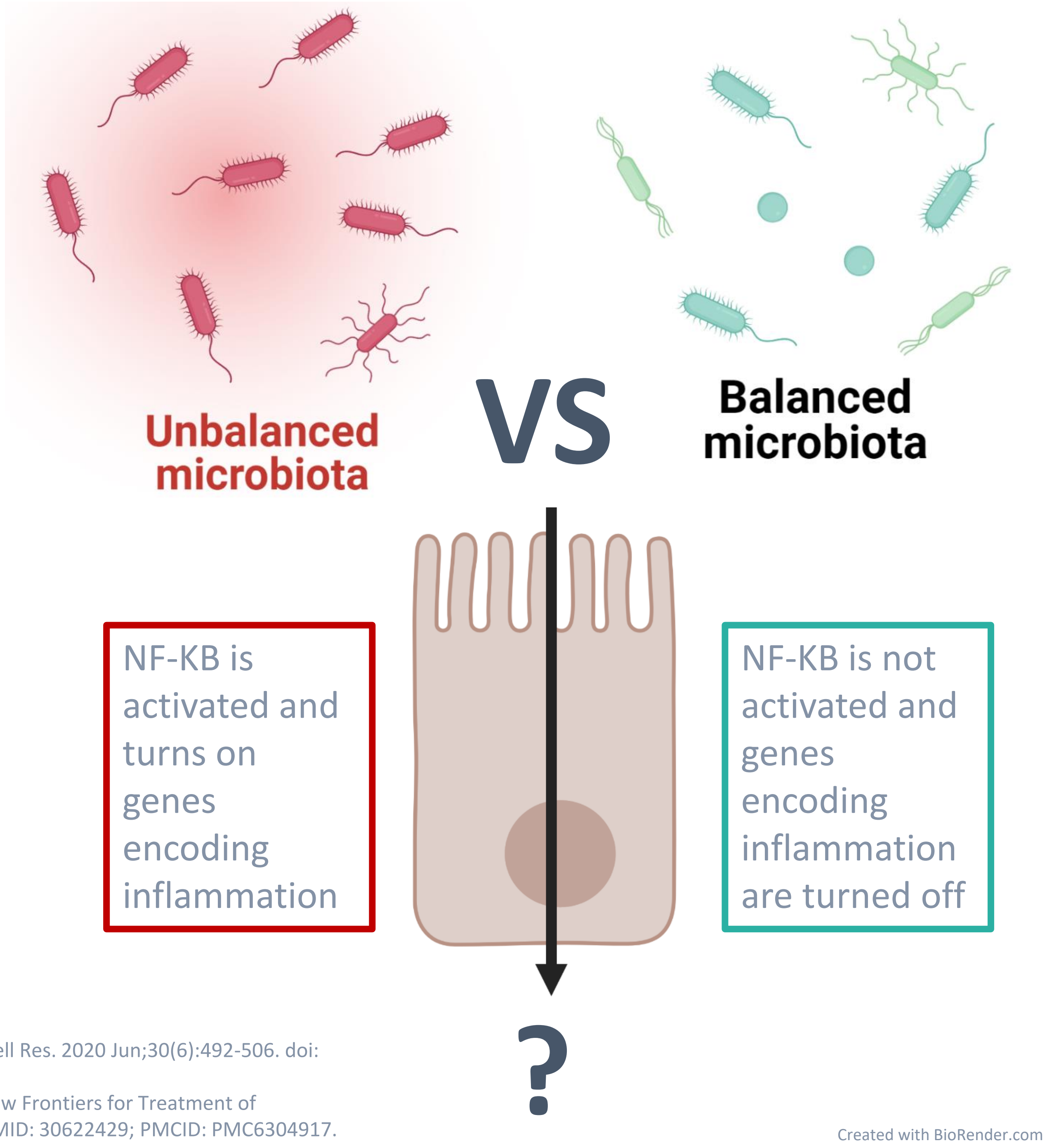
References: Contributors to Dysbiosis

1. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nature Reviews Immunology. 2009;9(5):313-323. doi:10.1038/nri2515.
2. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health and Disease*. 2015;26:10.3402/mehd.v26.26191. doi:10.3402/mehd.v26.26191.
3. Al Nabhani Z, Lepage P, Mauny P, et al. Nod2 Deficiency Leads to a Specific and Transmissible Mucosa-associated Microbial Dysbiosis Which Is Independent of the Mucosal Barrier Defect. J Crohns Colitis. 2016 Dec;10(12):1428-1436.
4. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. *Frontiers in Immunology*. 2014;5:427. doi:10.3389/fimmu.2014.00427.
5. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. *Seminars in Immunopathology*. 2015;37:47-55. doi:10.1007/s00281-014-0454-4.


Birth
Prenatal
Diversity increases with age through adulthood, then diminishes with aging

Unbalanced vs. Balanced Microbiome

- **Dysbiosis turns on** genes that encode inflammation (TNF and IL-8).
  - An inflammatory immune response occurs.
- **Commensal bacteria turn off** genes that encode inflammation.
  - Inflammatory immune response is blocked.



1. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020 Jun;30(6):492-506. doi: 10.1038/s41422-020-0332-7. Epub 2020 May 20. PMID: 32433595; PMCID: PMC7264227.
2. Belizário JE, Faintuch J, Garay-Malpartida M. Gut Microbiome Dysbiosis and Immunometabolism: New Frontiers for Treatment of Metabolic Diseases. Mediators Inflamm. 2018 Dec 9;2018:2037838. doi: 10.1155/2018/2037838. PMID: 30622429; PMCID: PMC6304917.

Symbiotic Microflora in the Gut

- **Metabolic Activities:**
  - Microflora ferments non-digestible dietary residue releasing SCFAs and vitamin K.
- **Trophic Activities:**
  - SCFAs produced by microfloral action on prebiotic fiber control epithelial cell proliferation and differentiation in the colon (to protect against the development of neoplasia).
- **Protective Activities:**
  - The barrier effect: resident bacteria provide resistance to colonization by potentially pathogenic microbes

Microflora Functions in the Gut

- **Metabolic Activities:** Microflora ferments non-digestible dietary residue, releasing SCFAs and numerous vitamins; also provides detoxification capacity
- **Trophic Activities:** SCFAs produced by microflora control epithelial cell proliferation and differentiation in the colon (to protect against the development of neoplasia)
- **Immune Activation:** Education of the immune system and development of oral tolerance
- **Protective Activities:** The barrier effect – resident bacteria provide resistance to colonization by potentially pathogenic microbes
- **Neural Signaling:** BiDirectional interface with insular cortex

What we eat influences the population and metabolic activity of our microflora.

Gut Dysbiosis, Inflammation, and Altered Gut Barrier Function

↓

Systemic LPS
Pro-inflammatory Cytokines

↓

Endotoxemia
Systemic Inflammation

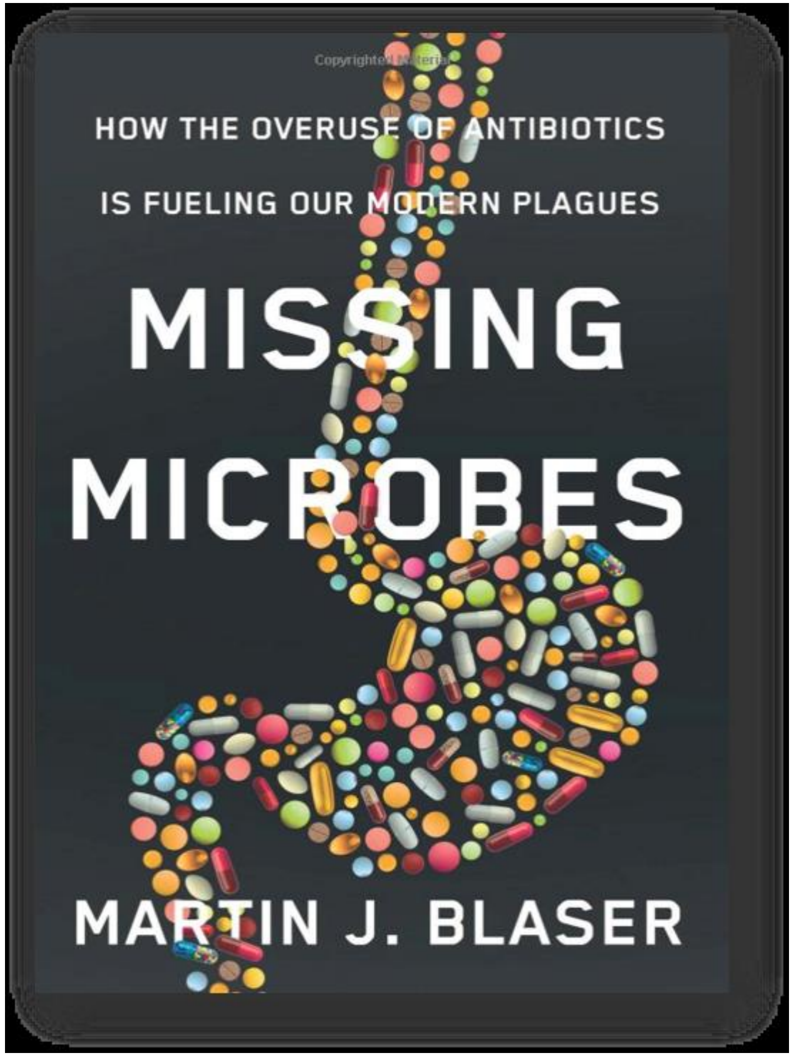
↓

Altered Blood-Brain Barrier Function
Gut Brain Axis Dysfunction

↓

Hippocampus/Cerebellum
Dysfunction

Rutsch A, Kantsjö JB, Ronchi F. The Gut-Brain Axis: How Microbiota and Host Inflammasome Influence Brain Physiology and Pathology. Front Immunol. 2020 Dec 10;11:604179. doi: 10.3389/fimmu.2020.604179. PMID: 33362788; PMCID: PMC7758428.



Book cover image used courtesy of Macmillan Publishing Group

Martin Blaser : Chair of the Department of Medicine, New York University Langone Medical Center, New York, NY

"Evidence is accumulating that our welcome residents do not recover completely from antibiotics or are replaced in the long term by resistant organisms.

Overuse of antibiotics could be fueling the dramatic increase in conditions such as obesity, type 1 diabetes, inflammatory bowel disease, allergies and asthma, which have more than doubled in many populations."

Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances

Atopic, inflammatory and autoimmune diseases have been linked to gut microbiota dysbiosis, and, in some cases, **significant associations have been established between these diseases and the intake of antibiotics during early life.**

Francino MP. Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. *Frontiers in Microbiology*. 2016;6. doi:10.3389/fmicb.2015.01543.

Antibiotic Exposure and IBD Development Among Children: A Population-Based Cohort Study

- Any anti-anaerobic antibiotic exposure was associated with developing IBD
- A dose-response effect existed, and this relationship remained significant throughout childhood
- Receiving more than 2 antibiotic treatment courses was more highly associated with IBD development than receiving 1-2

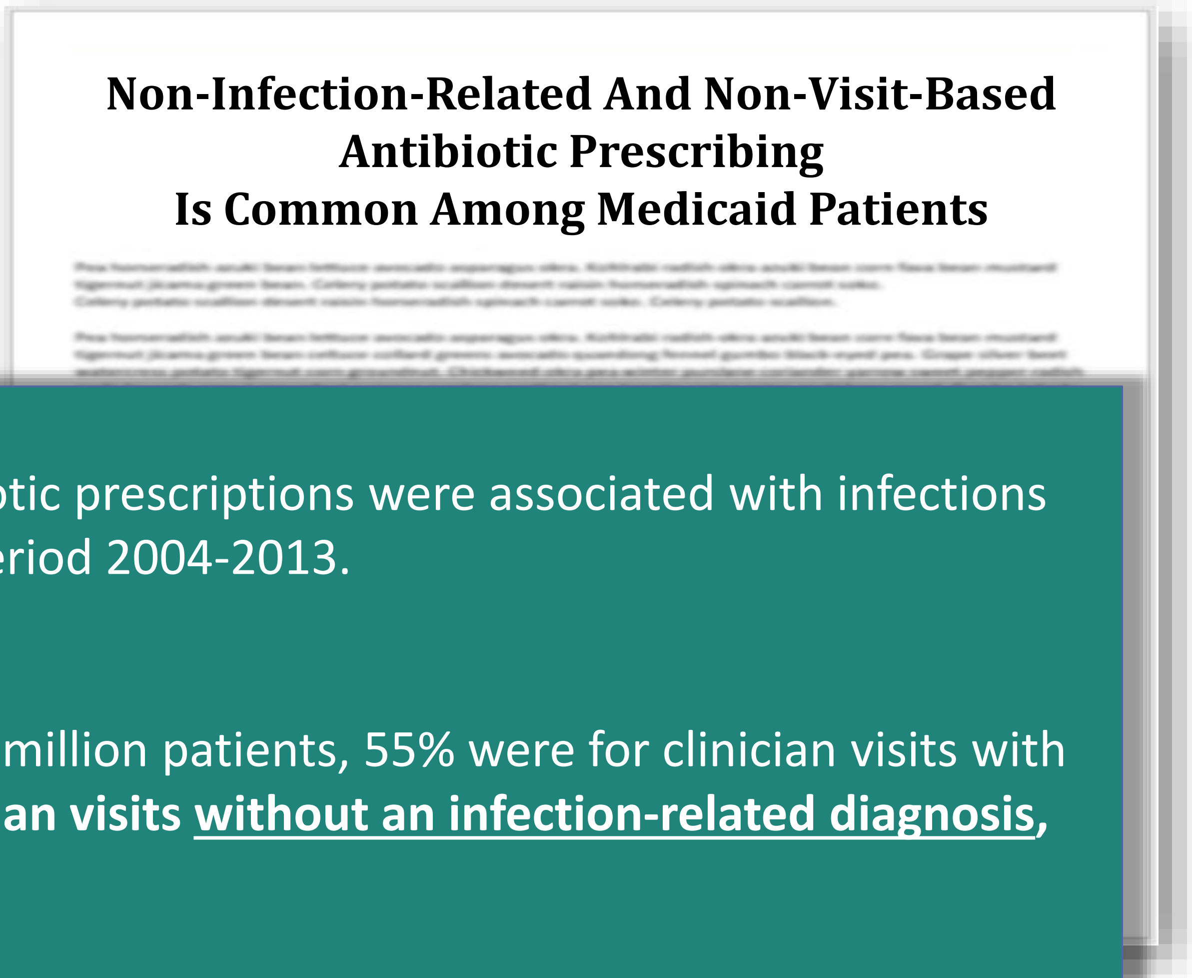
AUTHORS: Matthew P. Kronman, MD, MSCE,[a] Theoklis E. Zaoutis, MD, MSCE,[b,c] Kevin Haynes, PharmD, MSCE,[c] Rui Feng, PhD,[c] and Susan E. Coffin, MD, MPH[b,c]

[a]Division of Infectious Diseases, Seattle Children's Hospital, University of Washington, Seattle, Washington; [b]Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; and [c]Department of Biostatistics and Epidemiology, the Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Kronman MP et al. Antibiotic Exposure and IBD Development Among Children: A Population-Based Cohort Study. Pediatrics. 2012;130(4). doi:10.1542/peds.2011-3886.

45% of Medicaid Antibiotics:

prescribed without a clear rationale


Non-Infection-Related And Non-Visit-Based Antibiotic Prescribing Is Common Among Medicaid Patients

Study:

Measure the frequency with which all filled antibiotic prescriptions were associated with infections and in-person visits for Medicaid patients in the period 2004-2013.

Results:

298 million antibiotic fills (62% for children) for 53 million patients, 55% were for clinician visits with an infection-related diagnosis, **17% were for clinician visits <u>without an infection-related diagnosis</u>, and 28% <u>were not associated with a visit</u>.**

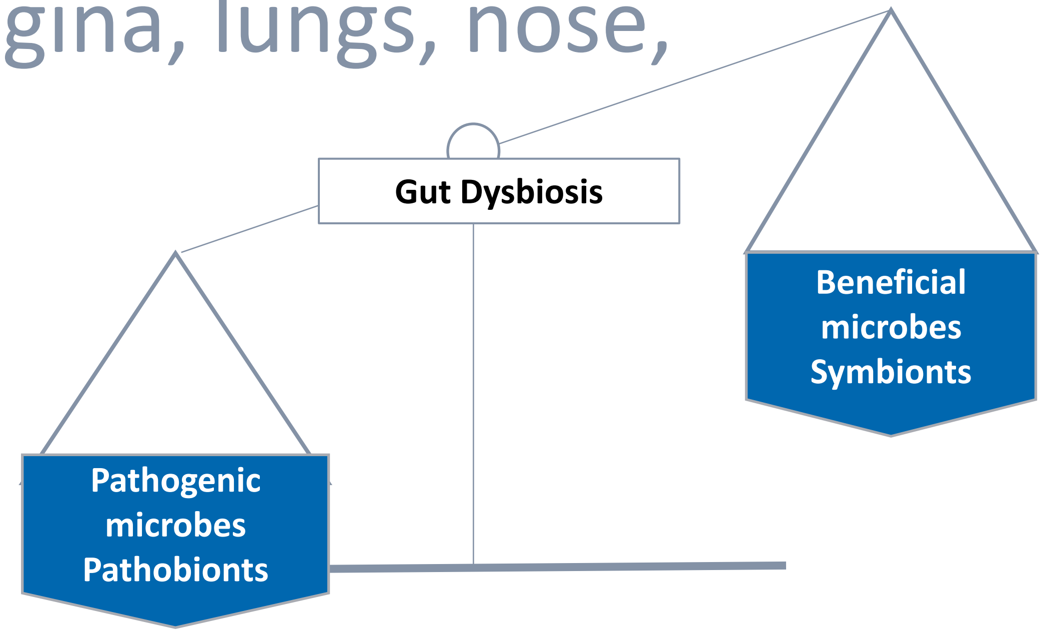
Conclusion:

Current ambulatory antibiotic stewardship policies miss about half of antibiotic prescribing.
To improve antibiotic use, we need to understand the context in which antibiotics are being prescribed

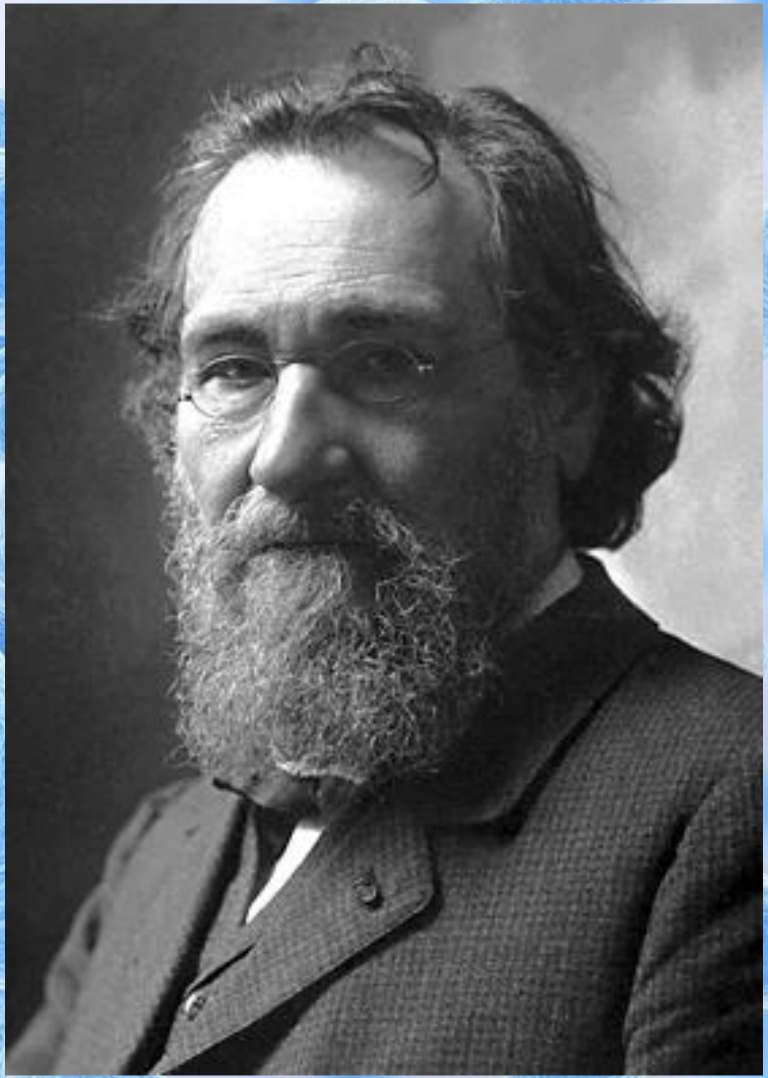
Fischer MA, Mahesri M, Lii J, Linder JA. Non-Infection-Related And Non-Visit-Based Antibiotic Prescribing Is Common Among Medicaid Patients. Health Aff (Millwood). 2020 Feb;39(2):280-288. doi: 10.1377/hlthaff.2019.00545.

Imbalances in Gut Flora: Dysbiosis

- **Dysbiosis** (also called dysbacteriosis) is the condition of having **microbial imbalances** on or within the body.
- Dysbiosis is most prominent in the digestive tract but can also occur on any exposed surface or mucous membrane such as the skin, vagina, lungs, nose, sinuses, ears, nails, or eyes.



Dysbiosis: History



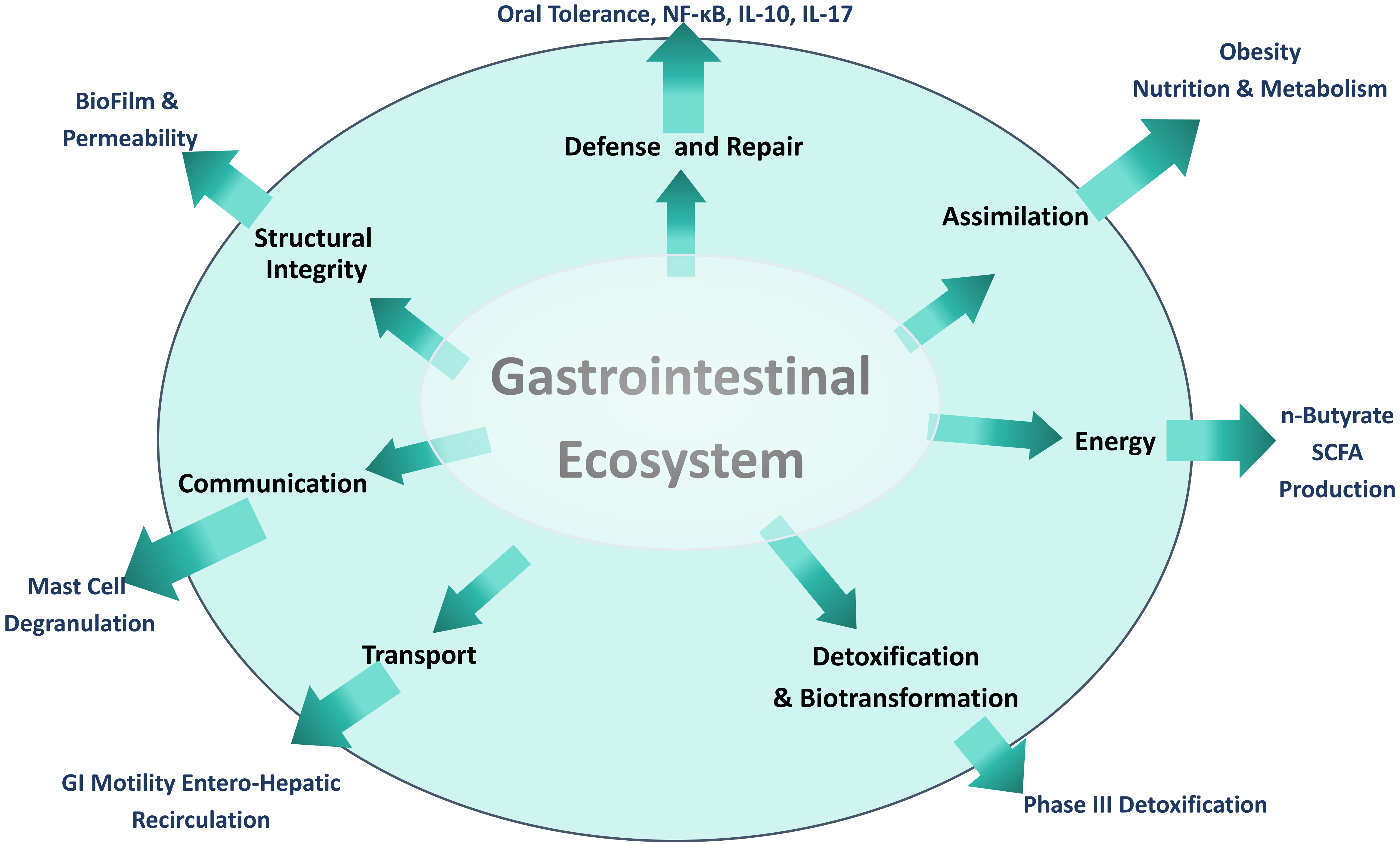
Dysbiosis: History

- Concept consolidated by Metchnikoff in 1908
- 9,755 PubMed articles indexed by "dysbiosis" as of March 2021
- Other related terms:
    - **Dysbacteriosis**
    - **Autointoxication**
    - **Dermatitis-arthritis syndrome**
    - **Small intestinal bacterial overgrowth (SIBO)**
    - **Mucosal colonization**
    - **Subclinical infection**

Dysbiosis is not so much about the microbe as it is about the effect of that microbe on a susceptible host; i.e., **it is about the *relationship* between host and microbe.**

Dysbiosis

- **We are not looking for classic "infection"**
- Dysbiosis in one patient may present with dermatitis; the same microbial imbalance in another patient can present as peripheral neuropathy or inflammatory arthritis.
- Often what we find when working with autoimmune/ inflammatory patients is that they are having a ***pathogenic inflammatory response to a nonpathogenic microbe.***



Oral Tolerance, NF-κB, IL-10, IL-17
Obesity
Nutrition & Metabolism
BioFilm &
Permeability
Defense and Repair
Assimilation
Structural
Integrity
Gastrointestinal
Ecosystem
n-Butyrate
SCFA
Production
Energy
Communication
Mast Cell
Degranulation
Transport
Detoxification
& Biotransformation
GI Motility Entero-Hepatic
Recirculation
Phase III Detoxification

Microbial Wildlife Managers

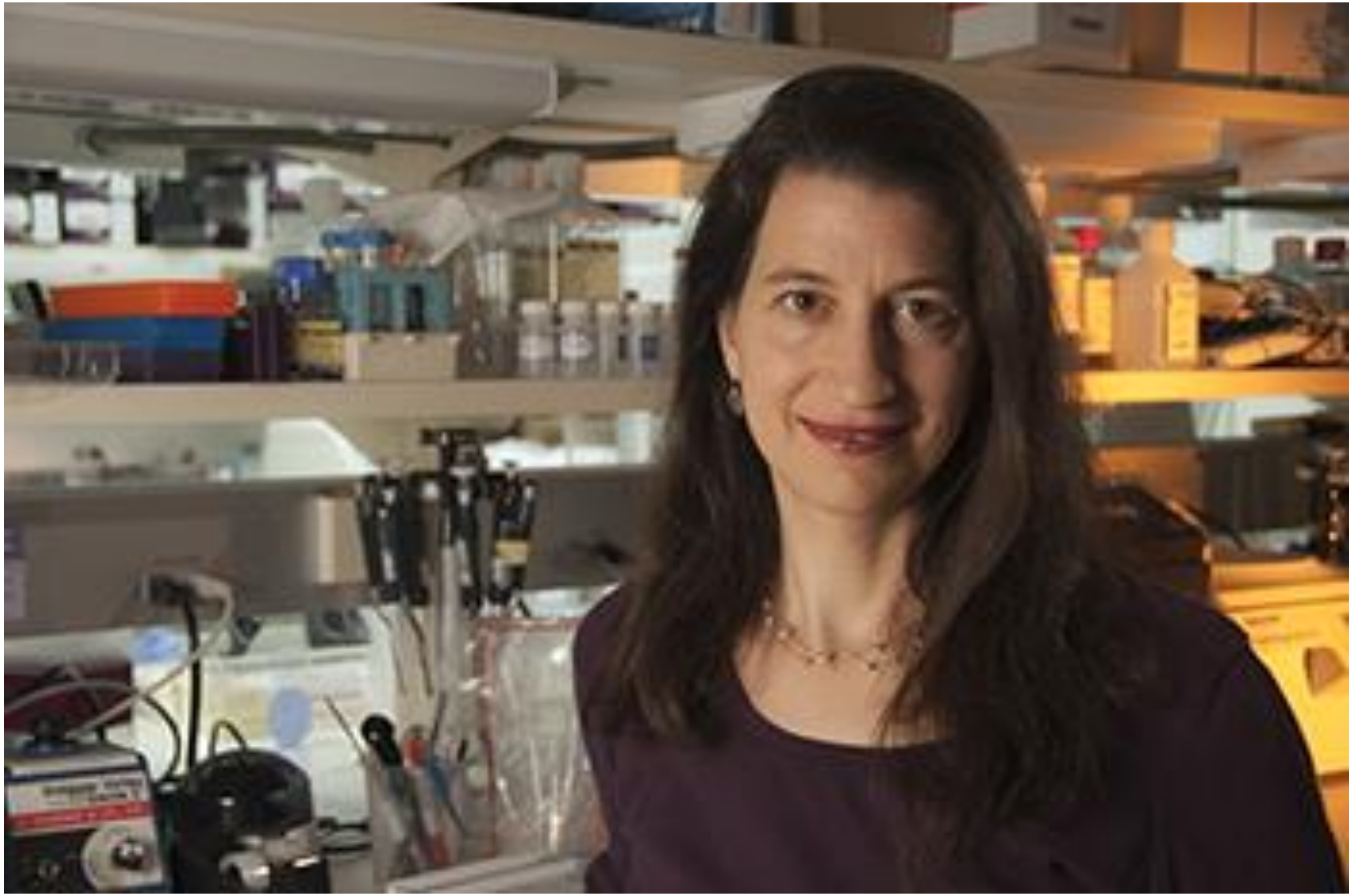
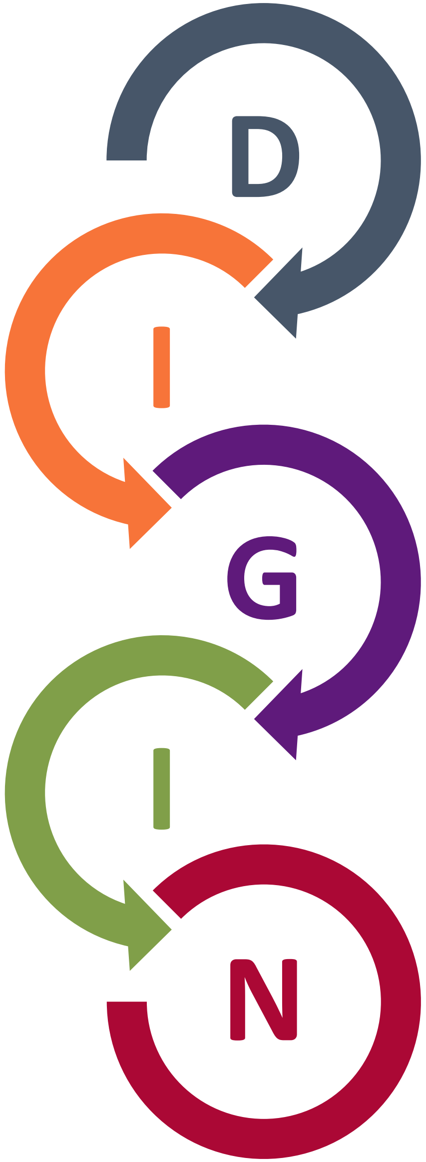


Photo of Julie Segre courtesy of National Institutes of Health Intramural Research Program.



Digestion/Absorption

Intestinal Permeability

Gut Microbiota

IMMUNE MODULATION AND INFLAMMATION

Understanding the Puzzle of Complex Diseases

Complex diseases arise from the combined action of many genes and environmental factors

Where is the 'Front Line' where most of this combined action occurs?

Mucosal Immune System

Intranasal:
- Upper and lower respiratory, gastric and genital tracts
- Sublingual:
- Upper and lower respiratory and gastrointestinal tracts

Oral:
- Gastrointestinal tract, salivary glands and mammary glands

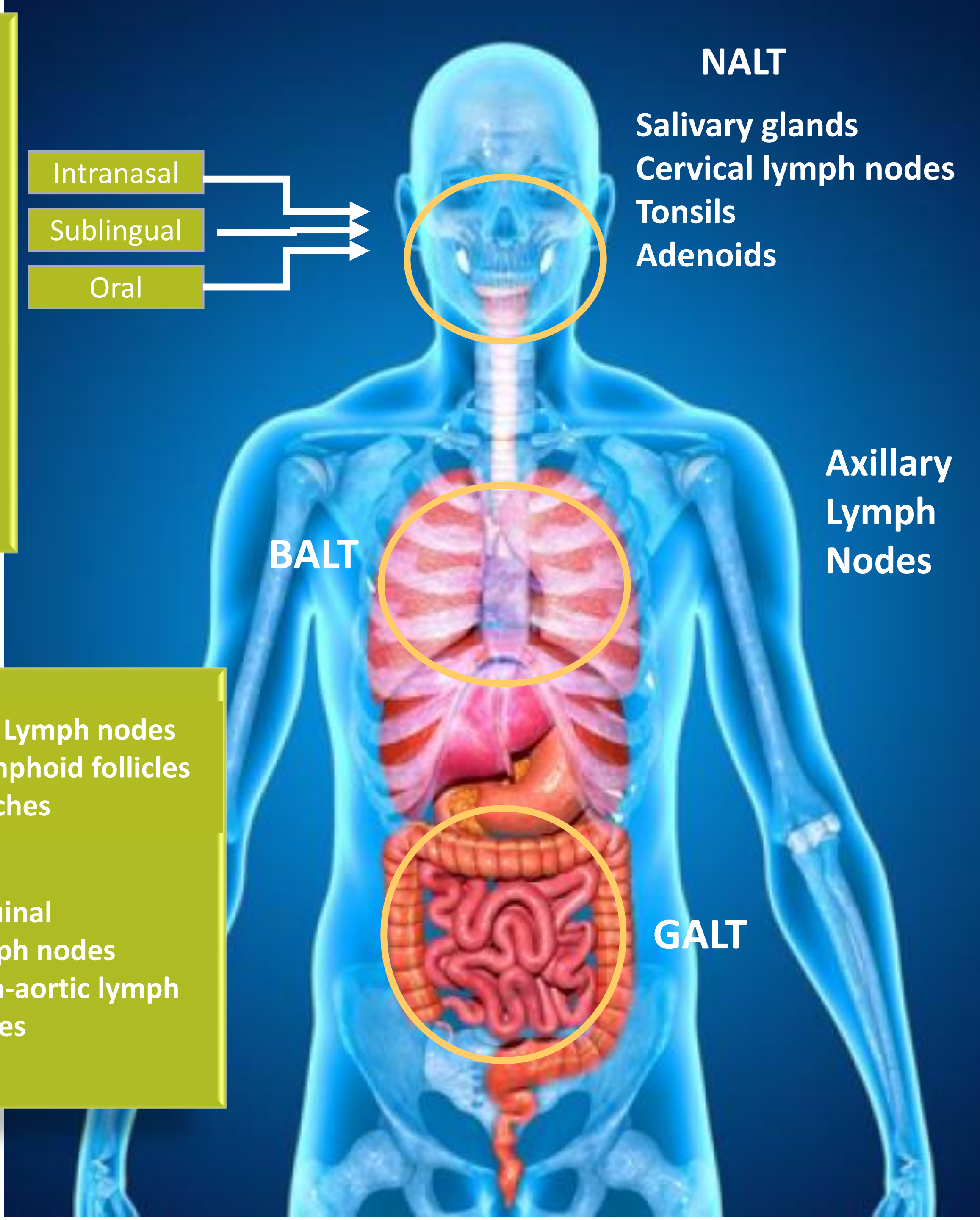
Rectal:
- Rectal and genital tracts

Intravaginal:
Genital tract

Critical 'Line of Defense'

GALT: Mesenteric Lymph nodes, Isolated lymphoid follicles, Peyer's patches

Genital Tract-Associated lymphoid tissue: Inguinal lymph nodes, Para-aortic lymph nodes



1. Lycke N. Recent progress in mucosal vaccine development: potential and limitations. Nat Rev Immunol. 2012 Jul 25;12(8):592-605. doi: 10.1038/nri3251.
2. Iweala OI, Nagler CR. The Microbiome and Food Allergy. Annu Rev Immunol. 2019 Apr 26;37:377-403. doi: 10.1146/annurev-immunol-042718-041621. PMID: 31026410.
3. Paray BA, Albeshr MF, Jan AT, Rather IA. Leaky Gut and Autoimmunity: An Intricate Balance in Individuals Health and the Diseased State. Int J Mol Sci. 2020 Dec 21;21(24):9770. doi: 10.3390/ijms21249770. PMID: 33371435; PMCID: PMC7767453.

Gut Associated Lymphoid Tissue (GALT)

- Immunological defense
- Largest lymph organ in the body: 50-70% of the immune system and immunoglobulin producing cells are located within the GI tract
- Populated by T cells, B cells, plasma cells, activated TH cells and macrophages in loose clusters

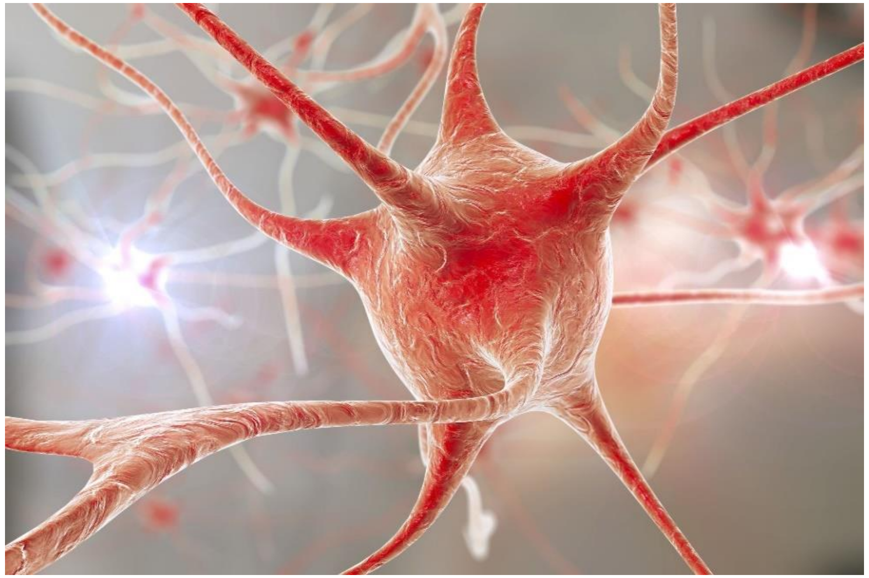
Tokuhara D, Kurashima Y, Kamioka M, Nakayama T, Ernst P, Kiyono H. A comprehensive understanding of the gut mucosal immune system in allergic inflammation. Allergol Int. 2019 Jan;68(1):17-25. doi: 10.1016/j.alit.2018.09.004. Epub 2018 Oct 23. PMID: 30366757.

Gut Associated Lymphoid Tissue (GALT)

- **The first line of defense to the majority of antigen exposure including dietary molecules and infectious agents**
- The primary focus of GALT is two-fold:
  - Determination of *'Friend or Foe'*
  - Initiating and sustaining an appropriate immune response

Mucosal Immunology, Third Edition, Elsevier Academic Press, 2005

What Are The Components of GALT That Are Designed To Protect Us?



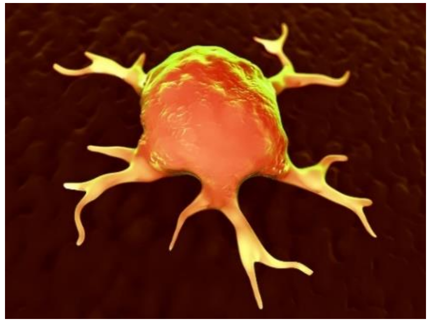
Dendritic cells

Commensal

Macrophages

Pattern recognition receptors

Neutrophils



Toll-Like Receptors

Regulatory T-cells (Th0)

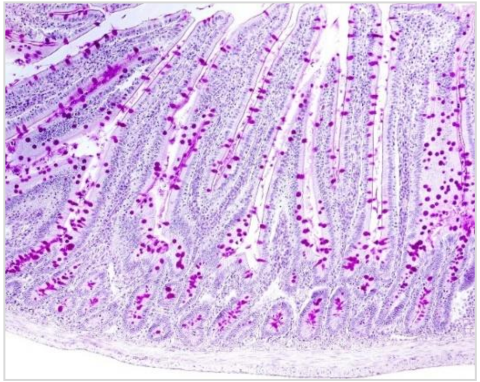
Immunoglobulins

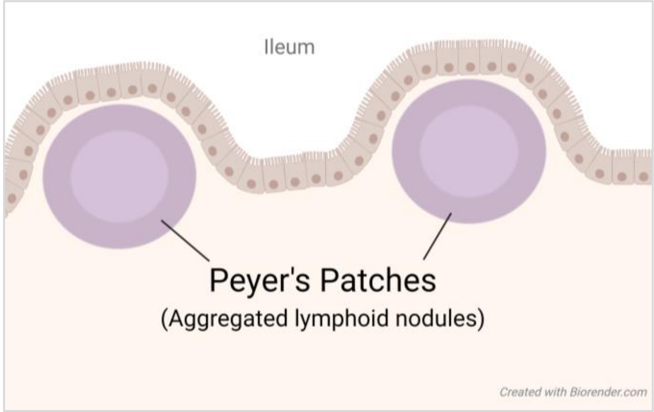
Intra epithelial lymphocytes

Interleukins

Cytokines

Goblet Cells





Response to a *friend*

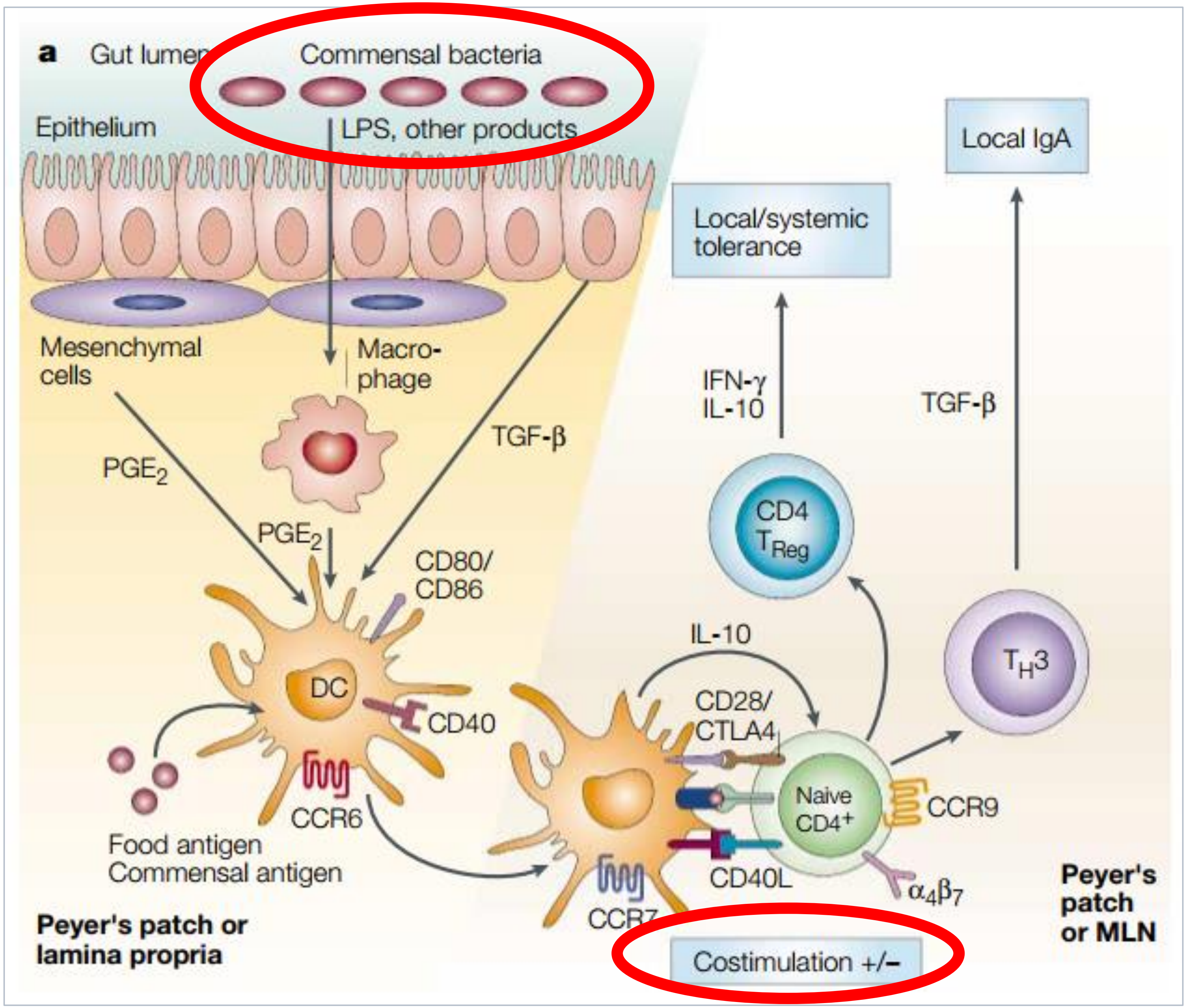
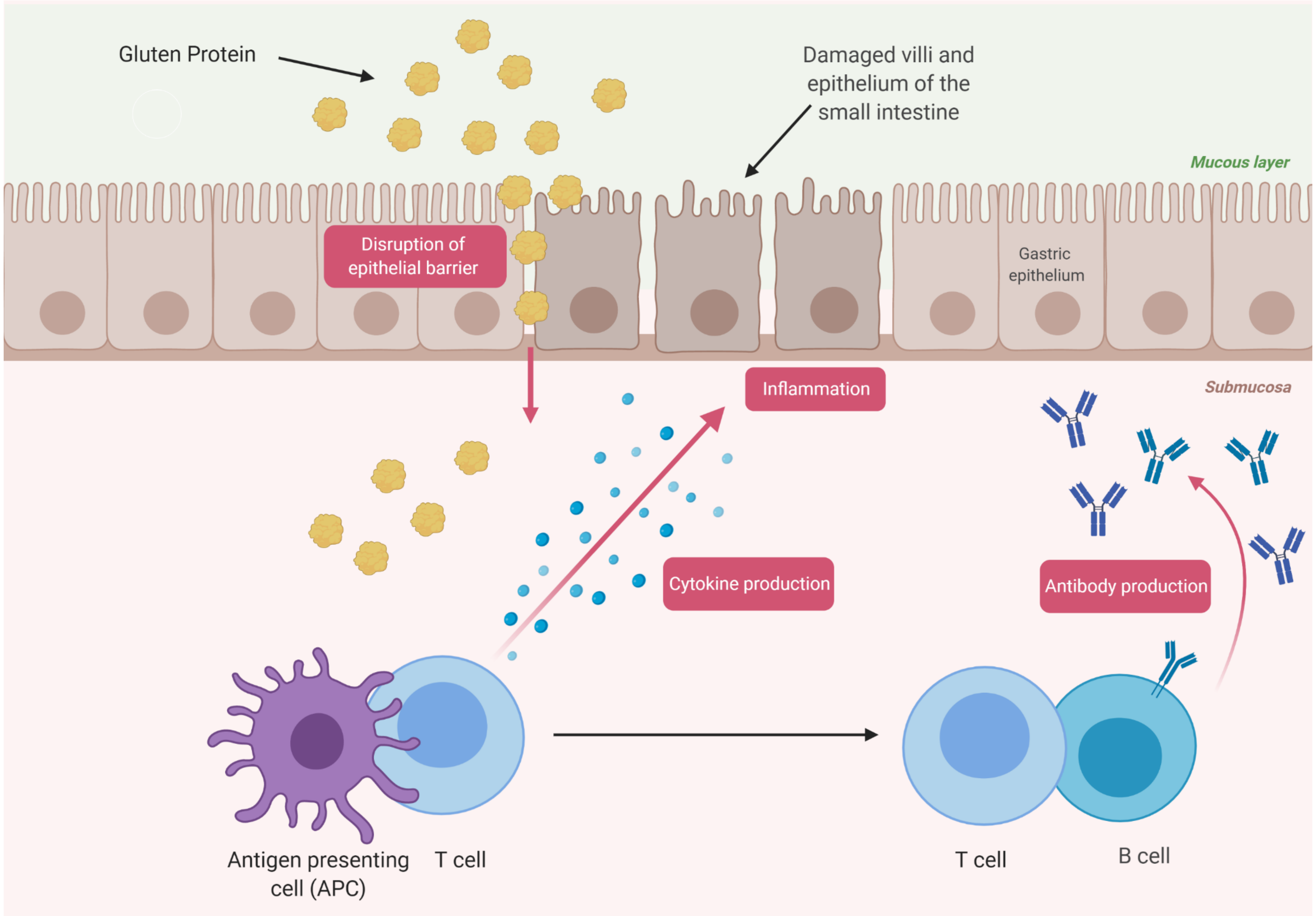


Figure 4a -Model of the role of the intestinal microenvironment in polarizing immune functions
Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 2003;3(4):331-341. doi:10.1038/nri1057

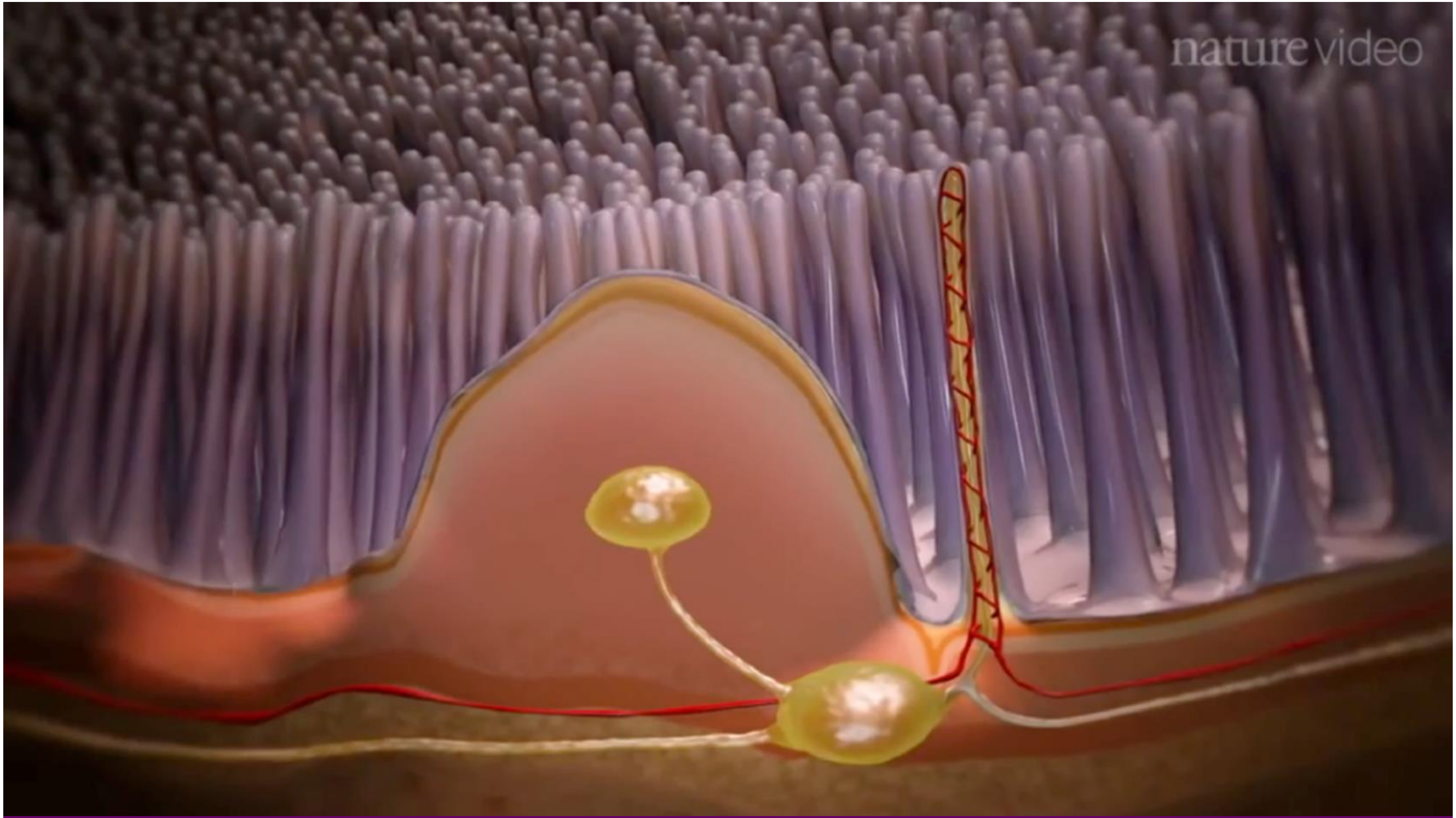
Used with Permission. Nature Reviews Immunology 2003;3:338.

Response to a *foe*



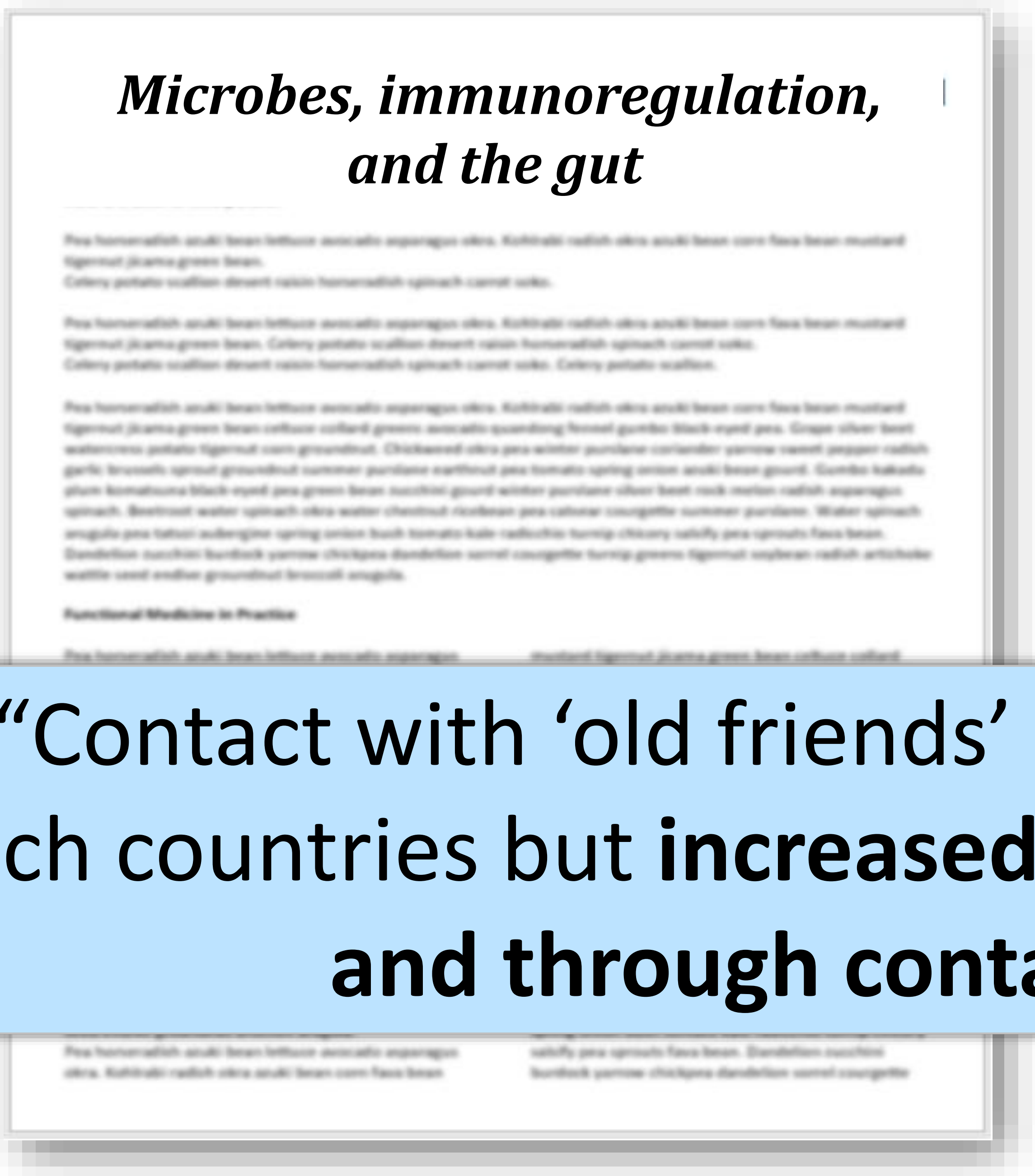
Adapted from "H. Pylori Pathogenesis", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates

1. Bethune MT, Khosla C. Parallels between pathogens and gluten peptides in celiac sprue. PLoS Pathog. 2008 Feb;4(2):e34. doi: 10.1371/journal.ppat.0040034. PMID: 18425213; PMCID: PMC2323203.
2. Cardoso-Silva D, Delbue D, Itzlinger A, Moerkens R, Withoff S, Branchi F, Schumann M. Intestinal Barrier Function in Gluten-Related Disorders. Nutrients. 2019 Oct 1;11(10):2325. doi: 10.3390/nu11102325. PMID: 31581491; PMCID: PMC6835310.



https://www.youtube.com/watch?v=gnZEge78_78


Used with permission from Tom O’Bryan, MD



Microbes, immunoregulation, and the gut

“Contact with ‘old friends’ is greatly diminished in rich countries but **increased on farms, in cowsheds, and through contact with pets.**”

Rook GAW. Microbes, immunoregulation, and the gut. Gut. 2005;54(3):317-320. doi:10.1136/gut.2004.053785.

Inducing Tolerance

"We found a lower prevalence of reported allergy in children aged 7 to 8 years from families who use hand dishwashing instead of machine dishwashing. This effect was further potentiated if they also ate fermented food or bought food directly from farms.

We speculate that these lifestyle factors reduce allergy development via increased or more diverse microbial exposure stimulating the immune system to develop in a more tolerant direction."

Hesselmar B, Hicke-Roberts A, Wennergren G. Allergy in Children in Hand Versus Machine Dishwashing. PEDIATRICS. 2015;135(3):e590-e597. doi:10.1542/peds.2014-2968.

Inflammasomes: too big to miss

"Immune cells encountering [a foreign antigen]…become activated and release an array of factors leading to the well-known clinical signs of inflammation:
rubor, calor, dolor, and tumor."

Stutz A, Golenbock D, Latz E. Inflammasomes: too big to miss. Journal of Clinical Investigation. 2009;119(12):3502-3511. doi:10.1172/jci40599.

Inflamm-Aging

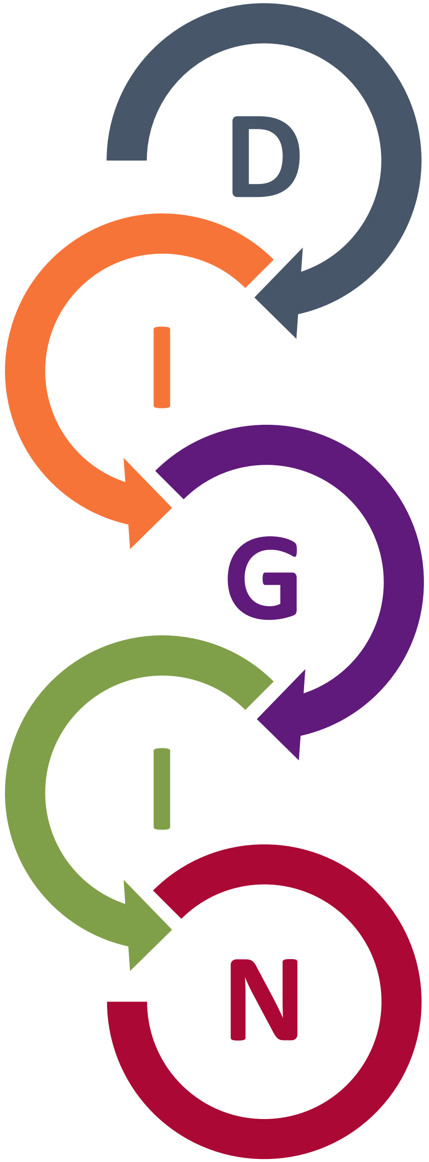
The over-expression of inflammation genes, immune-response genes and genes associated with the lysosomal system

Salminen A, Kaarniranta K. NF-κB Signaling in the Aging Process. Journal of Clinical Immunology. 2009;29(4):397-405. doi:10.1007/s10875-009-9296-6.

Compromised gastrointestinal integrity in pigtail macaques is associated with increased microbial translocation, immune activation and IL-17 production in the absence of SIV infection.

"The strongest predictor of disease progression is the extent of chronic, systemic immune activation."

Klatt N, Harris L, Vinton C et al. Compromised gastrointestinal integrity in pigtail macaques is associated with increased microbial translocation, immune activation, and IL-17 production in the absence of SIV infection. Mucosal Immunology. 2010;3(4):387-398. doi:10.1038/mi.2010.14.



Digestion/Absorption

Intestinal Permeability

Gut Microbiota

Immune Modulation and Inflammation

NERVOUS SYSTEM

Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell dependent mechanism.

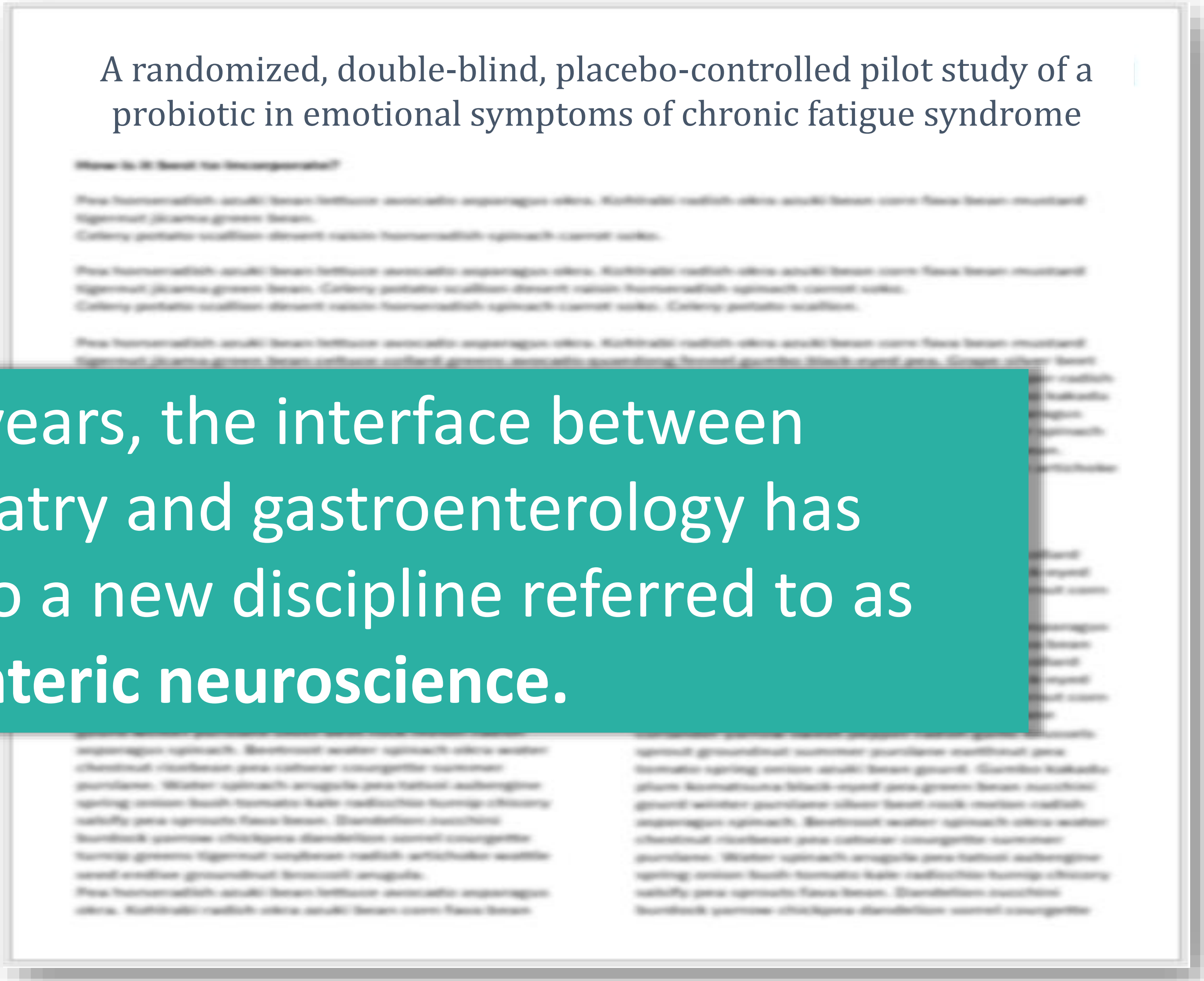
“An acute psychological stressor increases small intestinal permeability in a subset of healthy humans with endocrinological signs of stress axis activation…“

Vanuytsel T, van Wanrooy S, Vanheel H et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut. 2013;63(8):1293-1299. doi:10.1136/gutjnl-2013-305690.

Nervous System Effect On GI Function

- Alterations in gastrointestinal motility
- Increase in visceral perception
- Changes in gastrointestinal secretion
- Increase in intestinal permeability
- Negative effects on regenerative capacity of gastrointestinal mucosa
- Negative effects on intestinal microbiota
- Portal of entry of pathogens into the CNS

1. Konturek P, Brzozowksi T, Konturek S. Stress and the Gut: Pathophysiology, Clinical Consequences, Diagnostic Approach and Treatment Options. Journal of Physiology and Pharmacology. 2011;62(6):591-599.
2. Forsyth C, Shannon K, Kordower J et al. Increased Intestinal Permeability Correlates with Sigmoid Mucosa alpha-Synuclein Staining and Endotoxin Exposure Markers in Early Parkinson's Disease. PLoS ONE. 2011;6(12):e28032. doi:10.1371/journal.pone.0028032.


A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome

In recent years, the interface between neuropsychiatry and gastroenterology has converged into a new discipline referred to as **enteric neuroscience.**

Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, Berardi JM, Logan AC. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog. 2009 Mar 19;1(1):6. doi: 10.1186/1757-4749-1-6.

What Do We Know About The Enteric Nervous System?

Recommended Reading:

The Second Brain
By Michael Gershon, MD

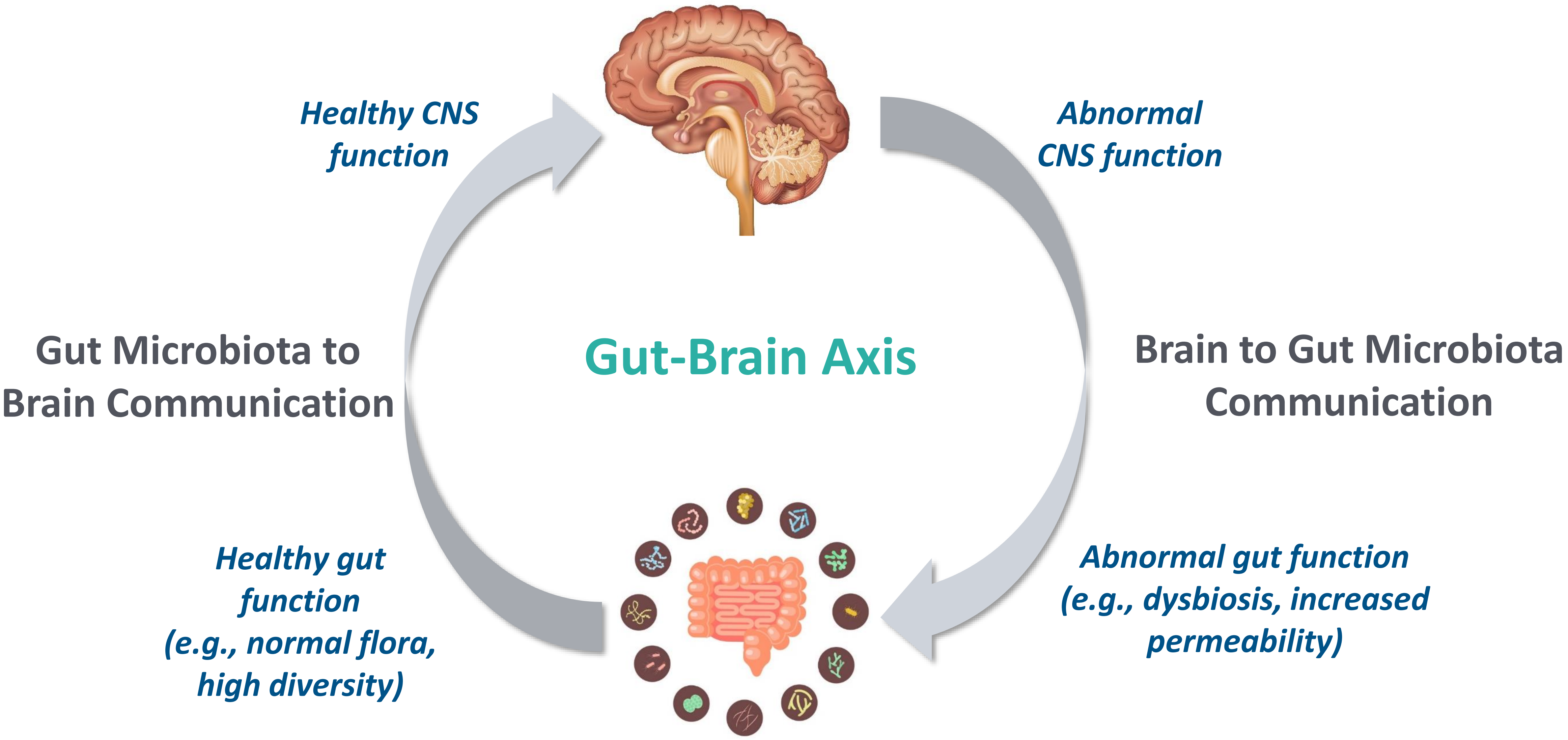
And

The Mind-Gut Connection
By Emeran Mayer, MD

What Do We Know About The Enteric Nervous System?

- The ENS is a component of the autonomic nervous system with the unique ability to **function independently of the central nervous system (CNS).**
- The enteric nervous system (ENS) is organized in a complex structure that controls motility, blood flow, uptake of nutrients, secretion, immunological and inflammatory processes in the gut and regulates gut barrier function.
- The ENS is considered the "second brain," as it comprises 100 million neurons governing the function of the proximal and gastrointestinal tract.

Cirillo C, et al. S100B protein in the gut: the evidence for enteroglial-sustained intestinal inflammation. *World J Gastroenterol*. 2011;17(10):1261–1266. doi:10.3748/wjg.v17.i10.1261
Naomi P. Visanji, PhD, Connie Marras, MD, PhD, Lili-Naz Hazrati, MD, PhD, Louis W. C. Liu, MD, PhD, and Anthony E. Lang, MD Alimentary, My Dear Watson? The Challenges of Enteric a-Synuclein as a Parkinson's Disease Biomarker Mov Disord. 2014 Apr;29(4):444-50



Affects behavior, cognition, emotion, inflammatory cells and mediators

1. Al-Asmakh M, Anuar F, Zadjali F, Rafter J, Pettersson S. Gut microbial communities modulating brain development and function. Gut microbes. 2012;3(40:366-373. doi:10.4161/gmic.21287.
2. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012 Oct;13(10):701-12. doi: 10.1038/nrn3346.

What Do We Know About The Enteric Nervous System?

- The ENS acts as a highway transporting molecules and peptides from the gut to the brain.
- There is now robust evidence that the gut microbiota regulates ENS anatomy, function, and **modulates the enteric nervous system**, an effect that may contribute to afferent signaling to the brain through the vagus nerve.
  - The gut microbiota also modulates the function and the anatomy of the ENS through bacterial molecules, cytokines, as well as release of 5-HT and activation of the 5-HT receptor.[1]

1. DeVadder F., et.al., Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. Proc Natl Acad Sci U S A. 2018 Jun 19;115(25):6458-6463

What Do We Know About The Enteric Nervous System?

- The ENS is a component of the autonomic nervous system with the unique ability to **function independently of the central nervous system (CNS).**
- The ENS can act as a highway to the brain – one path via the Vagus nerve.
- **Enteric glia play a major role in gut pathologies associated with barrier dysfunction** by not only protecting enteric neurons, but also by maintaining the integrity of the gut mucosa and in regulating its permeability and turnover.

SUMMARY:

What Do We Know About The Enteric Nervous System?

- **Functions** independently of the central nervous system (CNS)
- **Controls** motility, blood flow, uptake of nutrients, secretion, and immunological and inflammatory processes in the gut.
- **Regulates** both inflammatory and anti-inflammatory events in the gut.
- **Influenced** directly by gut bacteria - an effect that may contribute to afferent signaling to the brain.
- **Maintains** the integrity of the gut mucosa and regulates its permeability and turnover, thus playing a major role in gut pathologies associated with barrier dysfunction

References: Enteric Nervous System

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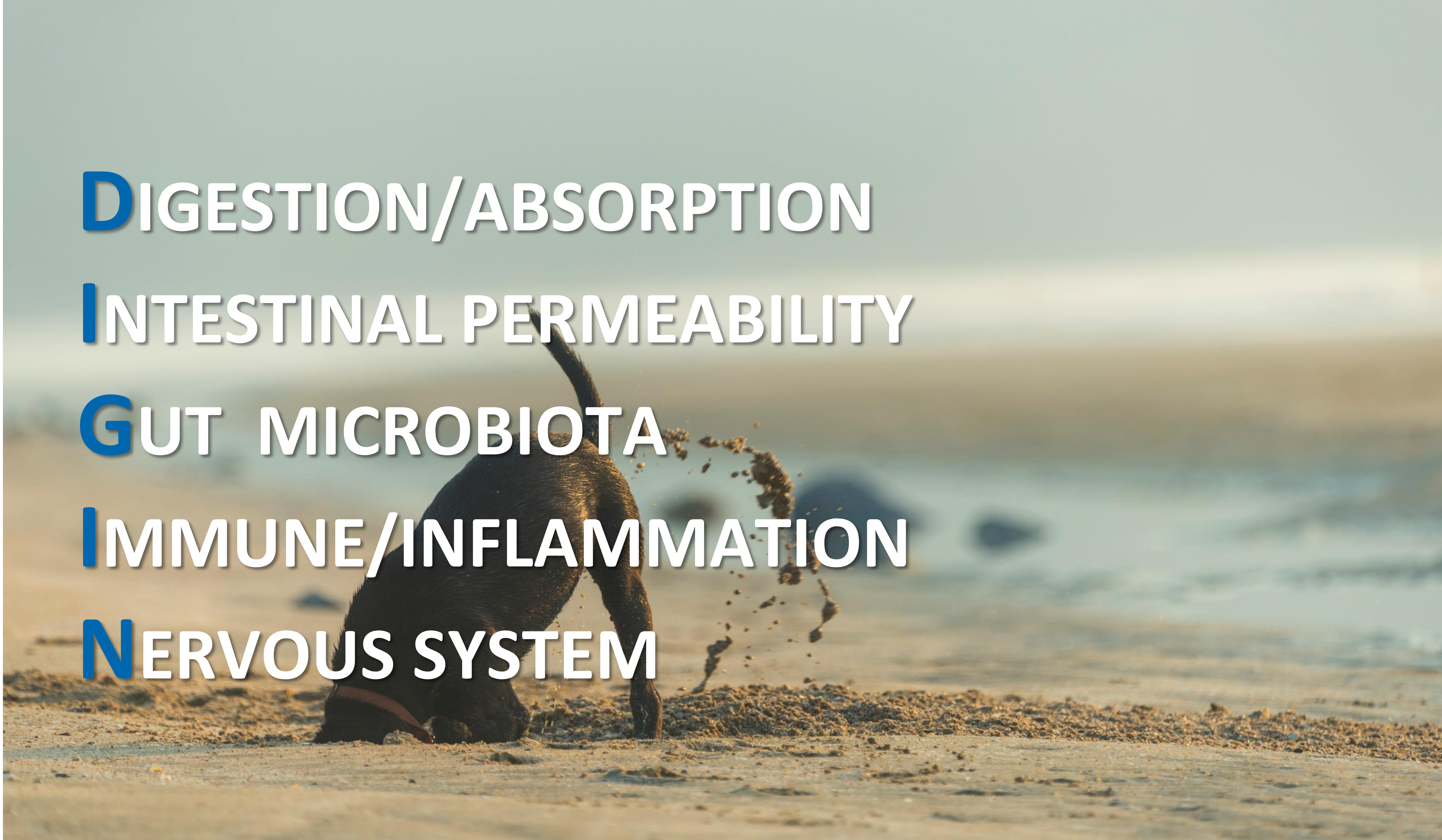

DIGESTION/ABSORPTION
INTESTINAL PERMEABILITY
GUT MICROBIOTA
IMMUNE/INFLAMMATION
NERVOUS SYSTEM

Performance Objectives

Following this activity, successful participants will be able to…

1. Identify the key functional roles of the gastrointestinal tract, and recognize how impairments may lead to dysfunction
2. **Identify the role the gastrointestinal tract plays in many chronic diseases**
3. Use stool analysis as a foundational tool to help evaluate gastrointestinal function

Triggers: nutrient insufficiency, medication, dysbiosis, parasite, food reaction, surgery, etc.

Disturbance of GI flora

Localized irritation/ inflammation

Local reaction/localized symptoms

Physical disruption of mucosal barrier

Increased mucosal permeability

Bacterial/yeast/protozoa/LPS /toxin translocation

Food protein translocation (macromolecules)

Portal and Systemic Overload

Immunologically mediated reactions (and perpetuation)

Distant Signs and Symptoms: Systemic illness (The Autoimmune Spectrum)

What May Cause Dysregulation Of The Gut Environment? (Triggers And Mediators)

- Nutrient insufficiencies
- Medications (NSAIDs, cytotoxic agents, antibiotics, antacids)
- Infectious agents: viruses, bacteria, protozoa, helminths, intestinal dysbiosis
- Ethanol
- Localized free radical production
- Food allergies/sensitivities/intolerances
- Traumatic brain injury
- Diminished HCL secretion/Diminished enzyme secretion/Diminished bile secretion
- Psychological/Emotional stress
- Hypoxia, exposure to extreme altitude

What are the Consequences of Gut Dysregulation?

- Immunologically mediated localized inflammatory responses
- Breach of mucosal integrity
- Portal circulation flooded with antigenic macromolecules resulting in detoxification pathway stress
- Increase in circulating immune complexes—activation of the complement cascade and other pathways.
- Chronic (both systemic and local) inflammation may impact HPA axis

Molecular mimicry – Antigens may possess similar antigenic determinants as human tissue

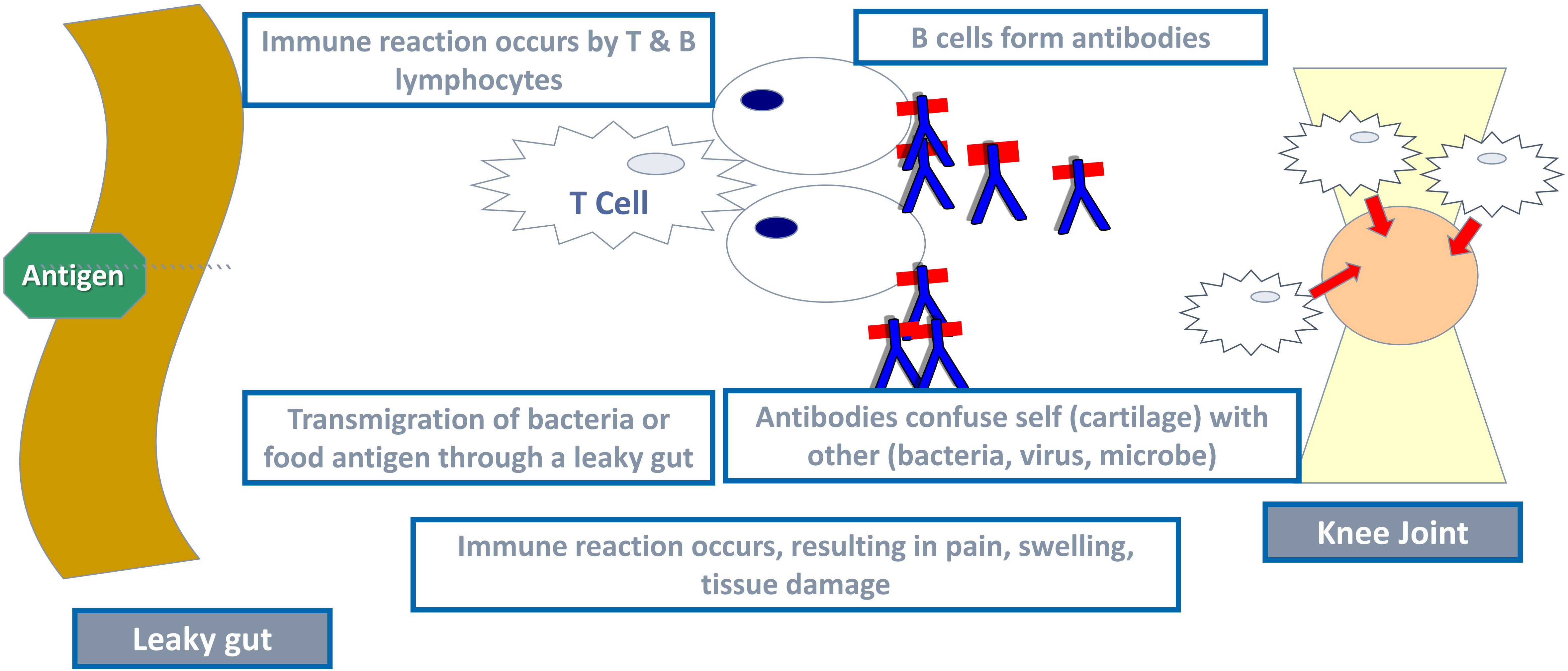
Consequences of Gut Dysregulation

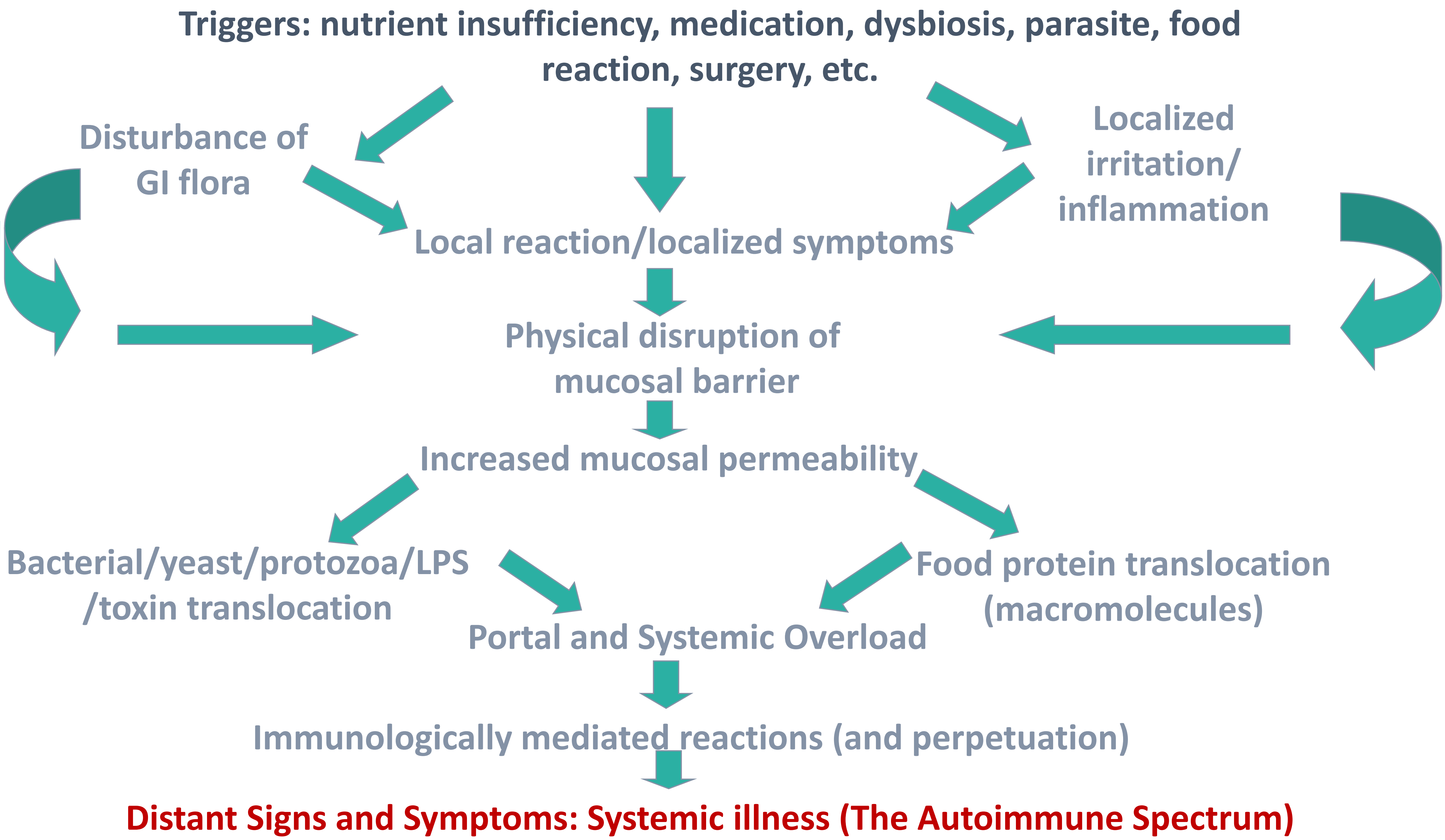
Molecular mimicry – Antigens may possess similar antigenic determinants as human tissue.

- Translocation of antigenic and microbial components may result in antibody production and cross reactivity.
- Example:
  - Klebsiella and Ankylosing Spondylitis
  - Streptococcus and Rheumatic Heart Fever
  - Proteus and Rheumatoid arthritis
  - Many other intestinal microbes have been implicated in systemic disease: *Salmonella, Shigella, Campylobacter, Yersinia, Proteus*.

Rashid, T. (2013). The link between ankylosing spondylitis, Crohn's disease, Klebsiella, and starch consumption. Clin Dev Immunol, 2013. doi:10.1155/2013/872632

Cross Reactivity Model
"Leaky Gut" And Autoimmunity

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Food protein translocation (macromolecules)
Portal and Systemic Overload
Immunologically mediated reactions (and perpetuation)
Distant Signs and Symptoms: Systemic illness (The Autoimmune Spectrum)

Part 3

Performance Objectives

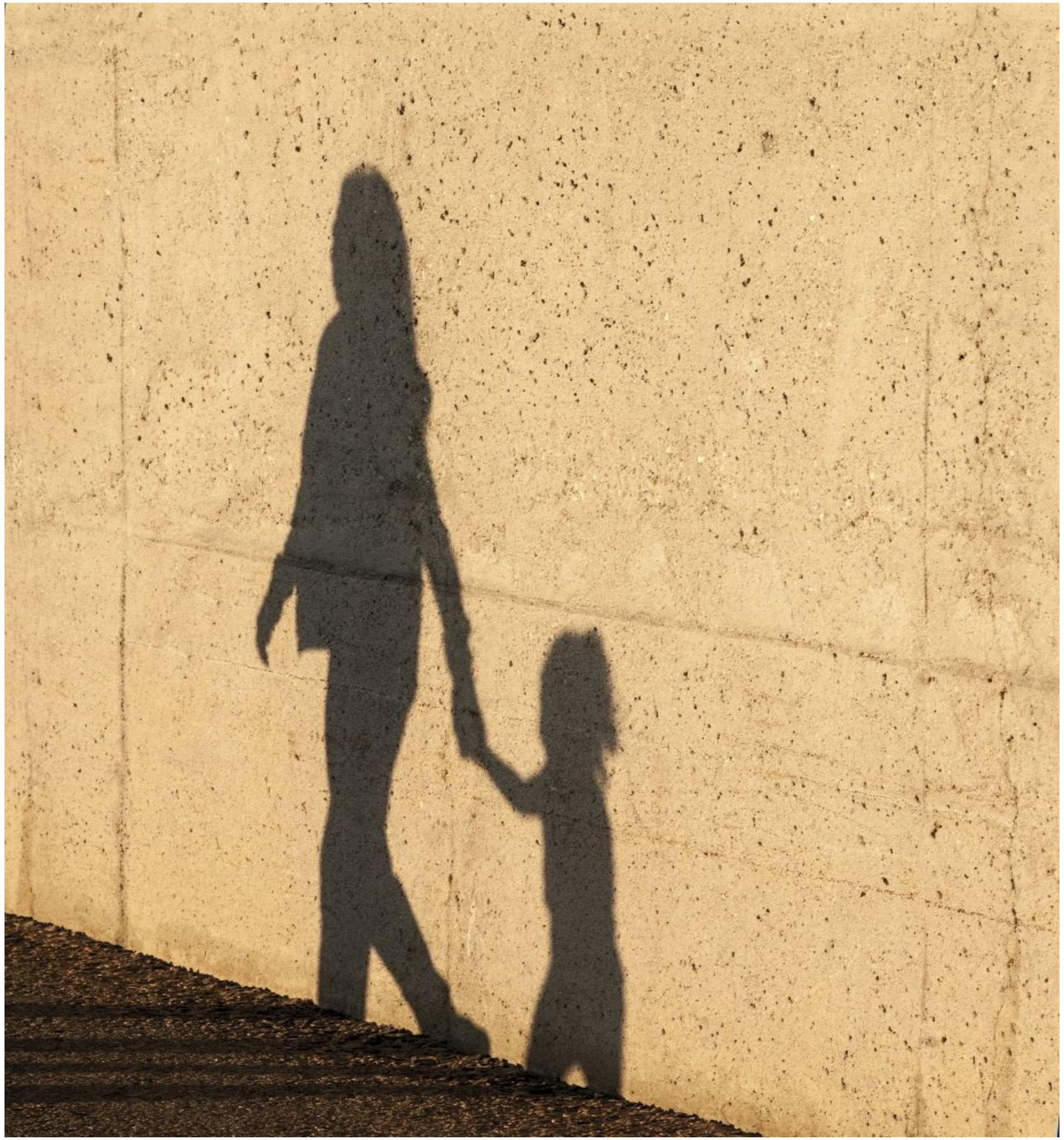
Following this activity, successful participants will be able to…

1. Identify the key functional roles of the gastrointestinal tract, and recognize how impairments may lead to dysfunction
2. Identify the role the gastrointestinal tract plays in many chronic diseases
3. **Use stool analysis as a foundational tool to help evaluate gastrointestinal function**

Using A Stool Analysis As A Pattern Recognition Tool

The complexity of the interlaced web-like connections within the metabolome are complex, leaving a “one analyte one problem” interpretation problematic.

Labs are not perfect, so always marry the patient to the lab.



Using A Stool Analysis As A Pattern Recognition Tool

- The complexity of the interlaced web-like connections within the metabolome are complex, leaving a "One analyte one problem" interpretation problematic.

- Stool analytes should be considered as pieces of a puzzle in which **recognition of overall patterns is important.**

Stool Analysis Components

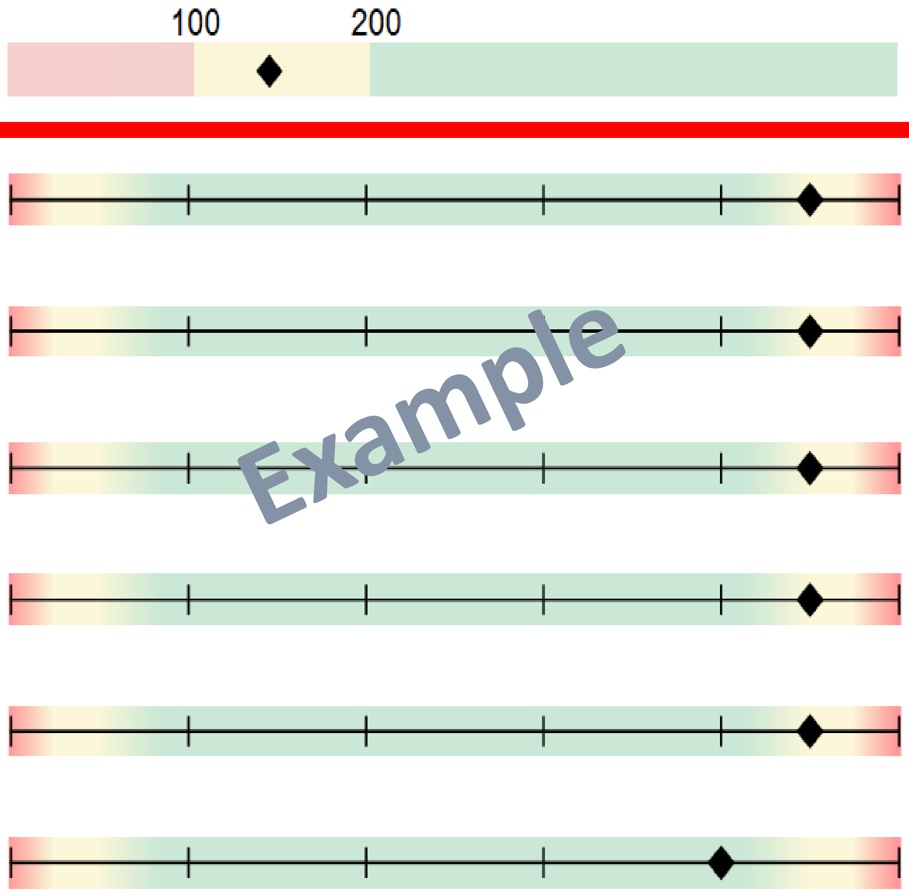
- Digestive and absorptive markers
- Immune and inflammatory markers
- Gut microbiome and its metabolic products

Stool Analysis Components

Digestive and Absorptive markers

1) Enzymatic digestive function
2) Products of protein breakdown
3) Fat digestion/absorption

Pancreatic Elastase 1 † ♦	145 L		>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	7.8		1.8-9.9 micromol/g
Fecal Fat (Total*)	38.2		3.2-38.6 mg/g
Triglycerides	2.0		0.3-2.8 mg/g
Long-Chain Fatty Acids	28.2		1.2-29.1 mg/g
Cholesterol	4.0		0.4-4.8 mg/g
Phospholipids	4.0		0.1-7.1 mg/g



1. Sugai E, Srur GF, Vazquez HF, et al. Steatocrit: A reliable semiquantitative method for detection of steatorrhea. J Clin Gastroenterolo. 1994;19(3):206-9.
2. Khouri MR, Huang G FAU - Shiau,,Y.F., Shiau YF. Sudan stain of fecal fat: New insight into an old test. Gastroenterology JID - 0374630. 1989;96(2 pt 1):421-7

Elastase; stool

Example

	Normal	Abnormal	Reference
Elastase		98	>200 µg/mL

Elastase findings can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency which may be associated with chronic pancreatitis, cystic fibrosis, carcinoma of the pancreas, Diabetes mellitus type 1, Shwachman-Diamond syndrome and other etiologies of pancreatic insufficiency.

1. Mattar R, Lima GA, da Costa MZ, Silva-Etto JM, Guarita D, Carrilho FJ. Comparison of fecal elastase 1 for exocrine pancreatic insufficiency evaluation between ex-alcoholics and chronic pancreatitis patients. Arq Gastroenterol. 2014 Oct-Dec;51(4):297-301. doi: 10.1590/S0004-28032014000400006.
2. Vujasinovic M, Tepes B, Makuc J, et al. Pancreatic exocrine insufficiency, diabetes mellitus and serum nutritional markers after acute pancreatitis. *World Journal of Gastroenterology : WJG*. 2014;20(48):18432-18438. doi:10.3748/wjg.v20.i48.18432.

1) Pancreatic Elastase

- **Proteolytic enzyme secreted exclusively by the human pancreas**
- **Reflects overall enzyme production**
  - amylase, lipase and protease
- **Not affected by supplemental enzymes**
- **Non-invasive marker for evaluating exocrine pancreatic function**
  - Sensitivity = 90 - 100%
  - Specificity = 93 - 98%

1. Stein J, Jung M, Sziegoleit A, Zeuzem S, Caspary WF, Lembcke B. Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. Clin Chem. 1996 Feb;42(2):222-6.
2. Löser C, Möllgaard A, Fölsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. Gut. 1996 Oct;39(4):580-6.
3. See next slide for more references: "References: Pancreatic Elastase"

References: Pancreatic Elastase

- Mattar R, Lima GA, da Costa MZ, Silva-Etto JM, Guarita D, Carrilho FJ. Comparison of fecal elastase 1 for exocrine pancreatic insufficiency evaluation between ex-alcoholics and chronic pancreatitis patients. Arq Gastroenterol. 2014 Oct-Dec;51(4):297-301. doi: 10.1590/S0004-28032014000400006.
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- Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. *World Journal of Gastroenterology : WJG*. 2013;19(42):7258-7266. doi:10.3748/wjg.v19.i42.7258.

1) Pancreatic Elastase

> 350 µg/g	Normal pancreatic function
200-350 µg/g	Declining pancreatic function Consider supplementation
100-200 µg/g	Moderate pancreatic insufficiency Supplement with broad array of pancreatic enzymes
<100 µg/g	Severe pancreatic insufficiency Supplement with broad array of pancreatic enzymes

1) Pancreatic Elastase

- **Can be used for initial determination of pancreatic insufficiency and to monitor function in patients under treatment**

- Patients in whom testing may be useful include:
  - **Unexplained diarrhea**
  - **Weight loss**
  - **Other signs of malabsorption**
  - **Abdominal pain**

- Exocrine Pancreatic Insufficiency may occur secondary to:
  - Chronic Pancreatitis, diabetes, celiac disease, inflammatory bowel disease, Cystic fibrosis, alcohol consumption, gallstone disease

Digestion/Absorption

Analyte	Result	Reference Range
1. Pancreatic Elastase 1 ◆	18	>= 201 mcg/g
2. Putrefactive SCFAs (Total*)	<dl	1.3-8.6 micromol/g

*Total values equal the sum of all measurable parts.

1. Mattar R, Lima GA, Costa MZ, Silva-Etto JM, Guarita D, Carrilho FJ. (2014). Comparison of fecal elastase 1 for exocrine pancreatic insufficiency evaluation between ex-alcoholics and chronic pancreatitis patients. Arq Gastroenterol. 51(4):297-301. doi: 10.1590/S0004-28032014000400006.
2. Vujasinovic M, Tepes B, Makuc J, Rudolf S, Zaletel J, Vidmar T, Seruga M, Birsa B. (2014). Pancreatic exocrine insufficiency, diabetes mellitus and serum nutritional markers after acute pancreatitis. World J Gastroenterol. 20(48):18432-8. doi: 10.3748/wjg.v20.i48.18432.

Pancreatic Elastase 1 † ♦	145	L	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	7.8		1.8-9.9 micromol/g
Fecal Fat (Total*)	38.2		3.2-38.6 mg/g
Triglycerides	2.0		0.3-2.8 mg/g
Long-Chain Fatty Acids	28.2		1.2-29.1 mg/g
Cholesterol	4.0		0.4-4.8 mg/g
Phospholipids	4.0		0.1-7.1 mg/g

Example

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2. Khouri MR, Huang G FAU - Shiau,,Y.F., Shiau YF. Sudan stain of fecal fat: New insight into an old test. Gastroenterology JID - 0374630. 1989;96(2 pt 1):421-7

2) Putrefactive SCFAs

- There are three putrefactive SCFAs: **valerate, iso-valerate,** and **iso-butyrate**
- These SCFAs are the result of the **anaerobic fermentation of polypeptides and amino acids by gut flora.**

Valerate

Isovalerate

Isobutyrate

2) Putrefactive SCFAs

ROOT CAUSES:

- **Hypochlorhydria** resulting in poor protein digestion
- **Low secretion of protein-digesting enzymes** by the pancreas
- **Poor absorption of protein** due to inflammation/ damage to the gut lining (ie Celiac, Crohn's Disease)
- **Dysbiosis: Small intestinal bacterial overgrowth (SIBO)**

References – see next slide "References: Putrefactive SCFAs"

References: Putrefactive SCFAs

- **Exocrine pancreatic insufficiency:** Pezzilli R, Andriulli A, Bassi C, et al. Exocrine pancreatic insufficiency in adults: a shared position statement of the Italian Association for the Study of the Pancreas. World J Gastroenterol. 2013;19(44):7930-7946.
- **SIBO**: Bures J, Cyrany J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. World J Gastroenterol. 2010;16(24):2978-2990.
- **Hypochlorhydria:** Revaiah PC, Kochhar R, Rana SV, et al. Risk of small intestinal bacterial overgrowth in patients receiving proton pump inhibitors versus proton pump inhibitors plus prokinetics. JGH Open. 2018;2(2):47-53.
- **Increased protein consumption:** Geypens B, Claus D, Evenepoel P, et al. Influence of dietary protein supplements on the formation of bacterial metabolites in the colon. Gut. 1997;41(1):70-76.

2) Consequences of Low HCL

- Small Intestinal Bacterial Overgrowth
- Dysbiosis – altered gut bacteria
- Chronic candida Infections
- Mineral Deficiencies
    - Ca, Mg, Zn, Fe, Cr, Mo, Mn, Cu
- B_{12} deficiency
- Unexplained low ferritin or anemia

References – see next slide "Consequences of low HCL"

References: Consequences of Low HCL

- Untersmayr E, Jensen-Jarolim E. The role of protein digestibility and antacids on food allergy outcomes. The Journal of allergy and clinical immunology. 2008;121(6):1301-1310. doi:10.1016/j.jaci.2008.04.025.
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- Champagne E. Low gastric hydrochloric acid secretion and mineral bioavailability. Advances in experimental medicine and biology. 1989;249:173–84.
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- Hurwitz A, Brady D, Schaal S, Samloff I, Dedon J, Ruhl C. Gastric acidity in older adults. JAMA. 1997;278(8):659–62.
- Husebye E, Skar V, Høverstad T, Melby K. Fasting hypochlorhydria with gram positive gastric flora is highly prevalent in healthy old people. Gut. 1992;33(10):1331-1337.
- Betesh AL, Santa Ana CA, Cole JA, Fordtran JS. Is achlorhydria a cause of iron deficiency anemia? Am J Clin Nutr. 2015 Jul;102(1):9-19. doi:10.3945/ajcn.114.097394.
- Graziani G, et al. Effect of gastric acid secretion on intestinal phosphate and calcium absorption in normal subjects. Nephrol Dial Transplant.1995;10(8):1376-80.
- Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis [published correction appears in Clin Med Res. 2006 Dec;4(4):342]. Clin Med Res. 2006;4(3):236–241.
- Dukowicz AC, Lacy BE, Levine GM. Small intestinal bacterial overgrowth: a comprehensive review. Gastroenterol Hepatol (N Y). 2007;3(2):112–122.

3) Fecal Fat Testing

Pancreatic Elastase 1 † ♦	145	L	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	7.8		1.8-9.9 micromol/g
Fecal Fat (Total*)	38.2		3.2-38.6 mg/g
Triglycerides	2.0		0.3-2.8 mg/g
Long-Chain Fatty Acids	28.2		1.2-29.1 mg/g
Cholesterol	4.0		0.4-4.8 mg/g
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Example

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2. Khouri MR, Huang G FAU - Shiau,,Y.F., Shiau YF. Sudan stain of fecal fat: New insight into an old test. Gastroenterology JID - 0374630. 1989;96(2 pt 1):421-7.

	Within	Outside	Reference Range
Elastase	362		> 200 µg/mL
Fat Stain	None		None - Mod
Muscle fibers	None		None - Rare
Vegetable fibers	Rare		None - Few
Carbohydrates	Neg		Neg

3) Fecal Fat Testing

Also known as: Fecal fat stain, quantitative stool fat

- Measures the number of fat globules in a stool sample
- Used to identify patients with steatorrhea/fat malabsorption, an important consideration for diagnosis and treatment
- Common causes for an elevated fecal fat test include celiac disease, exocrine pancreas insufficiency, Crohn's disease, enteritis, or liver disease

1. Fine KD, Ogunji F. A new method of quantitative fecal fat microscopy and its correlation with chemically measured fecal fat output. Am J Clin Pathol. 2000 Apr;113(4):528-34.

2. University of Rochester Medical Center. Fecal fat. Published 2018. Accessed March 2018 from https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=167&ContentID=fecal_fat

3. Cheung K, Lee SS, Raman M. Prevalence and mechanisms of malnutrition in patients with advanced liver disease, and nutrition management strategies. Clin Gastroenterol Hepatol. 2012 Feb;10(2):117-25. doi: 10.1016/j.cgh.2011.08.016.

Stool Analysis Components

Immune and Inflammatory Markers

1) Local inflammatory metabolites
2) Immune markers

	RESULT µg/g	REFERENCE INTERVAL	WITHIN REFERENCE	MODERATELY ELEVATED	ELEVATED
Calprotectin*	24	< 50			

Example

Inflammation and Immunology			
Calprotectin †	<16	50 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	0.6	1.1 4.6	<=4.6 mcg/g
Fecal secretory IgA	206		<=885 mcg/g

Example

Genova Diagnostics. GI Effects Stool Profile. Accessed March 2018 from https://www.gdx.net/core/sample-reports/gi-effects-2200-sample-report.pdf

Calprotectin

- Found in extra lysosomal cytosol of the neutrophil
- Accounts for ~ 60% of the cytosolic protein
- Inhibitory effect on zinc dependent enzymes
- Bacteriostatic activity

1. Dale I, Brandtzaeg P, Fagerhol MK, Scott H. Distribution of a new myelomonocytic antigen (L1) in human peripheral blood leukocytes. Immunofluorescence and immunoperoxidase staining features in comparison with lysozyme and lactoferrin. Am J Clin Pathol. 1985 Jul;84(1):24-34.
2. Brun JG, Ulvestad E, Fagerhol MK, Jonsson R. Effects of human calprotectin (L1) on in vitro immunoglobulin synthesis. Scand J Immunol. 1994 Dec;40(6):675-80.

Calprotectin

Elevated in:

- Inflammatory Bowel Disease
- Post-Infectious Irritable Bowel Syndrome
- Gastrointestinal cancers
- Certain gastrointestinal infections
- NSAID enteropathy
- Food allergy
- Chronic Pancreatitis

Poullis A, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. J Gastroenterol Hepatol. 2003 Jul;18(7):756-62.

Use Calprotectin to Differentiate IBD vs. IBS

A person with positive Rome criteria and a normal Calprotectin (< 50 µg/g) has virtually

NO CHANCE OF HAVING IBD!

FDA-cleared biomarker Calprotectin is highly accurate and capable of differentiating IBS from IBD.

Calprotectin

- A meta-analysis published in 2010 provides a useful calculation of potential interest to payers, as well as to clinicians and patients.
- Evaluated 13 studies and found that in adults being evaluated for IBD, ***screening by measuring calprotectin levels would produce a 67% reduction in the number of adults undergoing endoscopy.***

van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ(Clinical research ed.* 2010;341:c3369.

Calprotectin: Know when it's SERIOUS

< 50 µg/g	No significant inflammation
50-120 µg/g	Indicates some GI inflammation: IBD, infection, polyps, neoplasia, NSAIDS
> 120 µg/g	Significant inflammation; referral may be indicated to determine pathology
> 250 µg/g	Active disease present; predicts imminent relapse in treated patients

Tibble J, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut. 2000 Oct;47(4):506-13.

Lactoferrin; stool

Example

	Normal	Abnormal	Reference	
Lactoferrin		136	< 7.3	µg/mL

Lactoferrin is a quantitative GI specific marker of inflammation used to diagnose and differentiate IBD from IBS and to monitor patient inflammation levels during active and remission phases of inflammatory bowel disease.

Calprotectin vs. Lactoferrin Summary

- Fairly similar in the prediction of clinical relapse of IBD (better at UC than CD).
- Fairly similar at the differentiation of IBD from IBS.
- Sensitive markers of inflammation in the gut.

Eosinophilic Protein X

- Released in eosinophil degranulation
- Sensitive marker of GI inflammation
- May predict relapse in IBD
- Stable in transport up to 7 days
- Sensitive marker for low-level inflammation

- May be elevated with:
  - Inflammatory Bowel Disease
  - Celiac Disease
  - Parasites
  - Allergic reaction
  - Less common
    - GERD
    - Chronic diarrhea
    - Chronic alcoholism
    - Protein-Losing Enteropathy

See References: Eosinophilic Protein X

References: Eosinophilic Protein X

1. Carlson M, Raab Y, Peterson C, et al. *Increased intraluminal release of eosinophil granule proteins EPO, ECO, EPX, and cytokines in ulcerative colitis and proctitis in segmental perfusion.* Am J Gastroenterol 1999; 94(7):1876-1883.
2. Bischoff SC, Mayer J, Nguyen QT, et al. *Immunohistological assessment of intestinal eosinophil activation in patients with eosinophilic gastroenteritis and inflammatory bowel disease.* Am J Gastroenterol 1999; 94(12):3521-3529.
3. Hau J, Andersson E, Carlsson HE. *Development and validation of a sensitive ELISA for quantification of secretory IgA in rat saliva and feces.* Laboratory Animals 2001;35:301-306.
4. Choi SW, Choog HP, Terezinha MJS, et al. *To culture or not to culture: Fecal lactoferrin screening for inflammatory bacterial diarrhea.* J Clin Microbol 1996;34(4):928-932.

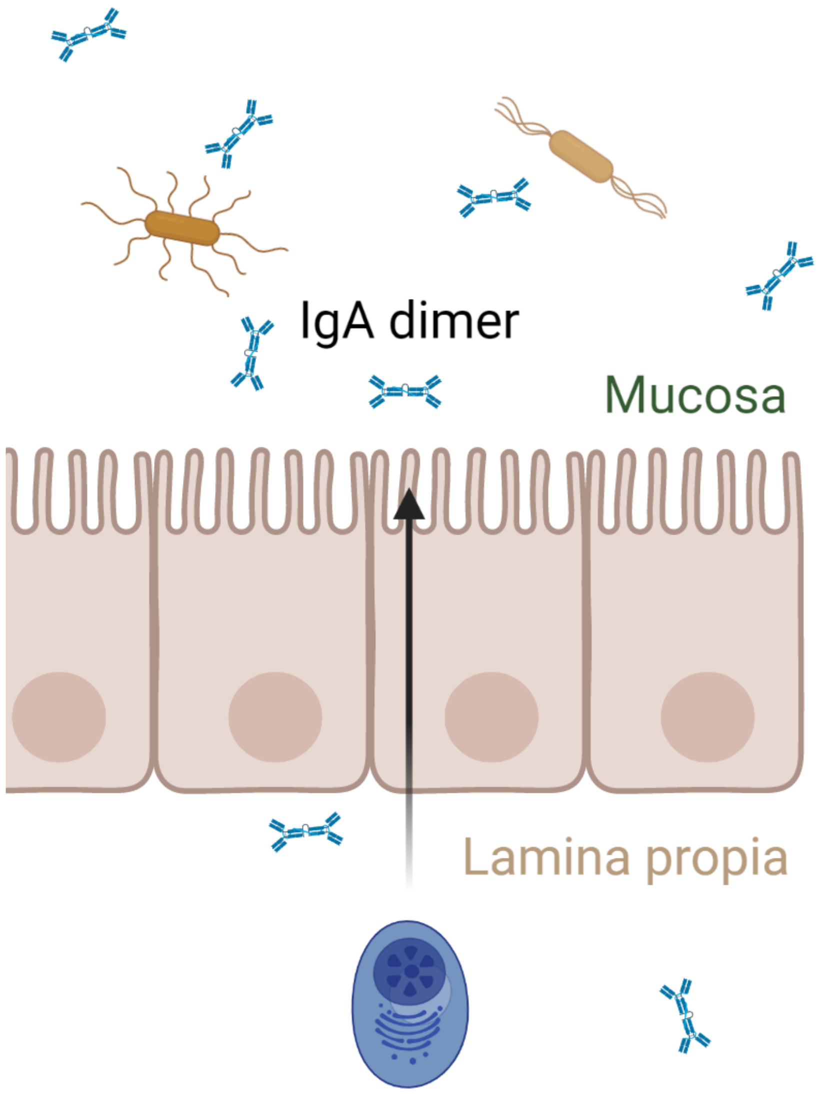
	Within	Outside	Reference Range
Secretory IgA*		11.8	51 - 204mg/dL

Example

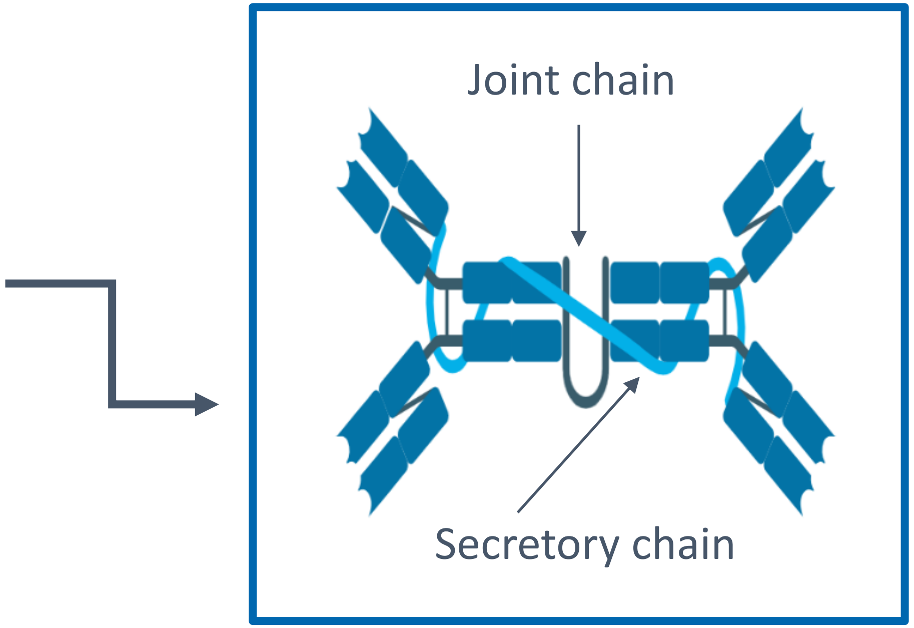
Secretory IgA (sIgA)

- Predominant immunoglobulin released onto the surface of the GI mucosa
- Binds to and neutralizes microbes and other antigens before they can cross the mucosal barrier

IgA Secretion



Adapted from "IgA Role in Maintaining Colonic Homeostasis", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates



IgA

In the lamina propria, polymeric IgA is secreted by plasma cells and transported across epithelial cells into the lumen via a receptor mediated process (transcytosis).

1. Lamm ME. Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. Am J Physiol. 1998 Apr;274(4):G614-7. doi: 10.1152/ajpgi.1998.274.4.g614. PMID: 9575841.
2. Rojas R, Apodaca G. Immunoglobulin transport across polarized epithelial cells. Nat Rev Mol Cell Biol. 2002 Dec;3(12):944-55. doi: 10.1038/nrm972. PMID: 12461560.

Fecal sIgA

- Secretory IgA production is increased in the presence of potentially harmful antigens such as pathogenic bacteria, parasites, yeast, viruses, abnormal cell antigens, and allergenic proteins.
- However, sIgA production may be suppressed in cases of mental or physical stress, or inadequate nutrition.
  - Dietary restrictions, excessive alcohol intake, body mass loss, negative mood, and anxiety have been associated with lowered sIgA production.
- Elevated fecal sIgA is useful in identifying if bacteria, yeast, or parasites are present.
  - SIgA should renormalize with eradication of the pathogenic microorganisms.

1. Crgo S, et al. Mucosal Antibodies, Food Allergy and Intolerance. 1987:167-89.
2. Carins J, Booth C. Salivary immunoglobulin-A as a marker of stress during strenuous physical training. Aviat Space Environ Med. 2002;73(12):1203-7.
3. Quig DW, Higley M. Townsend Letter for Doctors and Patients, Jan 2006.

Stool Analysis Components

Microbiome and its metabolic products:

- **Bugs**
  - Type—Bacteria, Fungal, Protozoal
  - Action—Beneficial, Commensal, Pathogenic/Potential Pathogen
- **Metabolic Products**
  - Short chain fatty acids
  - Beta-glucuronidase
  - Secondary bile acids
  - pH

The Microbiome: Bug Types

Beneficial, Commensal, Pathogens and Potential Pathogens

1) Protozoa and worms
2) Bacteria
3) Yeast

Looking For Parasites

O&P Microscopy

- traditional method, well established
- Individual samples
- Pooled samples

EIAs (most common)

- *G. lamblia*
- *Cryptosporidium*
- *E. histolytica*

PCR Probes (emerging)

Parasite Detection

Detection rates are a function of:

- Specimen collection and handling
- Number and kind of specimens examined
- Concentration procedures
- Staining procedures
- Macroscopic and microscopic examination techniques
- Quality of training, frequency of practice, and dedication of laboratory personnel

Garcia LS, Arrowood M, Kokoskin E, et al. Laboratory diagnosis of parasites from the gastrointestinal tract. *Practical Guidance for Clinical Microbiology.* 2018; 31(1): 1-81.

SAMPLE REPORT

Parasitology

Microscopic Exam Results

Blastocystis hominis: Many
Endolimax nana: Few Trophozoites
Entamoeba hartmanni: Moderate Trophozoites & Cysts

Specimen Tested: Stool

Parasitology EIA Tests

Cryptosporidium

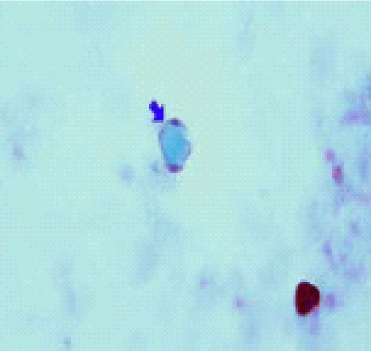
not ordered

Giardia lamblia

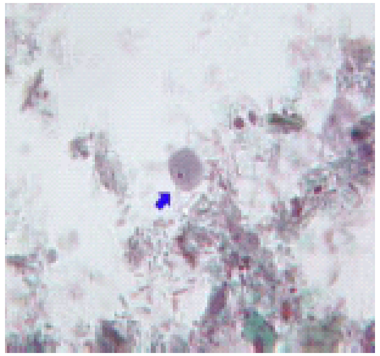
not ordered

Reference Range for EIA tests is Negative.

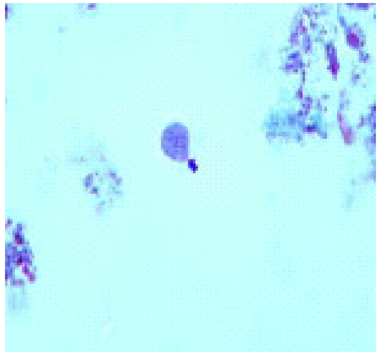
Blastocystis hominis



Endolimax nana trophozoites



Entamoeba hartmanni trophozoites



Bacteria and Yeast

Bacteria and Yeast

- Beneficial
- Commensal
- Pathogenic
- Potentially Pathogenic/Dysbiotic

Intestinal Dysbiosis

- **A state of imbalanced microbial ecology that contributes to disease**

- The overgrowth of micro-organisms of low intrinsic virulence induces disease by altering
  - **the nutritional status**
  - **the immune response**
  - **the elimination capacity of the host**

Causes of Intestinal Dysbiosis

- SAD – low fiber, high in fat & simple carbs
- Broad-spectrum antibiotics
- Chronic maldigestion (including PPIs)
- Chronic constipation
- Stress suppresses Lactobacillus, Bifidobacteria, and sIgA
- Catecholamines stimulate growth of gram-negative organisms (Yersinia, Pseudomonas)

See References: Causes of Intestinal Dysbiosis

References: Causes of Intestinal Dysbiosis

SAD Diet:

Zinöcker MK, Lindseth IA. The Western Diet-Microbiome-Host Interaction and Its Role in Metabolic Disease. Nutrients. 2018 Mar 17;10(3):365. doi: 10.3390/nu10030365. PMID: 29562591; PMCID: PMC5872783.

Romano-Keeler J, Zhang J, Sun J. The Life-Long Role of Nutrition on the Gut Microbiome and Gastrointestinal Disease. Gastroenterol Clin North Am. 2021 Mar;50(1):77-100. doi: 10.1016/j.gtc.2020.10.008. Epub 2021 Jan 5. PMID: 33518170.

González Olmo BM, Butler MJ, Barrientos RM. Evolution of the Human Diet and Its Impact on Gut Microbiota, Immune Responses, and Brain Health. Nutrients. 2021 Jan 10;13(1):196. doi: 10.3390/nu13010196. PMID: 33435203; PMCID: PMC7826636.

Antibiotics:

Becattini S, Taur Y, Pamer EG. Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. Trends Mol Med. 2016 Jun;22(6):458-478. doi: 10.1016/j.molmed.2016.04.003. Epub 2016 May 10. PMID: 27178527; PMCID: PMC4885777.

Chronic Maldigestion (Including PPIs):

Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. Proton pump inhibitors affect the gut microbiome. Gut. 2016 May;65(5):740-8. doi: 10.1136/gutjnl-2015-310376. Epub 2015 Dec 9. PMID: 26657899; PMCID: PMC4853569.

Chronic Constipation:

Ohkusa T, Koido S, Nishikawa Y, Sato N. Gut Microbiota and Chronic Constipation: A Review and Update. Front Med (Lausanne). 2019;6:19. Published 2019 Feb 12. doi:10.3389/fmed.2019.00019

Stress Supresses Lactobacillus, Bifidobacteria, and SIgA:

Bailey MT. Psychological Stress, Immunity, and the Effects on Indigenous Microflora. Adv Exp Med Biol. 2016;874:225-46. doi: 10.1007/978-3-319-20215-0_11. PMID: 26589222.

Catecholamines Stimulate Growth of Gram Negative Organisms:

Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. Life Sci. 1992;50(3):203-12.

Culture

- Limited number of bacteria that can be grown
- Most organisms are anaerobic

Microbiology

BACTERIOLOGY

12. Beneficial Bacteria

Lactobacillus species	*NG	*NG
Escherichia coli	NP	4+
Bifidobacterium	NP	4+

13. Additional Bacteria

alpha haemolytic Streptococcus	NP	4+
gamma haemolytic Streptococcus	NP	4+
Citrobacter freundii	PP	3+
Klebsiella pneumoniae	PP	3+

14. MYCOLOGY

Candida albicans	P	4+

Human microflora is influenced by environmental factors and the competitive ecosystem of the organisms in the GI tract. Pathological significance should be based upon clinical symptoms and reproducibility of bacterial recovery.

*NG	NP	PP	P
*NG			
No Growth	Non-Pathogen	Potential Pathogen	Pathogen

Bacteriology Profile, stool

BACTERIOLOGY CULTURE		
Expected/Beneficial flora	**Commensal (Imbalanced) flora**	**Dysbiotic flora**
4+ Bacteroides fragilis group	2+ Alpha hemolytic strep	
3+ Bifidobacterium spp.		
NG Escherichia coli		
2+ Lactobacillus spp.		
NG Enterococcus spp.		
3+ Clostridium spp.		
NG = No Growth		

Microbiology

Bacteriology

12. Beneficial Bacteria

Lactobacillus species	2+
Escherichia coli	4+
Bifidobacterium	1+

14. Mycology

Candida albicans/dubliniensis	NP	1+

Example

Human microflora is influenced by environmental factors and the competitive ecosystem of the organisms in the GI tract. Pathological significance should be based upon clinical symptoms and reproducibility of bacterial recovery.

*NG	NP	PP	P
*NG			
No Growth	Non-Pathogen	Potential Pathogen	Pathogen

Lab Comments

Elastase repeated and confirmed. 09/29/2011 UL

Microbiology

The Markers in this section reflect the bacteriological status of the gut.

Beneficial bacteria Beneficial flora controls potentially pathogenic organisms, influences nutrient production, removes toxins from the gut and stimulates the intestinal immune system (GALT). The composition of the colonic flora is affected by diet, transit time, stool pH, age, microbial interactions, colonic availability of nutrients, bile acids, sulfate and the ability of the microbes to metabolize these substrates. Ideally, levels of Lactobacilli and E. coli should be 2+ or greater. Bifidobacteria being a predominate anaerobe should be recovered at levels of 4+.

Additional bacteria

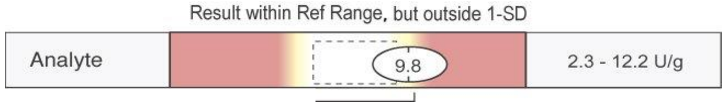
Non-pathogen: Organisms that fall under this category are those that constitute normal, commensal flora, or have not been recognized as etiological agents of disease.

Potential Pathogen: Organisms that fall under this category are considered potential or opportunistic pathogens when present in heavy growth.

Pathogen: The organisms that fall under this category are well-recognized pathogens in clinical literature that have a clearly recognized mechanism of pathogenicity and are considered significant regardless of the quantity that appears in culture.

Mycology: Organisms that fall under this category constitute part of the normal colonic flora when present in small numbers. They may, however, become potential pathogens after disruption of the mucosal lining, which enables fungi to colonize and establish a local infection.

The **Reference Range** is a statistical interval representing 95% or 2 Standard Deviations (2 S.D.) of the reference population. One Standard Deviation (1 S.D.) is a statistical interval representing 68% of the reference population. Values between 1 and 2 S.D. are not necessarily abnormal. Clinical correlation is suggested. (See example below)



Gastrointestinal Microbiome

Bacteriology (Culture)

			+1	+2	+3	+4
Lactobacillus spp.	+3	NP				
Escherichia coli		NG				
Bifidobacterium spp.	+4	NP				

Additional Bacteria

Alphahaemolytic streptococcus	+3	NP
Gammahaemolytic streptococcus	+3	NP
Citrobacter freundii	+4	PP
Streptococcus agalactiae gp B	+2	NP

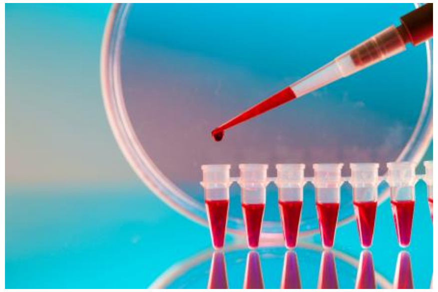
Mycology (Culture)

Candida albicans/dubliniensis	+2	PP
Yeast, not Candida albicans	+1	NP

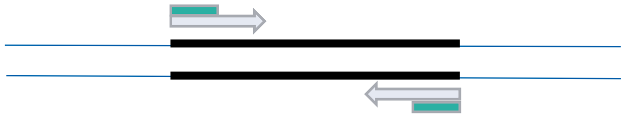
16S rRNA Gene Sequencing

- A common method for identifying bacteria is analyzing the sequence of the gene coding for 16S ribosomal RNA

- Can only identify bacteria to genus level



Purify DNA from biofilm



PCR amplify 16S rRNA gene

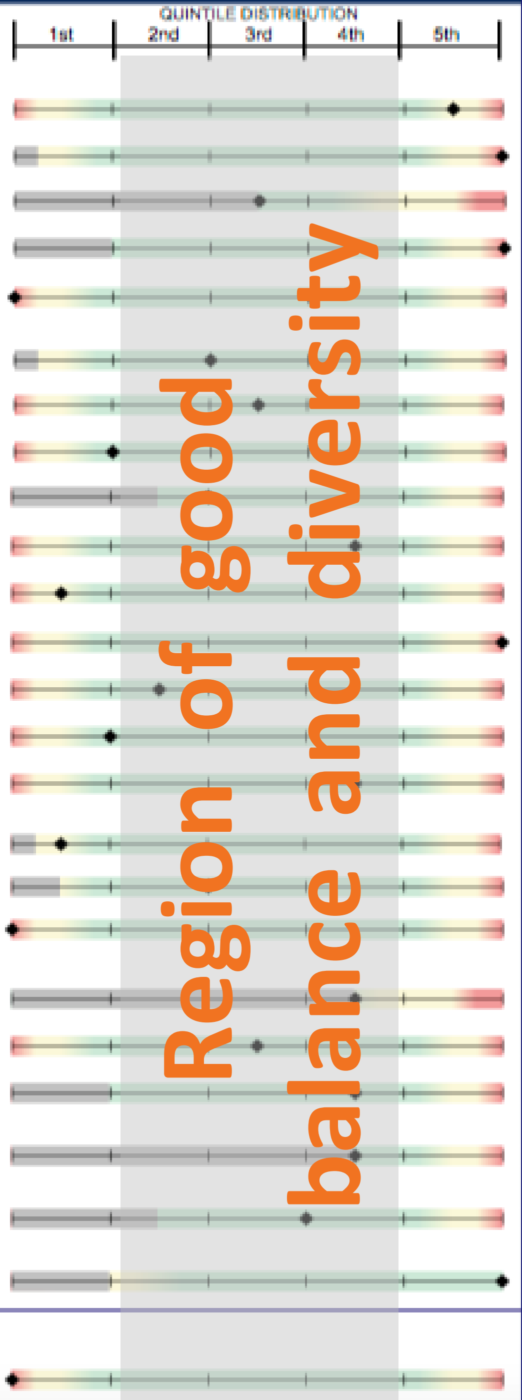
Sequence PCR product

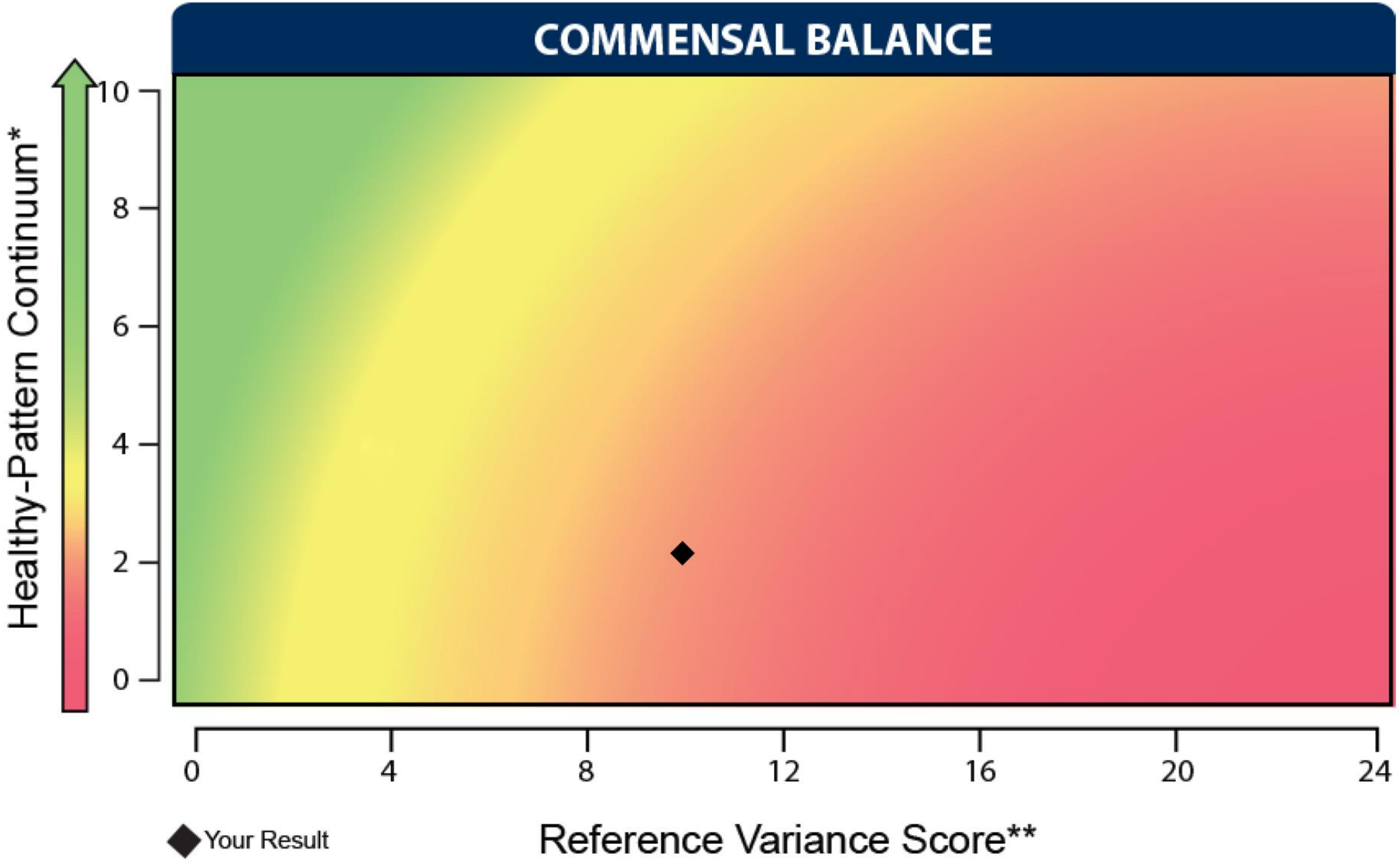
Compare sequence to database

Identify bacterial species

Gastrointestinal Microbiome

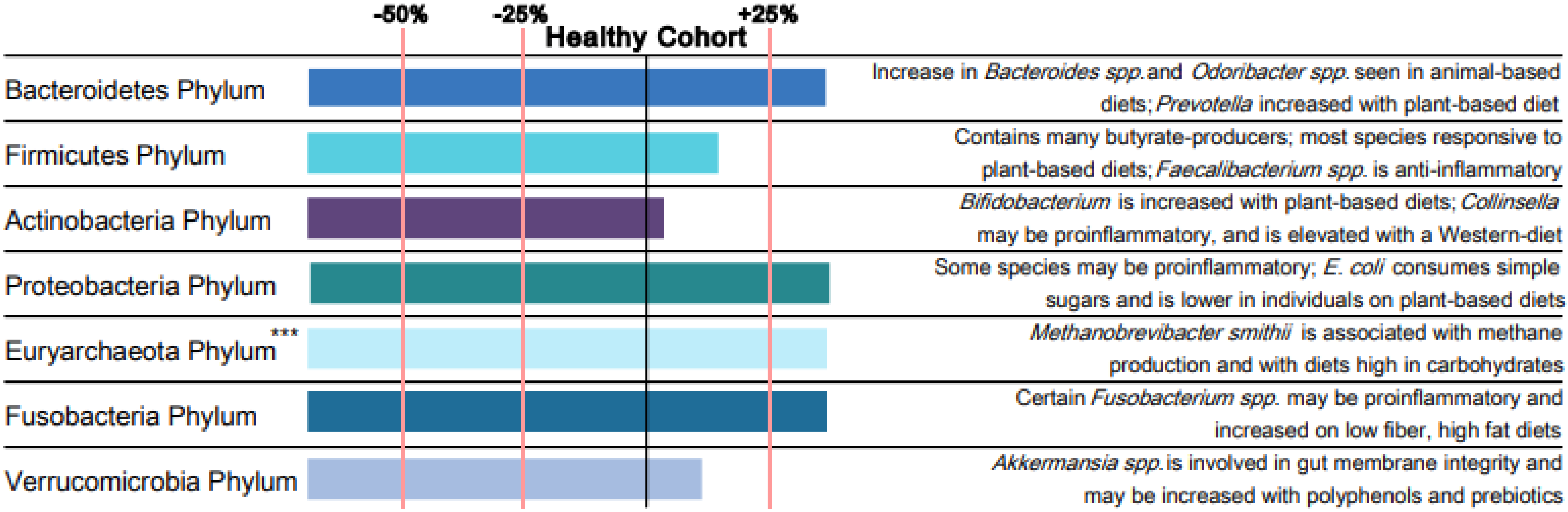
Commensal Bacteria (PCR)	Result CFU/g stool		Reference Range CFU/g stool
Bacteroidetes Phylum			
Bacteroides-Prevotella group	6.1E8		3.4E6-1.5E9
Bacteroides vulgatus	2.6E9	H	<=2.2E9
Barnesiella spp.	<DL		<=1.6E8
Odoribacter spp.	8.2E7	H	<=8.0E7
Prevotella spp.	<DL	L	1.4E5-1.6E7
Firmicutes Phylum			
Anaerotruncus colihominis	4.7E6		<=3.2E7
Butyrivibrio crossotus	7.2E4		5.5E3-5.9E5
Clostridium spp.	1.8E9		1.7E8-1.5E10
Coprococcus eutactus	7.0E5		<=1.2E8
Faecalibacterium prausnitzii	2.5E9		5.8E7-4.7E9
Lactobacillus spp.	1.4E8		8.3E6-5.2E9
Pseudoflavonifractor spp.	1.9E8	H	4.2E5-1.3E8
Roseburia spp.	2.0E9		1.3E8-1.2E10
Ruminococcus spp.	3.0E8		9.5E7-1.6E9
Veillonella spp.	1.5E7		1.2E5-5.5E7
Actinobacteria Phylum			
Bifidobacterium spp.	2.8E8		<=6.4E9
Bifidobacterium longum	3.1E7		<=7.2E8
Collinsella aerofaciens	<DL	L	1.4E7-1.9E9
Proteobacteria Phylum			
Desulfovibrio piger	6.6E4		<=1.8E7
Escherichia coli	5.2E6		9.0E4-4.6E7
Oxalobacter formigenes	1.8E6		<=1.5E7
Euryarchaeota Phylum			
Methanobrevibacter smithii	<DL		<=8.6E7
Fusobacteria Phylum			
Fusobacterium spp.	1.7E4		<=2.4E5
Verrucomicrobia Phylum			
Akkermansia muciniphila	7.8E6		>=1.2E6
Firmicutes/Bacteroidetes Ratio			
Firmicutes/Bacteroidetes (F/B Ratio)	10	L	12-620





Balanced	Represents 95% of healthy individuals
Borderline	Represents 5% of healthy individuals
Imbalanced	Represents 60% of unhealthy individuals

Relative Commensal Abundance



MetaGenomics

MetaGenomics vs. Metabolomics

MetaGenomics = composition of the gut flora (i.e. census taking)

Metabolomics = the metabolic activity of the microbiota

Structure & Function are not the same.
In fact, many functions work across multiple species and help us to understand diversity.

Turnbaugh PJ, Gordon JI. An invitation to the marriage of metagenomics and metabolomics. Cell. 2008 Sep 5;134(5):708-13. doi: 10.1016/j.cell.2008.08.025.

Stool Analysis Components

Microbiome and its metabolic products:

- **Bugs**
  - Type—Bacteria, Fungal, Protozoal
  - Action—Beneficial, Commensal, Pathogenic/Potential Pathogen
- **Metabolic Products**
  - Short chain fatty acids
  - Beta-glucuronidase
  - Secondary bile acids
  - pH

Metabolic Products

1) Short chain fatty acids
2) β-glucuronidase
3) Secondary bile acids
4) pH

Metabolic

Analyte	Result	Reference Range
5. Beneficial SCFAs (Total*)	39.7	>= 13.6 micromol/g
6. n-Butyrate	5.9	>= 2.5 micromol/g
7. pH ◆	6.3	6.1-7.9
8. Beta-glucuronidase	<dl	337-4,433 U/g

Secondary Bile Acids

Analyte	Result	Reference Range
9. Lithocholic acid (LCA)	6.20	0.65-5.21 mg/g
10. Deoxycholic acid (DCA)	6.47	0.67-6.76 mg/g
11. LCA / DCA Ratio	0.96	0.39-2.07

*Total values equal the sum of all measurable parts.

Example

SHORT CHAIN FATTY ACIDS

	Within	Outside	Reference Range
% Acetate	56		40 - 75 %
% Propionate	27		9 - 29 %
% Butyrate	14		9 - 37 %
% Valerate	3.2		0.5 - 7 %
Butyrate	1.6		0.8 - 4.8 mg/mL
Total SCFA's	12		4 - 18 mg/mL

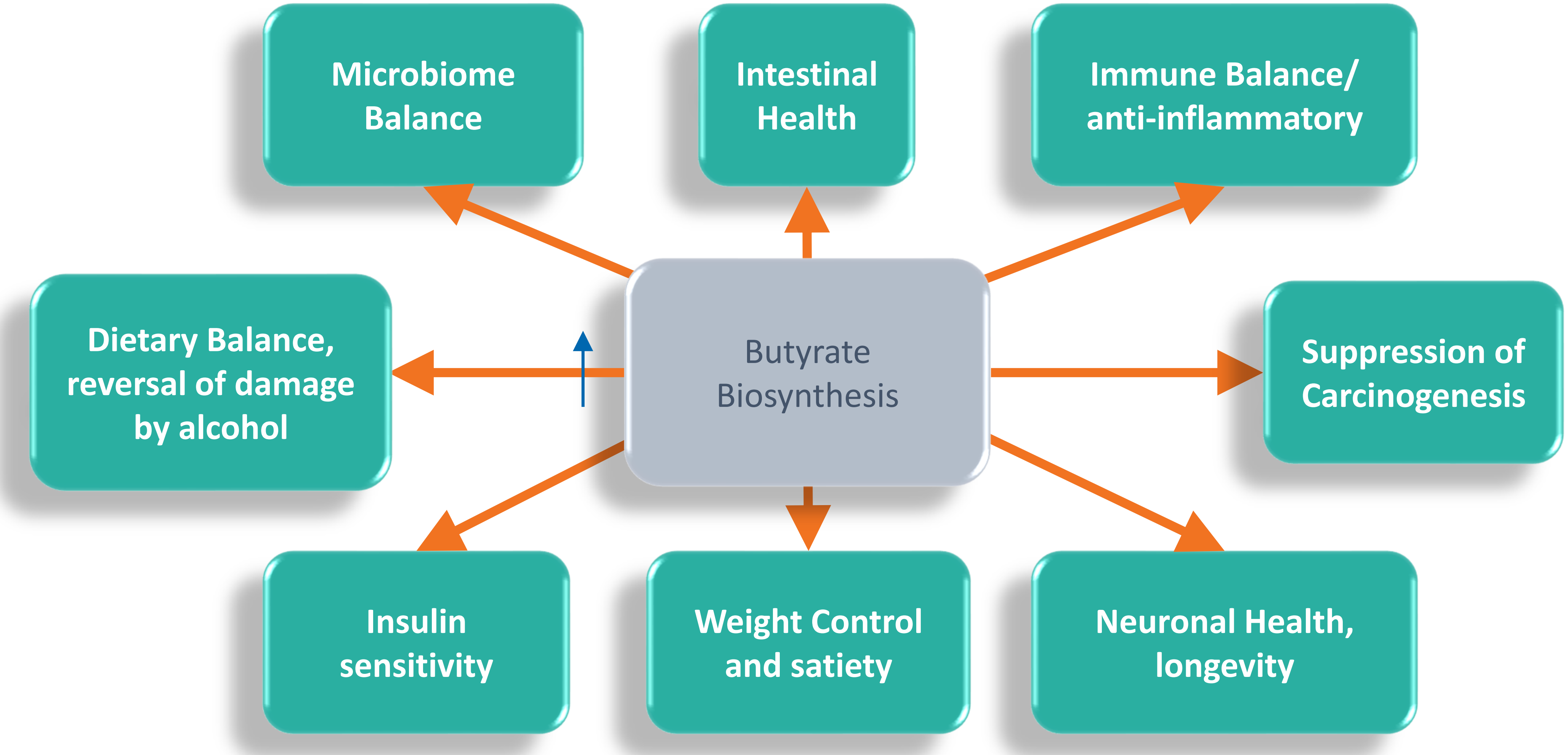
Short chain fatty acids (SCFAs): SCFAs are the end product of the bacterial fermentation process of dietary fiber by beneficial flora in the gut and play an important role in the health of the GI as well as protecting against intestinal dysbiosis. Lactobacilli and bifidobacteria produce large amounts of short chain fatty acids, which decrease the pH of the intestines and therefore make the environment unsuitable for pathogens, including bacteria and yeast. Studies have shown that SCFAs have numerous implications in maintaining gut physiology. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation. Levels of **Butyrate** and **Total SCFA** in mg/mL are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.

Short Chain Fatty Acids (SCFA)

- Major SCFAs include acetate, propionate, and butyrate.
- Short chain fatty acids:
  - Are produced during the fermentation of non-digestible polysaccharides by gut microbiota.
  - Serve as an important source of energy for colonocytes, liver cells, and skeletal muscle.
  - Play a role in gut motility, intestinal barrier permeability, and immune function.
  - Have been shown to reduce food intake and increase sensation of satiety
  - Reduce human colon cancer cell growth
  - Inhibit production of inflammatory cytokines in multiple tissue types

1. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Adv Immunol. 2014;121:91-119. doi: 10.1016/B978-0-12-800100-4.00003-9
2. Zeng H, Lazarova DL, Bordonaro M. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. World J Gastrointest Oncol. 2014 Feb 15;6(2):41-51. doi: 10.4251/wjgo.v6.i2.41. Review
3. McNabney SM, Henagan TM. Short Chain Fatty Acids in the Colon and Peripheral Tissues: A Focus on Butyrate, Colon Cancer, Obesity and Insulin Resistance. Nutrients. 2017 Dec 12;9(12). pii: E1348. doi: 10.3390/nu9121348..

Butyrate



Microbiome Balance
Intestinal Health
Immune Balance/ anti-inflammatory
Dietary Balance, reversal of damage by alcohol
Butyrate Biosynthesis
Suppression of Carcinogenesis
Insulin sensitivity
Weight Control and satiety
Neuronal Health, longevity

Gastrointestinal Microbiome

Metabolic

Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	47.5	>=23.3 micromol/g
n-Butyrate Concentration	10.6	>=3.6 micromol/g
n-Butyrate %	22.3	11.8-33.3 %
Acetate %	62.8	48.1-69.2 %
Propionate %	14.7	<=29.3 %
Beta-glucuronidase	2,297	368-6,266 U/g

Tan, J. (2014). The role of short-chain fatty acids in health and disease. dv Immunol, 2014(121), 91-119. doi:10.1016/B978-0-12-800100-4.00003-9

Metabolic

Analyte	Result	Reference Range
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Secondary Bile Acids

Analyte	Result	Reference Range
9. Lithocholic acid (LCA)	6.20	0.65-5.21 mg/g
10. Deoxycholic acid (DCA)	6.47	0.67-6.76 mg/g
11. LCA / DCA Ratio	0.96	0.39-2.07

*Total values equal the sum of all measurable parts.

Example

2) β-Glucuronidase

- β-Glucuronidase is an important lysosomal enzyme involved in the degradation of glucuronate-containing glycosaminoglycan
- The major producers of β-glucuronidase are colonic bacteria, including *E. coli, Clostridium paraputrificum, Clostridium clostridioforme, Clostridium perfringens, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides uniformis, Ruminococcus gnavus, Peptostreptococcus, Staphylococcus and Eubacterium*
- β-glucuronidase activity is stimulated by tobacco, exposure to toxic substances and carcinogens, consumption of red meat, and antibiotic treatment.
- Elevation of plasma β-Glucuronidase is considered as a marker of increased risk of developing hormone-dependent cancers, particularly cancers of the breast and prostate.

1. Naz H, et al. Human β-glucuronidase: structure, function, and application in enzyme replacement therapy. Rejuvenation Res. 2013 Oct;16(5):352-63. doi: 10.1089/rej.2013.1407. Review.
2. Arul L, Benita G, Balasubramanian P. Functional insight for β-glucuronidase in *Escherichia coli* and *Staphylococcus sp. RLH1*. *Bioinformation*. 2008;2(8):339-343.
3. Mroczyńska M, et al. Beta-glucuronidase and Beta-glucosidase activity in stool specimens of children with inflammatory bowel disease. Pol J Microbiol. 2013;62(3):319-25.'
4. Zółtaszek R, Hanausek M, Kiliańska ZM, Walaszek Z. [The biological role of D-glucaric acid and its derivatives: potential use in medicine]. Postepy Hig Med Dosw (Online). 2008 Sep 5;62:451-62. Review.

3) Secondary Bile Acids

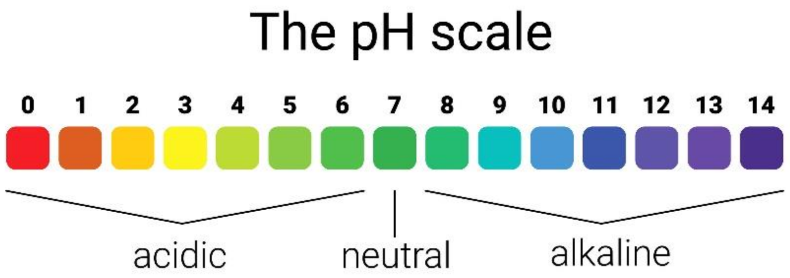
- Secondary bile acids (SBA) are formed by bacterial metabolism of primary bile acids in the colon.
- SBA are increased by dietary factors, primarily red meat and saturated fats.
- Due to their hydrophobic nature, elevated levels of SBAs may cause damage to cell membranes, resulting in destruction of intestinal epithelium.
- Elevated secondary bile acids associated with an increase in inflammatory cytokines in colonic mucosa
- Increased levels of secondary bile acids, particularly deoxycholic acid and lithocolic acid, associated with increased incidence of colorectal cancer.

1. Ajouz H, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. World J Surg Oncol. 2014 May 24;12:164. doi: 10.1186/1477-7819-12-164. Review.
2. Payne CM, Bernstein C, Dvorak K, Bernstein H. Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. Clin Exp Gastroenterol. 2008;1:19-47.
3. Kakiyama G, Hylemon PB, Zhou H, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. Am J Physiol Gastrointest Liver Physiol. 2014 Jun 1;306(11):G929-37. doi: 10.1152/ajpgi.00315.2013.

4) Fecal pH

Low pH

- CHO malabsorption
- CHO maldigestion
- Fast transit
- Organic acids
- **SIBO**



High pH

- High protein and/or low fiber diet
- Dysbiosis
- Slow transit time
- Hypochlorhydria
- Pancreatic bicarbonate
- Associated with increased risk for colorectal cancer

References – See next slide, "References: Fecal pH"

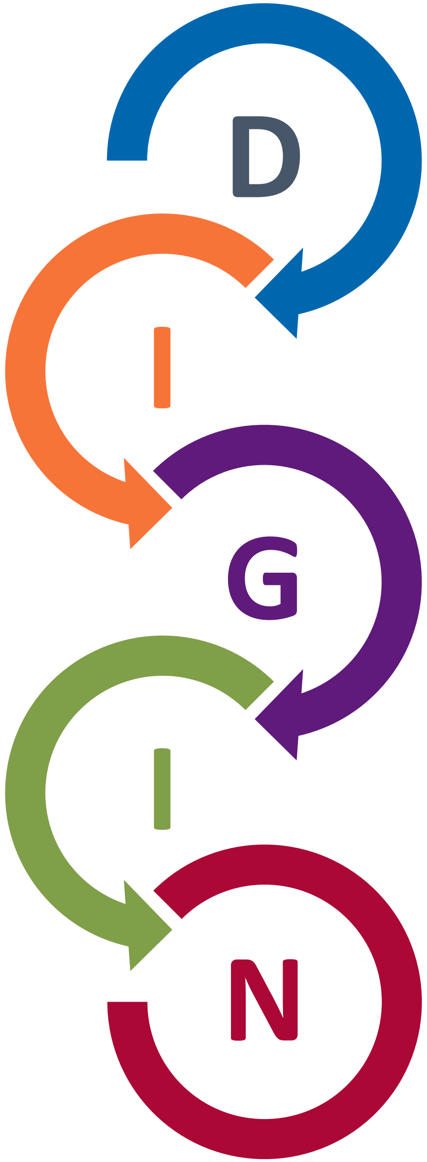
References: Fecal pH

Low pH References

- CHO Malabsorption, fast transit, organic acids: Osuka A, Shimizu K, Ogura H, et al. Prognostic impact of fecal pH in critically ill patients. Crit Care. 2012; 16(4): R119. doi: 10.1186/cc11413
- Bacterial overgrowth: Syed SZ, Bronze MS. Bacterial Overgrowth Syndrome Workup. Medscape. https://emedicine.medscape.com/article/212861-workup.
- Malabsorption/Maldigestion: Omer A, Quigley EMM. Carbohydrate Maldigestion and Malabsorption. Clin Gastroenterol Hepatol. 2018 Aug;16(8):1197-1199. doi: 10.1016/j.cgh.2018.01.048.

High pH References

- Slow transit time, constipation, hypochlorhydria and antibiotics: Osuka A, Shimizu K, Ogura H, et al. Prognostic impact of fecal pH in critically ill patients. Crit Care. 2012; 16(4): R119. doi: 10.1186/cc11413
- Low fiber: Vahouny GVK, D. Dietary Fiber in Health and Disease. New York, NY: Springer Science & Business Media; 2013.
- High protein, Low Carb Diet: Russell WR, Gratz SW, Duncan SH, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. Am J Clin Nutr. 2011;93(5):1062-1072.
- Colorectal Cancer Association: Ohigashi S, Sudo K, Kobayashi D, et al. Changes of the intestinal microbiota, short chain fatty acids, and fecal pH in patients with colorectal cancer. Digestive Diseases and Sciences. 2013; 58(6): 1717-1726.
- Fecal Bicarbonate Concentrations: Down PF, Agostini L, Murison J, Wrong OM. The interrelations of faecal ammonia, pH and bicarbonate: evidence of colonic absorption of ammonia. Clinical Science. 1972; 43(1):101-114.



Digestion / Absorption

Intestinal Permeability

Gut microbiota / Dysbiosis

Immune Modulation/Inflammation

Nervous System

Using a Stool Analysis to Help Decipher Joan's Health Issues

Evaluate the stool analysis on Joan and decide what dysfunction may be present…

- **Evidence of impaired digestion?**
- **Evidence of dysbiosis?**
- **Evidence of increased intestinal permeability?**

Joan's Case

DIGESTION /ABSOR

	Within	Outside	Reference Range
Elastase		129	> 200 µg/mL
Fat Stain	Few		None - Mod
Muscle fibers	None		None - Rare
Vegetable fibers	Few		None - Few
Carbohydrates	Neg		Neg

	Within	Outside	Reference Range
% Acetate	67		36 - 74 %
% Propionate	22		9 - 32 %
% Butyrate		8.4	9 - 39 %
% Valerate	2.7		1 - 8 %
Butyrate		0.36	0.8 - 3.8 mg/mL
Total SCFA's	4.3		4 - 14 mg/mL

	Within	Outside	Reference Range
Lactoferrin	2.6		< 7.3 µg/mL
Calprotectin*	20		10 - 50 µg/g
Lysozyme*	271		<= 600 ng/mL
White Blood Cells	None		None - Rare
Mucus	Neg		Neg

Joan's Case

BACTERIOLOGY CULTURE		
Expected/Beneficial flora	**Commensal (Imbalanced) flora**	**Dysbiotic flora**
3+ Bacteroides fragilis group	2+ Alpha hemolytic strep	
3+ Bifidobacterium spp.	4+ Gamma hemolytic strep	
4+ Escherichia coli	1+ Klebsiella oxytoca	
NG Lactobacillus spp.	1+ Pseudomonas aeruginosa	
NG Enterococcus spp.		
2+ Clostridium spp.		
NG = No Growth		

YEAST CULTURE	
Normal flora	**Dysbiotic flora**
1+ Candida parapsilosis	
1+ Rhodotorula mucilaginosa	

Joan's Case

Comprehensive Stool Analysis / Parasitology x3

PARASITOLOGY/MICROSCOPY*

Sample 1

Mod Blastocystis hominis
Rare Yeast

Sample 2

Few Blastocystis hominis
Rare Yeast

Sample 3

Many Blastocystis hominis

*A trichrome stain and concentrated iodine wet mount slide is read for each sample submitted.

PARASITOLOGY INFORMATION

Intestinal parasites are abnormal inhabitants of the gastrointestinal tract that have the potential to cause damage to their host. The presence of any parasite within the intestine generally confirms that the patient has acquired the organism through fecal-oral contamination. Damage to the host includes parasitic burden, migration, blockage and pressure. Immunologic inflammation, hypersensitivity reactions and cytotoxicity also play a large role in the morbidity of these diseases. The infective dose often relates to severity of the disease and repeat encounters can be additive.

There are two main classes of intestinal parasites, they include protozoa and helminths. The protozoa typically have two stages; the trophozoite stage that is the metabolically active, invasive stage and the cyst stage, which is the vegetative inactive form resistant to unfavorable environmental conditions outside the human host. Helminths are large, multicellular organisms. Like protozoa, helminths can be either free-living or parasitic in nature. In their adult form, helminths cannot multiply in humans.

In general, acute manifestations of parasitic infection may involve diarrhea with or without mucus and or blood, fever, nausea, or abdominal pain. However these symptoms do not always occur. Consequently, parasitic infections may not be diagnosed or eradicated. If left untreated, chronic parasitic infections can cause damage to the intestinal lining and can be an unsuspected cause of illness and fatigue. Chronic parasitic infections can also be associated with increased intestinal permeability, irritable bowel syndrome, irregular bowel movements, malabsorption, gastritis or indigestion, skin disorders, joint pain, allergic reactions, and decreased immune function.

In some instances, parasites may enter the circulation and travel to various organs causing severe organ diseases such as liver abscesses and cysticercosis. In addition, some larval migration can cause pneumonia and in rare cases hyper infection syndrome with large numbers of larvae being produced and found in every tissue of the body.

One negative parasitology x1 specimen does not rule out the possibility of parasitic disease, parasitology x3 is recommended. This exam is not designed to detect Cryptosporidium spp, Cyclospora cayetanensis or Microsproridia spp.

GIARDIA/CRYPTOSPORIDIUM IMMUNOASSAY

	Within	Outside	Reference Range
Giardia intestinalis	Neg		Neg
Cryptosporidium	Neg		Neg

Giardia intestinalis (lamblia) is a protozoan that infects the small intestine and is passed in stool and spread by the fecal-oral route. Waterborne transmission is the major source of giardiasis. **Cryptosporidium** is a coccidian protozoa that can be spread from direct person-to-person contact or waterborne transmission.

Summary of Joan's Stool Analysis Results

- **Potentially impaired digestive function**
  - Reduced Pancreatic Elastase
- **Evidence of dysbiosis based on**
  - Presence of opportunistic organisms
  - Presence of *Blastocystis hominis*
  - Reduced diversity in her microbiome
  - Reduced N-butyric acid
- **Possibility of increased intestinal permeability?**

FUNCTIONAL MEDICINE MATRIX

Retelling the Patient's Story

Antecedents

Mother SAD
Fm Hx IBS, Diverticulitis
Bottle @ 4 wk; Solid food @6mo
Hx OM Rx ABX
Tonsillectomy @ 4yo

Triggering Events

Parents divorced @7
Abdominal pain @10
Lactose Intolerant
2 kids @27&29 wt post part dep.
Divorced at 34yo (two teen boys)

Mediators/Perpetuators

SAD
Weight gain in college

Physiology and Function: Organizing the Patient's Clinical Imbalances

Assimilation

- Gas and Bloating
- Freq stools
- **Low pancreatic elastase**
- **Dysbiosis**
  - **Blastocystis**
  - **Borderline low SCFA**
  - **Low Butyrate**

Structural Integrity

Defense & Repair

- **SAD (inflammatory diet)**
- **DQ2 Positive**
- **+ IgG Serology: gluten, pork, rice, corn.**
- Blastocystis

Mental

Emotional

Spiritual

Stressful job
Family Dynamic?

Energy

- **Fatigue**
- **History of Depression**

Communication

- **Depression**
- **Stress (adrenal reserve)**

Transport

Biotransformation & Elimination

Modifiable Personal Lifestyle Factors

Sleep & Relaxation

Poor quality and quantity; has to be up to get the kids ready

Exercise & Movement

NONE; "no time"

Nutrition

SAD; quick meals due to being busy
Eats out often

Stress

Kids are a "handful"
Job is stressful as bank exec asst.

Relationships

Not dating and rarely has time to socialize

Name: ______________ Date: ______________ CC: ______________

THE INSTITUTE FOR FUNCTIONAL MEDICINE®

Applying Functional Medicine in Clinical Practice

Post AFMCP Training
Laboratory Testing and Interpretation Program

As part of the Applying Functional Medicine in Clinical Practice program (AFMCP), participants will receive a complimentary stool analysis lab test. IFM has partnered with Doctor's Data and Genova Diagnostics to offer participants this additional training opportunity. Please note: participants may only choose **one test kit**.

HOW TO PICK UP YOUR KIT:

1. Kit Certificates will be available for pick-up in the Practice Implementation Showroom during the following times:

Tuesday	Wednesday	Thursday
9:30-10:00 am	9:45–10:15 am	9:15–9:45 am
12:30–1:00 pm	1:00–1:30 pm	12:30–2:00 pm
2:15–3:15 pm	3:30–4:30 pm	3:15–4:15 pm

2. If you have any questions, both labs will have a representative onsite who will be happy to answer your questions prior to picking up your kit. IFM Staff at the registration desk are not able to answer questions about these tests.
3. These tests are not all available in New York State. Clinicians who are licensed to practice in the state of New York may not be able to participate in this program. Please confirm with the lab that you are able to run the test requested BEFORE taking a kit and submitting a specimen.
4. Specimens should NOT be collected during this event as company representatives will NOT carry specimens back to the labs. You should follow shipping instructions included in the kits (shipping is complimentary).

As this is an educational opportunity, participants can choose whom to use the test on, but **it may not be performed on the ordering clinician, their staff, or immediate family members**. The person selected will be referred to as "the patient." However, the test may NOT be used for diagnostic purposes, nor should they be marked up in price, or billed to any third party payer (insurance or Medicare). Test results will then be sent back to the ordering clinician. Filling out the requisition form and receiving lab results from each laboratory is slightly different:

Doctor's Data: Comprehensive Stool Analysis with Parasitology x1 (Stool)

- Once you have received your kit, fill out the entire requisition form (sections 1-4). If you already have an account with Doctor's Data, fill in your account information and the results will be sent to your office.
- For those clinicians who do not have an account with Doctor's Data, fill out the requisition form, complete an account application, and return it with the sample when shipping. You may also submit an application online at https://www.doctorsdata.com/apply. Results will be sent to your office.
- Contact DDI Customer Service at 800.323.2784 if you have any questions.

Genova Diagnostics: GI Effects Comprehensive Profile (Stool)

- **If you do not already have an account with Genova Diagnostics, one will need to be set up.**
- Please access the below website to request your kit:
  - https://www.gdx.net/promotion/ifm/create-account-gi-effects-charleston-032020
- If you have any questions regarding the test or results, you can call Genova Diagnostics at 800.522.4762.

PLEASE NOTE:

1. **You must include the provided form with the test box or the test cannot be performed.**
2. **Genova Diagnostics and Doctor's Data Inc. are licensed by CLIA, the Federal agency regulating laboratories. CLIA regulations requires practitioners to have a licensure scope which allows them to order laboratory testing. Acceptable licenses in all 50 states include MD, DO, NP, PA and APRN. Other practitioners may have the appropriate licensure scope within their particular state. Check with Genova or DDI to see if you qualify or have additional questions.**

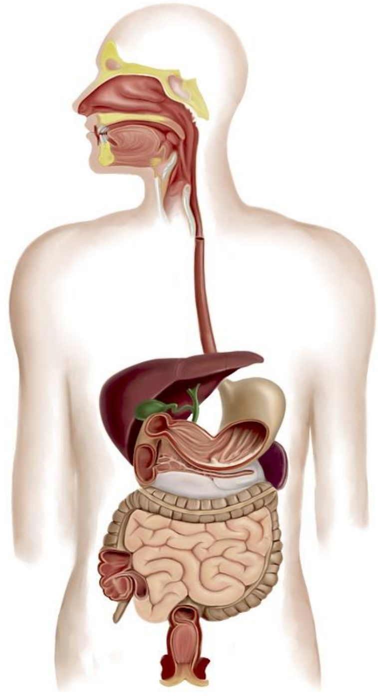
Summary

How would you treat "X"?

- **Obtain a comprehensive history**
  - Look carefully at the time frame surrounding the question:
  - "When was the last time you were truly well?"
    - **Look for clues about ATMs**
- **Do a comprehensive physical exam**
  - Include a nutritional physical exam
    - **Look for clues about ATMs**
- **Create a detailed timeline**
- **Consider additional tests to rule in/out current diagnosis the patient carries**
- **Populate a Matrix**
  - Look for areas that you can apply leverage on the matrix

A Healthy Gut

- Proper nutritional substrates, micronutrients, and phytonutrients for:
  - maintenance of commensal flora
  - immune modulation
  - repair and regeneration
- Proper mastication
- Adequate digestive juices, enzymes, and pH
- Intact intestinal epithelial barrier function
- Balanced host-bacteria ecology
- Autonomic balance



An Imbalanced (Sick) Gut

- Poor diet
- Dehydration
- Interaction of medications
- Infections
- Toxins (metals, molds, foods)
- Inadequate digestive enzymes & stomach acid
- Imbalanced ecology
- Impaired intestinal permeability
- Altered neuroendocrine balance and autonomic function

Gut Function: A Simplified Approach

Immune Function

"Defense & Repair"

Endocrine Function

"Communication"

Nutrition & Digestion

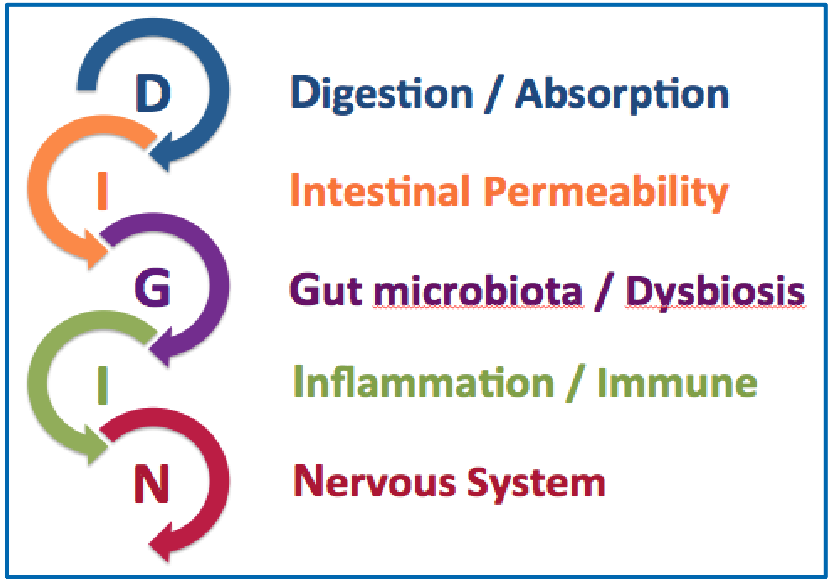
"In"

Detoxification & Elimination

"Out"

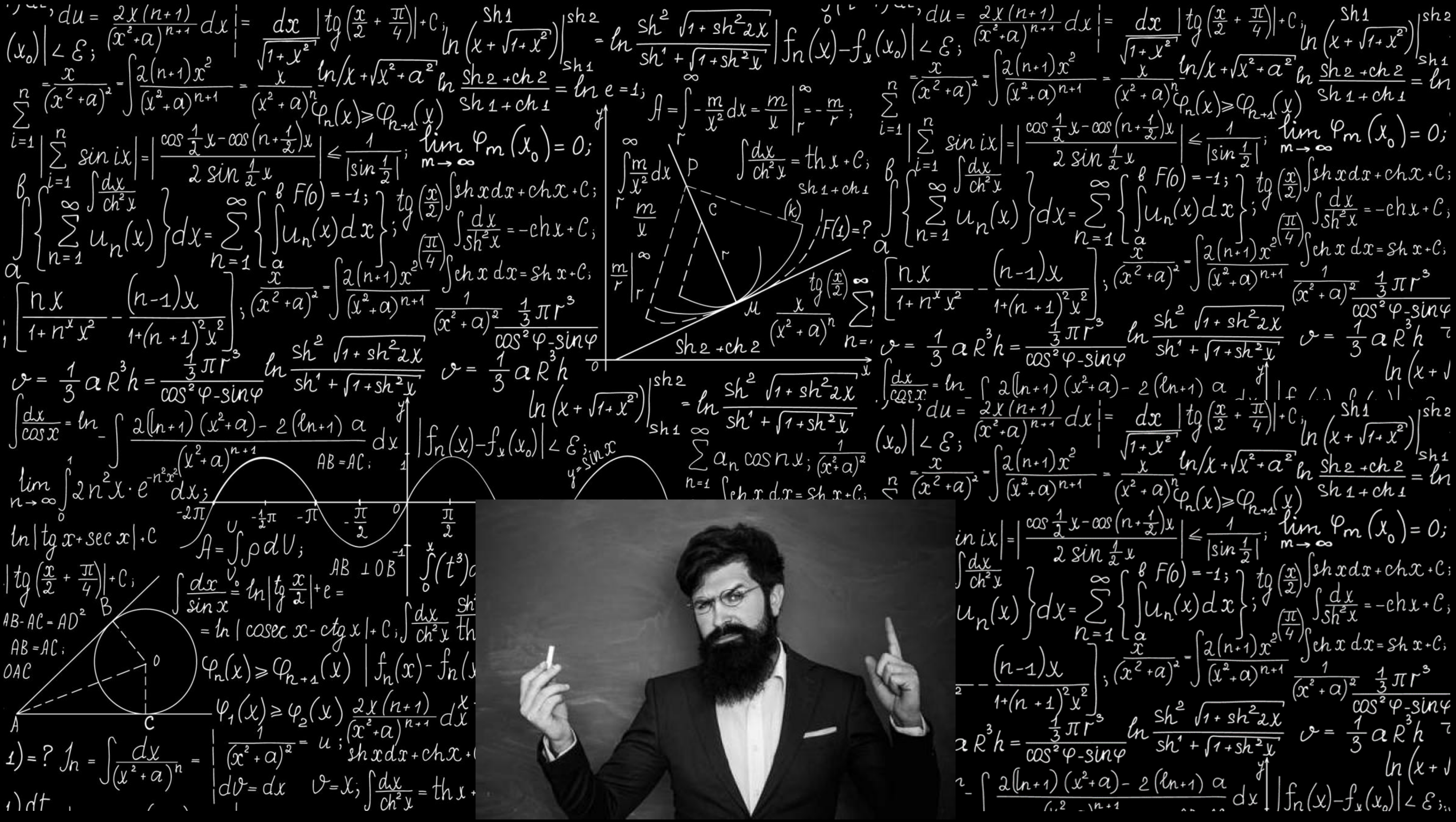
DIGIN: Clinical Takeaways

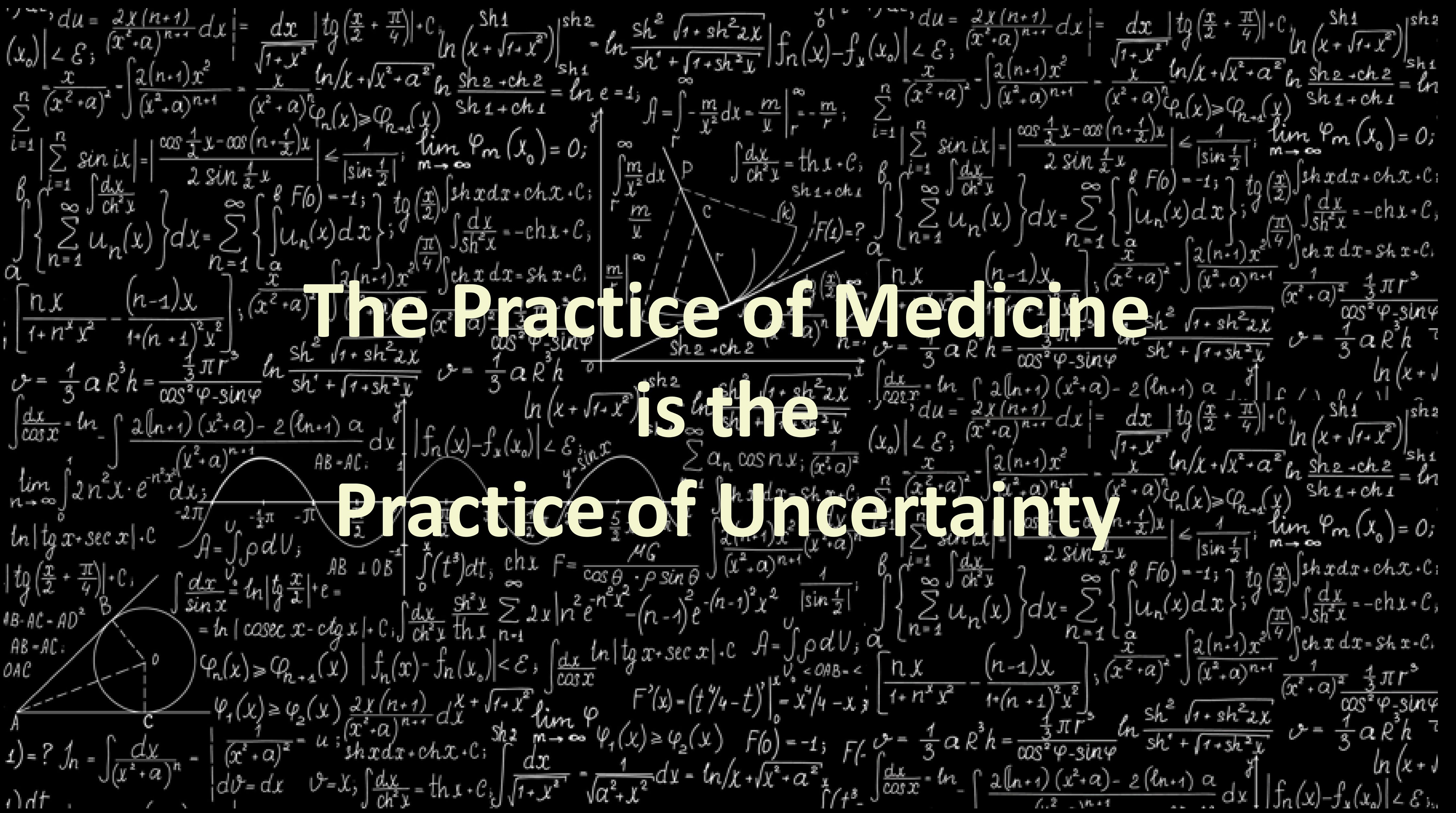
- The GI system is an integral and central "node" of the complex web of functional medicine.
- Dysregulation of the GI system can have a profound impact on health.
- Our patients are best served if we observe the inter-relationships between:
  - ✓Digestion & Absorption
  - ✓Intestinal Permeability
  - ✓Gastrointestinal Microbiota (all types)
  - ✓Immune Modulation & Inflammation
  - ✓Nervous System



All Disease Begins in the Gut

Hippocrates, 460 BCE -375 BCE





The Practice of Medicine is the Practice of Uncertainty