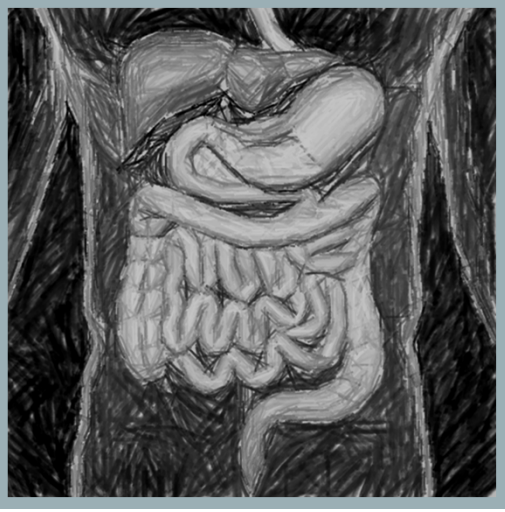
OVERVIEW OF THE DIGESTIVE, SENSING, BARRIER, AND IMMUNE FUNCTIONS OF THE GUT



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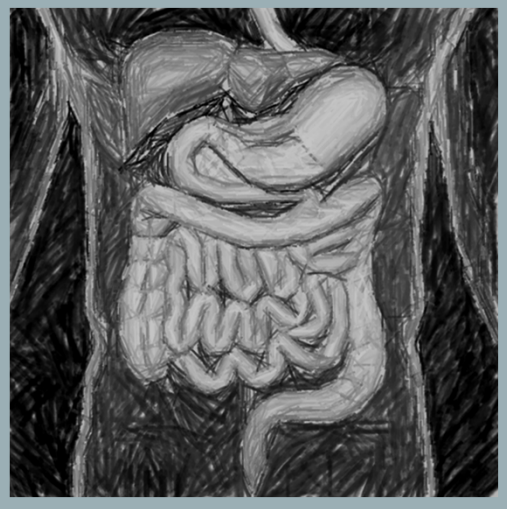
ADJ. Asst. Prof. U-Wisconsin School of Pharmacy

Clinical Instructor- George Washington University

Founder/Director- The Point Institute



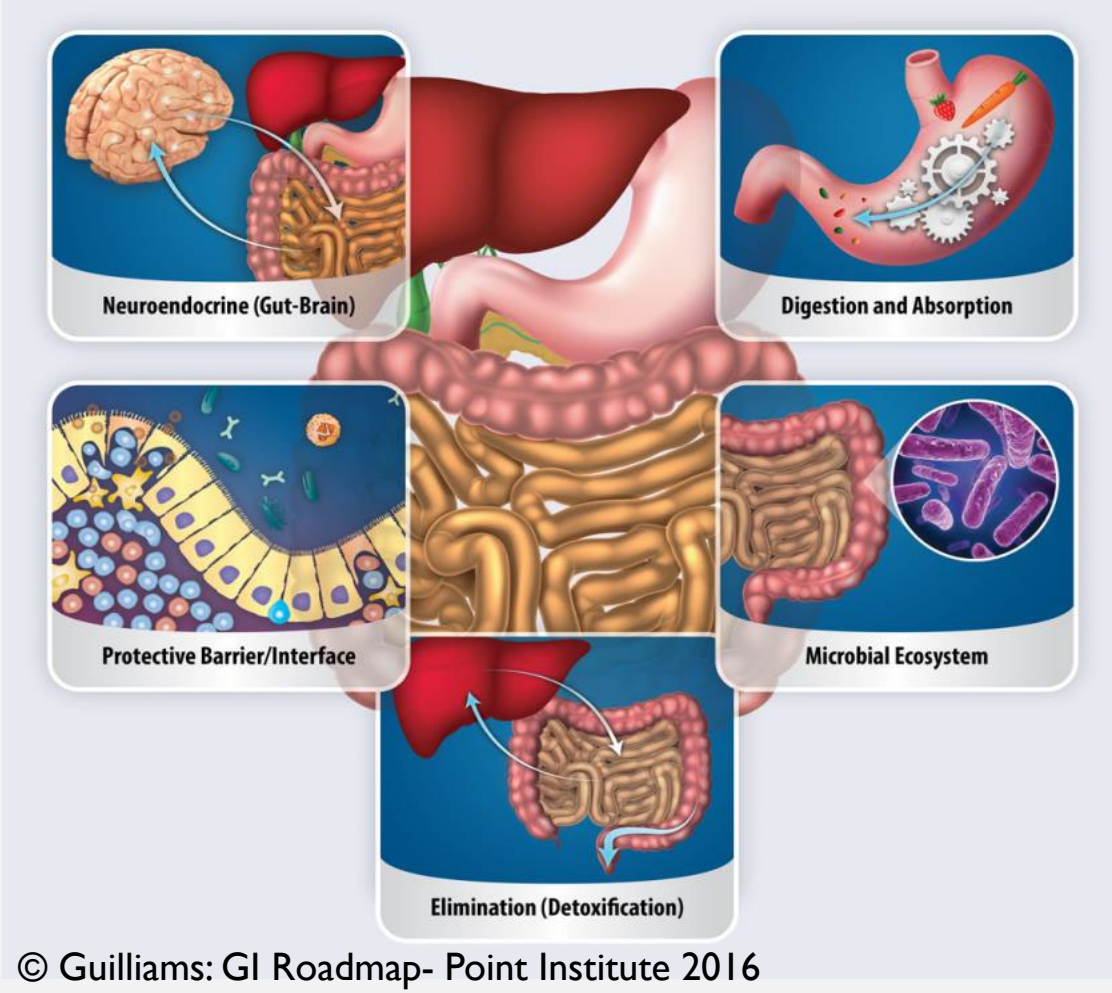
DISCLOSURE OF FINANCIAL RELATIONSHIPS

- Ortho Molecular Products
  - Consulting Fees
- Genova
  - Speaker Fees
- Off-Label Usage
  - None

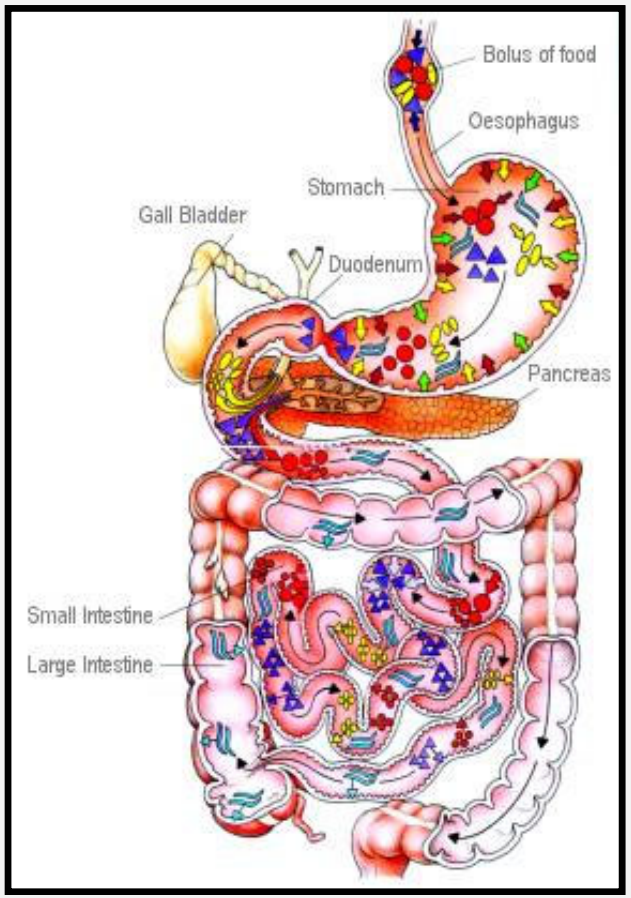
LEARNING OBJECTIVES

- Understand the nomenclature and framework of GI functions as a foundation to an integrative approach to successful therapies.
- Appreciate the inter-relationship between the various GI functions and how those functions support (or create vulnerabilities) for one another.
- Review simple methods of evaluating basic digestive, barrier and microbial environmental function of the GI tract
- Understand the importance of the GI immune system and the necessary education (tolerance) it provided for the whole immune system

ANOTHER WAY TO DESCRIBE THE CORE FUNCTIONS OF GI

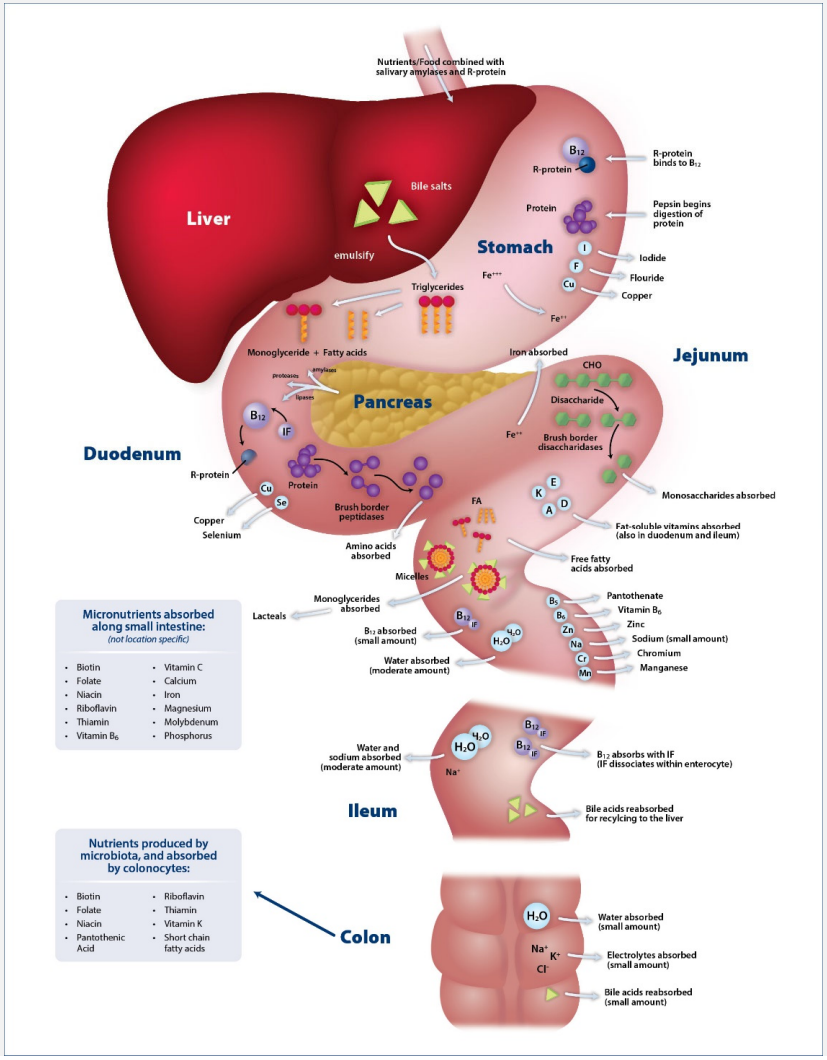


THE CHALLENGE OF DIGESTION & ABSORPTION



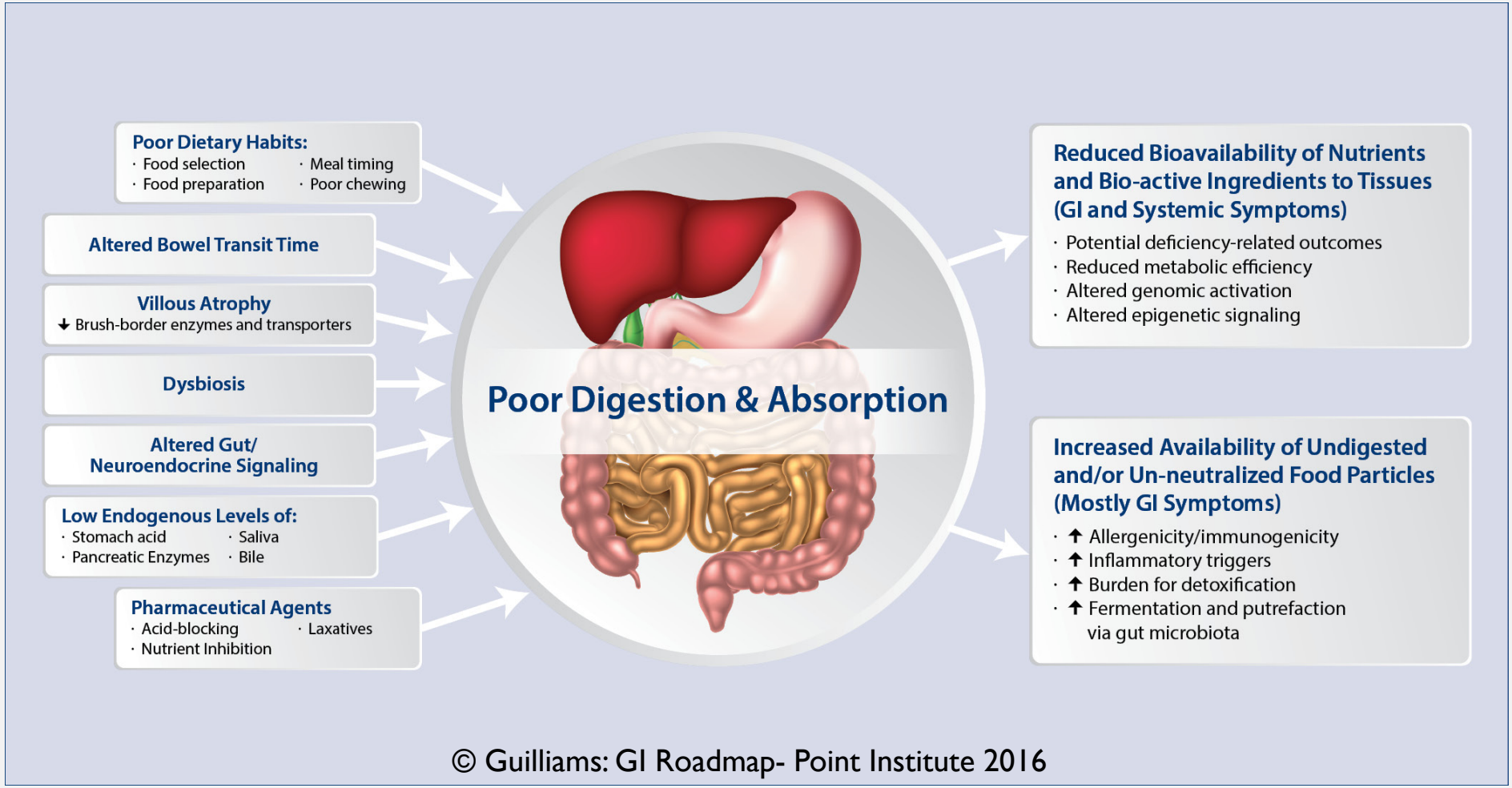
- Breakdown complex foods into basic constituents
- Divide Macronutrients into basic units
- Release Micronutrients from food matrix
- Selectively absorb nutrients
- Transform and/or activate nutrients and phytonutrients
- While maintaining a barrier against entry of unwanted particles

TIMING AND LOCATION IS IMPORTANT

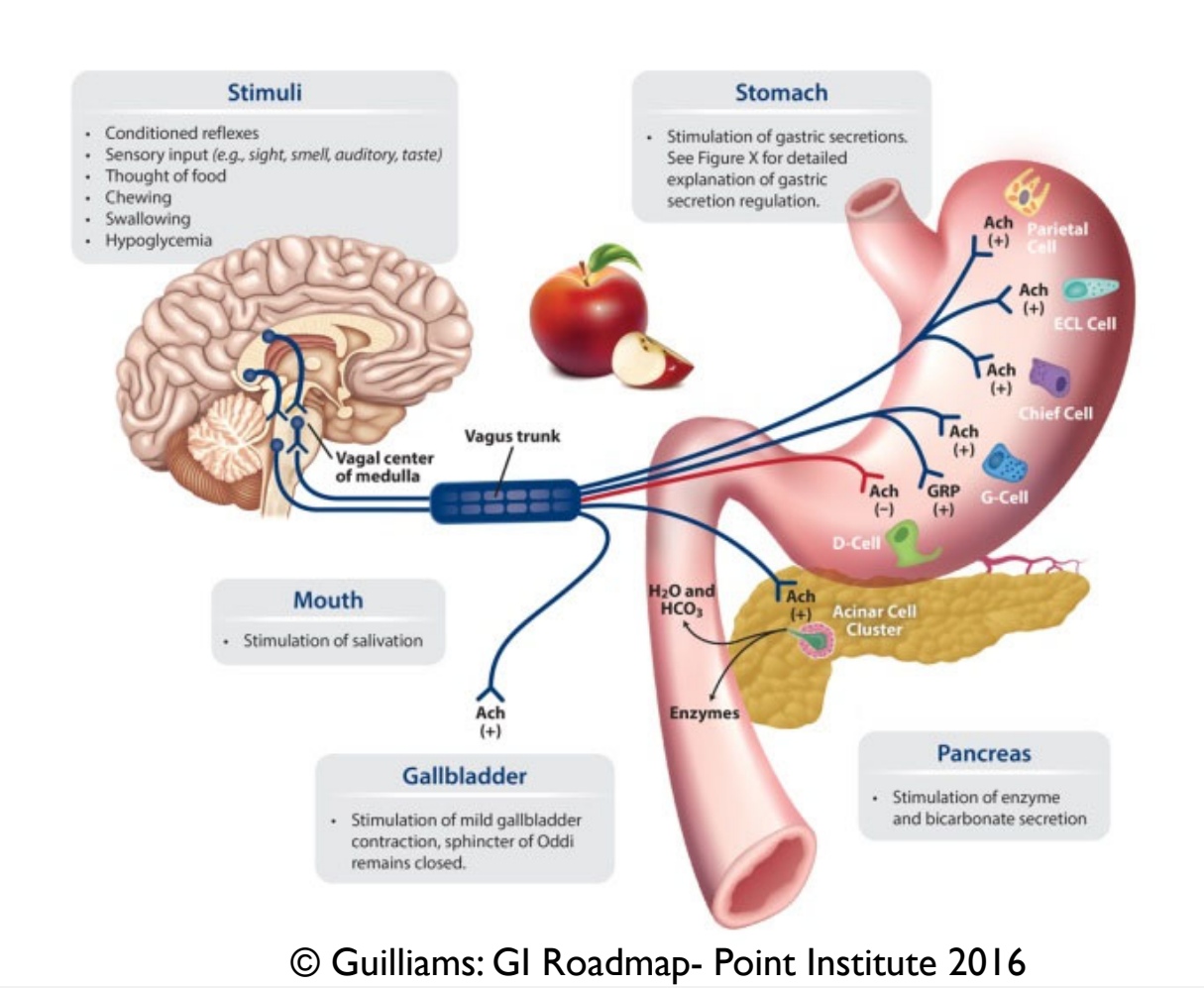


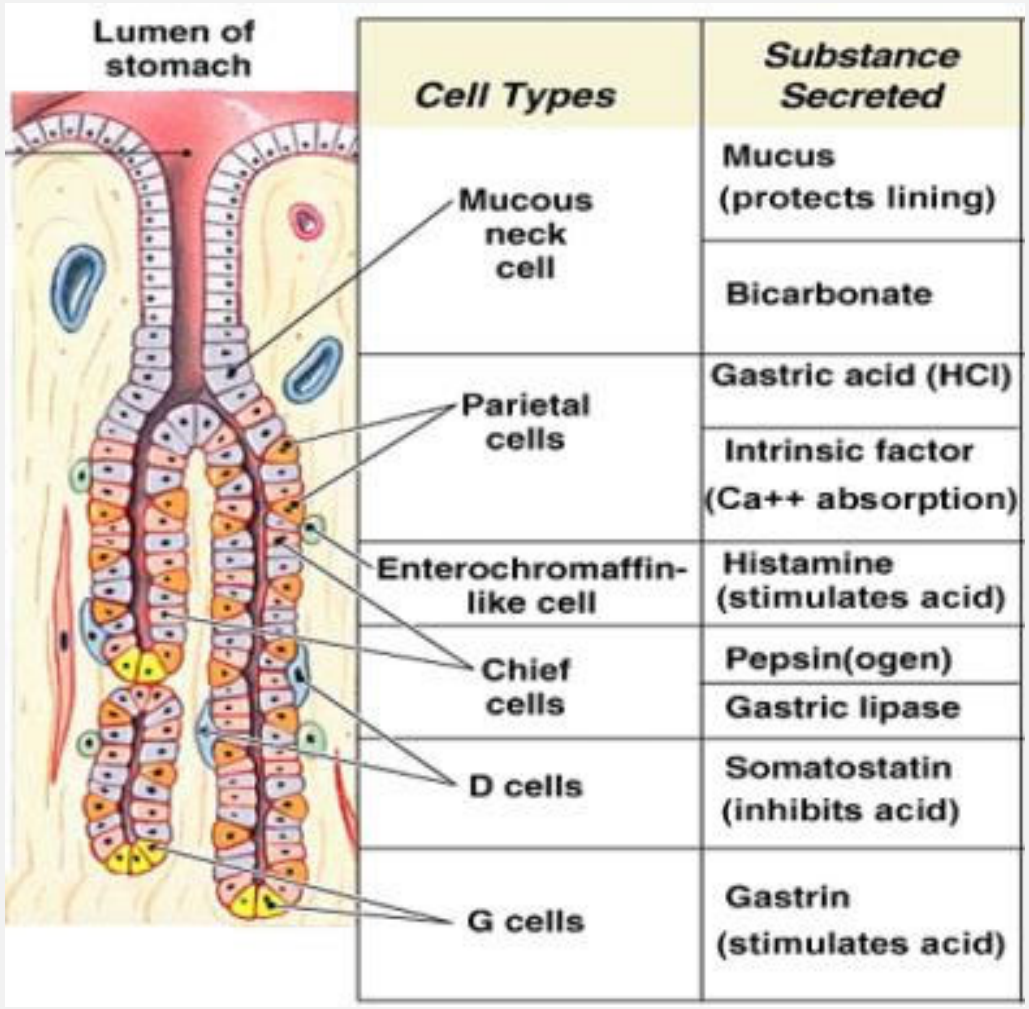
- Nutrients are not absorbed uniformly, there are specific locations where the available transporters or necessary processes are found.
- Appropriate timing and sequence is also important- bowel transit time can adversely affect this
- Transporters and enzyme capacity can be overwhelmed/saturated, reducing the effective benefit of dietary nutrients
- Nutrients produced by colonic bacteria may have limited human bioavailability, though may benefit colonocytes and microbes.

DIGESTION AND ABSORPTION
WHAT CAN GO WRONG?

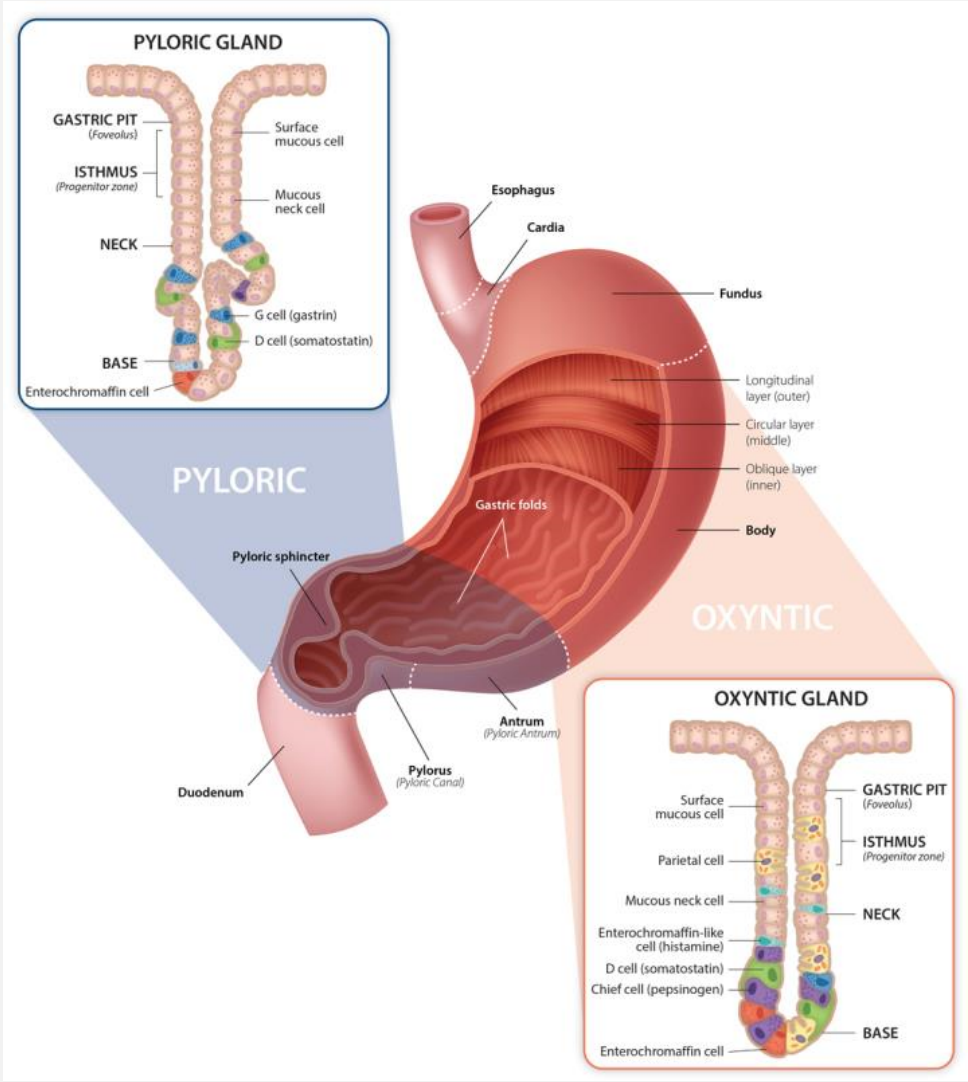


DIGESTION AND ABSORPTION
THE CEPHALIC PHASE





Cell Types	Substance Secreted
Mucous neck cell	Mucus (protects lining)
	Bicarbonate
Parietal cells	Gastric acid (HCl)
	Intrinsic factor (Ca++ absorption)
Enterochromaffin-like cell	Histamine (stimulates acid)
Chief cells	Pepsin(ogen)
	Gastric lipase
D cells	Somatostatin (inhibits acid)
G cells	Gastrin (stimulates acid)



ENTEROENDOCRINE CELLS (EEC)

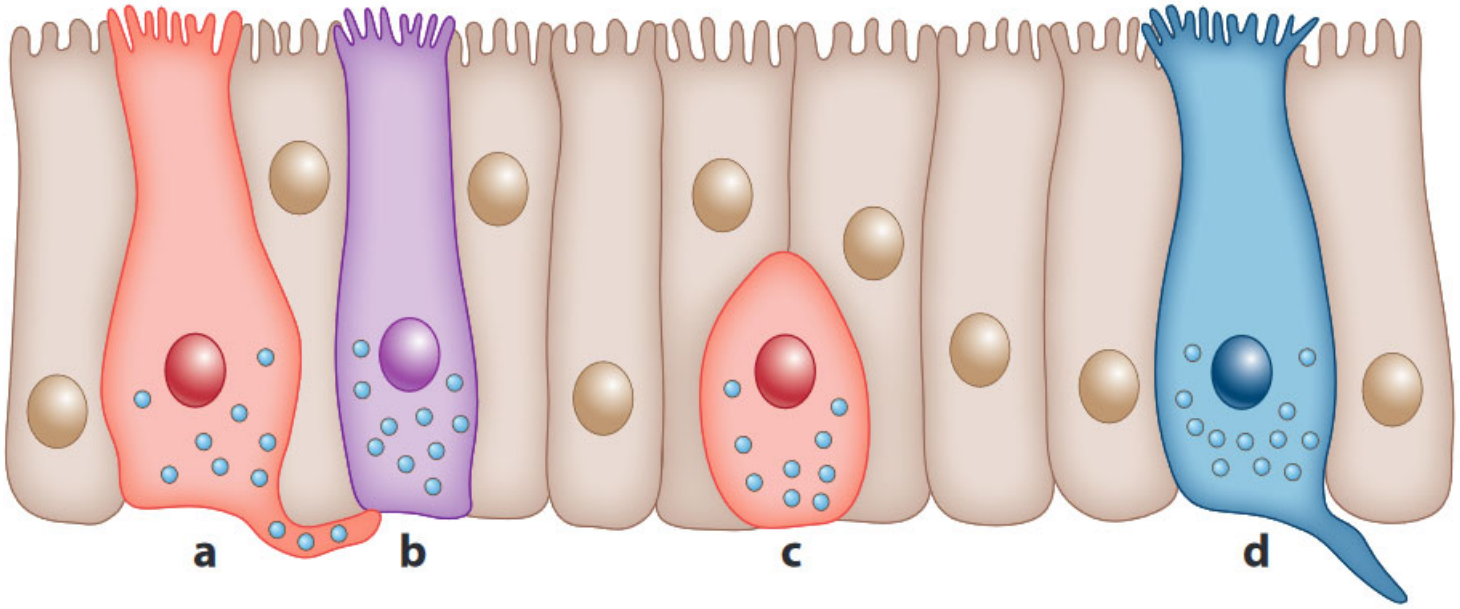
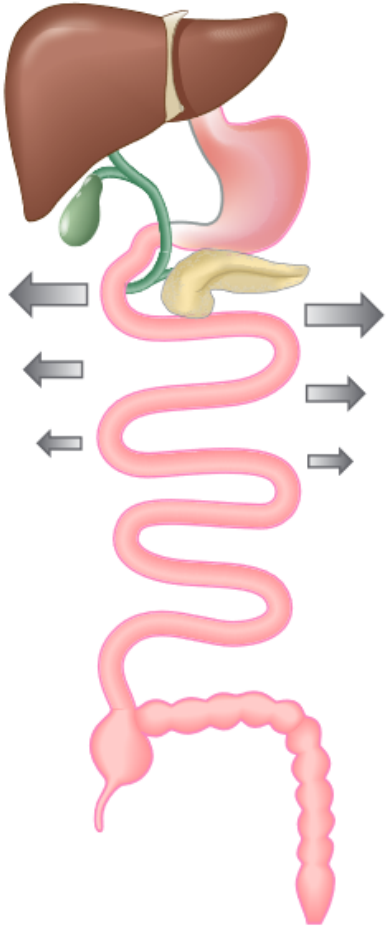


Figure 1

Gut epithelium showing different representative enteroendocrine cell (EEC) types. (*a*) Gastric somatostatin-producing D-cell with basolateral process that communicates with (*b*) a neighboring gastrin-producing G-cell. (*c*) Closed-type EEC and (*d*) small intestinal/colonic-type open EEC with neuropod basolateral extension.

Gribble FM, Reimann F. Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. *Annu Rev Physiol*. 2016;78:277-299. doi:10.1146/annurev-physiol-021115-105439

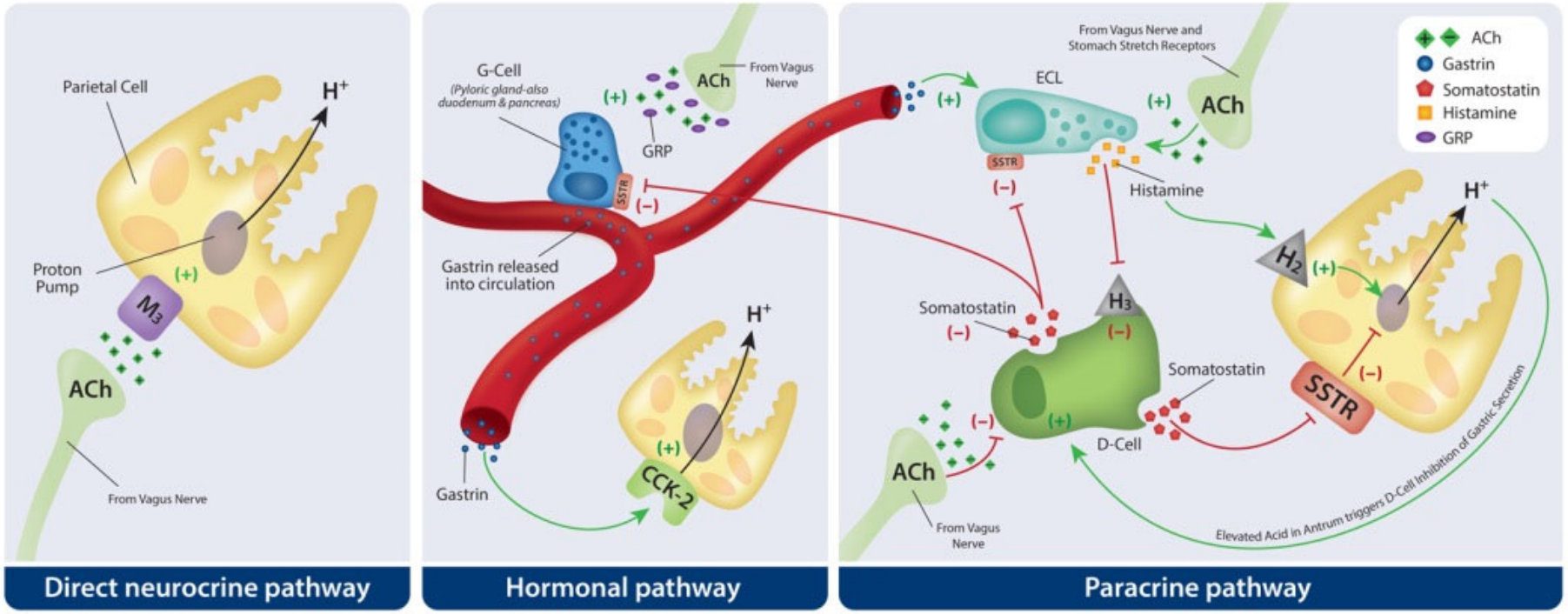
Table 1 Key hormones, possible secretory stimuli, and physiological processes occurring along the gut axis



Gut region	Intestinal processes	Luminal stimuli of EECs	Principal gut hormones
Stomach	Acid secretion Mechanical disruption	Acid Digested protein	SST, histamine, 5-HT, ghrelin, gastrin
Duodenum Jejunum Proximal ileum	Release of bile acids, pancreatic and intestinal enzymes, bicarbonate Digestion Absorption (➡)	Monosaccharides Free fatty acids Monoacylglycerols Amino acids Di/tripeptides Bile acids	Duodenum: GIP, ghrelin, CCK, 5-HT, SST Jejunum, ileum: GLP-1, GLP-2, PYY, 5-HT, Nts
Terminal ileum	Bile acid reabsorption	Bile acids Unabsorbed nutrients	GLP-1, GLP-2, PYY, Nts, 5-HT
Colon Rectum	Bacterial metabolism	Short-chain fatty acids Indole Secondary bile acids	GLP-1, GLP-2, PYY, Nts, Insl5, 5-HT

The details are based on mouse data and are discussed in the text. Abbreviations: 5-HT, 5-hydroxy-tryptamine (serotonin); CCK, cholecystokinin; EECs, enteroendocrine cells; GIP, glucose-dependent insulinotropic polypeptide; GLP-1 and GLP-2, glucagon-like peptides 1 and 2; Insl5, insulin-like peptide 5; Nts, neurotensin; PYY, peptide YY; SST, somatostatin.

CONTROLLING ACID PRODUCTION

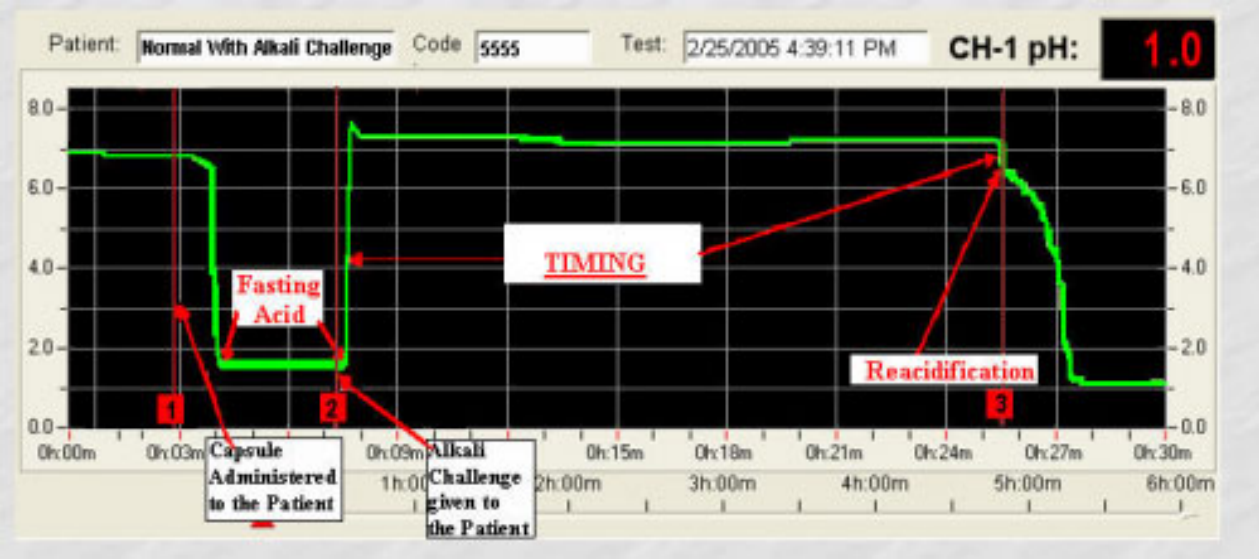


HOW MUCH ACID DO WE NEED?

- Low Stomach acid contributes to:
  - Reduced protein digestion (denaturing)
  - Increases protein allergenicity
  - Reduced solubility/absorption of key nutrients like calcium, iron, folic acid, vitamins B6 and B12
  - Increases SIBO, C. diff and related harmful bacteria
- But how low is too low?
  - This is a very debatable question and one that is not well studied…..

MEASURING STOMACH ACID:

- Heidelberg radio-telemetric capsule
  - Best used for alkali challenge/reacidification

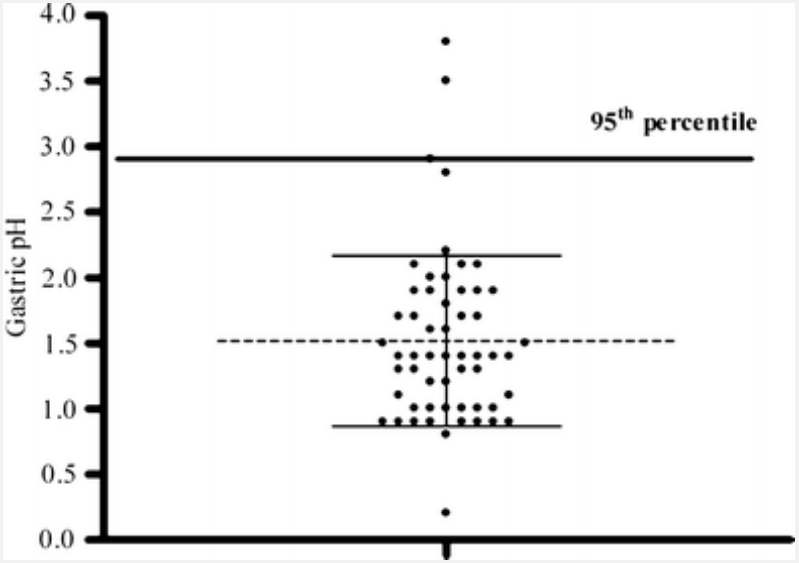


Some research also uses pH sensitive tablets and follow collection of metabolites in urine (i.e. riboflavin).

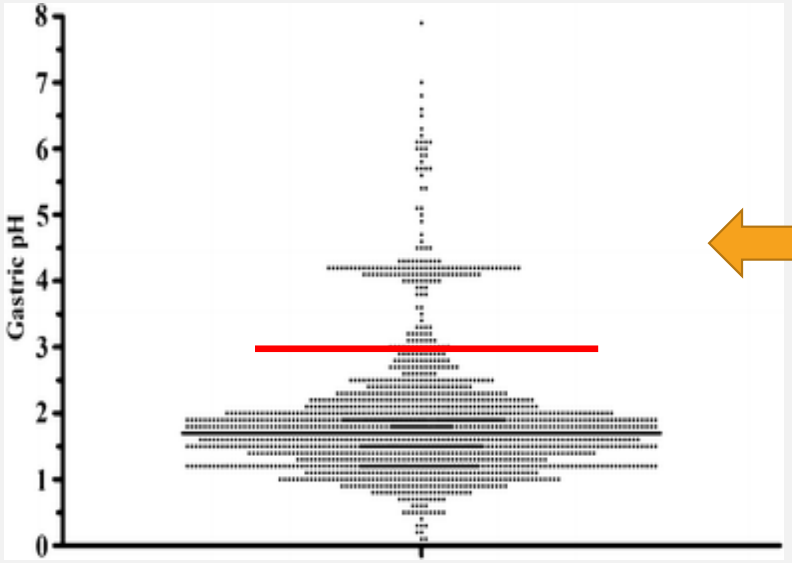
HYPOCHLORHYDRIA AND ACHLORHYDRIA

- It is generally assumed that **fasting pH** below 3.0 is considered "normal"
- Achlorhydria (no stomach acid) results in fasting pH of about 7 (neutral) or above.
- This is common in subjects with atrophic gastritis.
- Fasting Hypochlorhydria is considered to be present in about 10% of the aging American population, though upwards of 60% in older Japanese adults.
- Low stomach acid is not uncommon in patients with GERD-like symptoms

GERD IS NOT ASSOCIATED WITH EXCESSIVE ACID PRODUCTION!



Control Subjects (N=54)



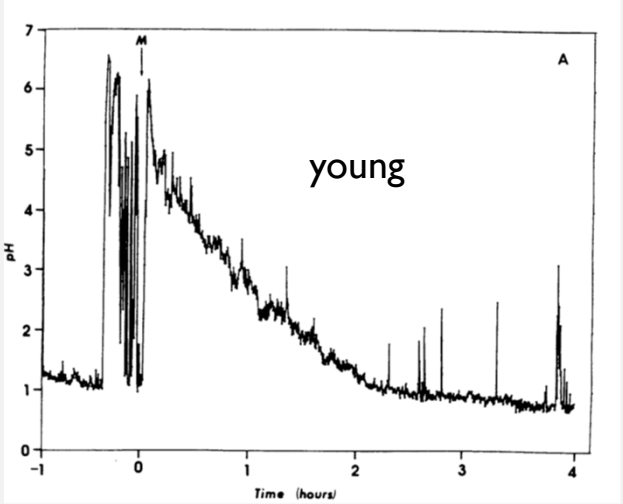
11% Hypo-HCl

Subjects with GERD (N=1,582)

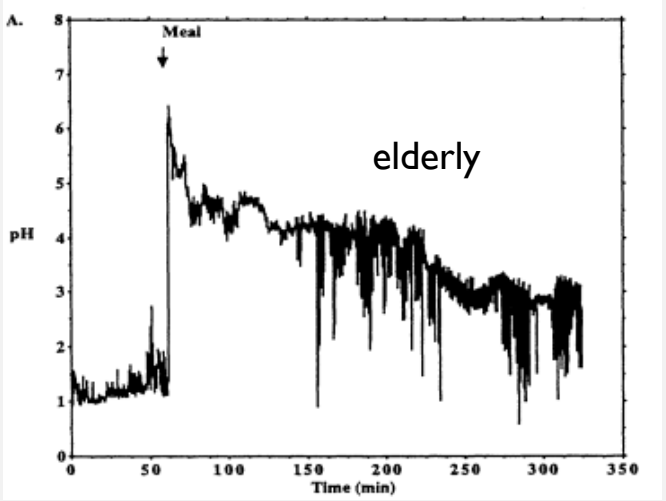
Measurement of gastric pH in ambulatory esophageal pH monitoring. Surg Endosc. 2009 Sep;23(9):1968-73

FUNCTIONAL HYPOCHLORHYDRIA

- Our body secretes acid primarily to digest food, therefore, fasting levels are not nearly as important as prandial levels (when you eat)- measured by re-acidification after eating food.
- If fasting gastric acid production goes down with aging, what happens to gastric pH during a meal?

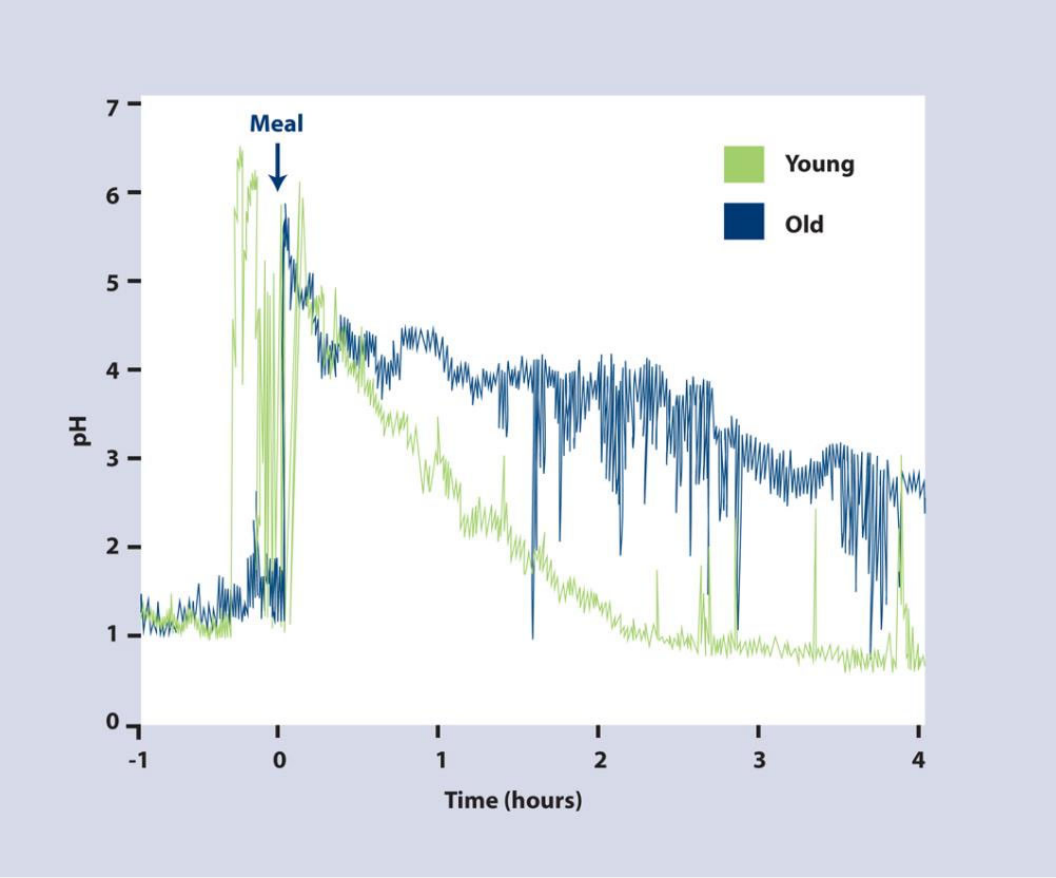


Pharm Res. 1990 Jul;7(7):756-61.



Pharm Res. 1993 Feb;10(2):187-96.

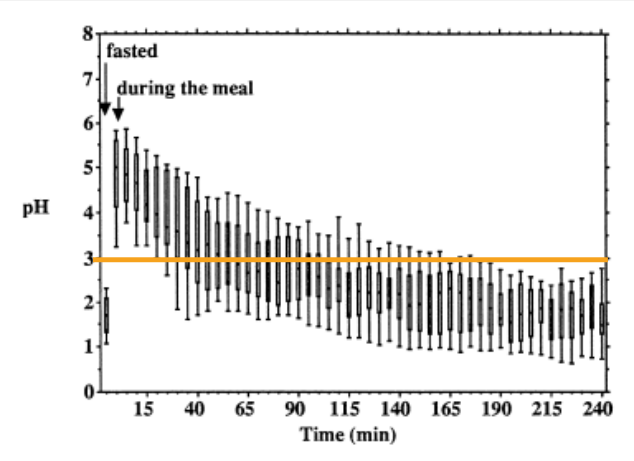
DOES THIS REPRESENT A FUNCTIONAL DIFFERENCE?

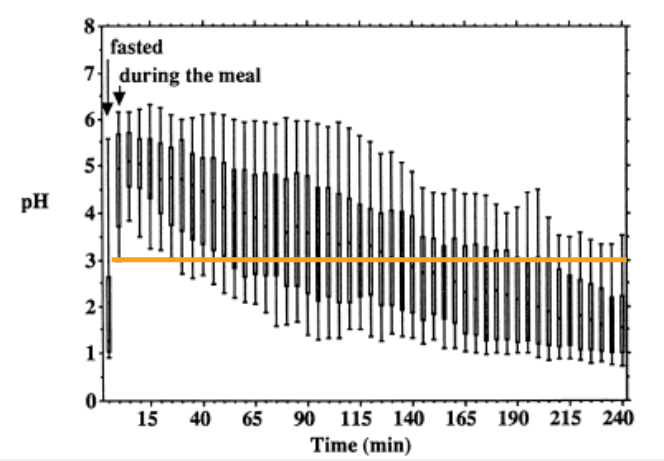


OLDER SUBJECT TAKE LONGER TO ACIDIFY STOMACH AFTER A MEAL!

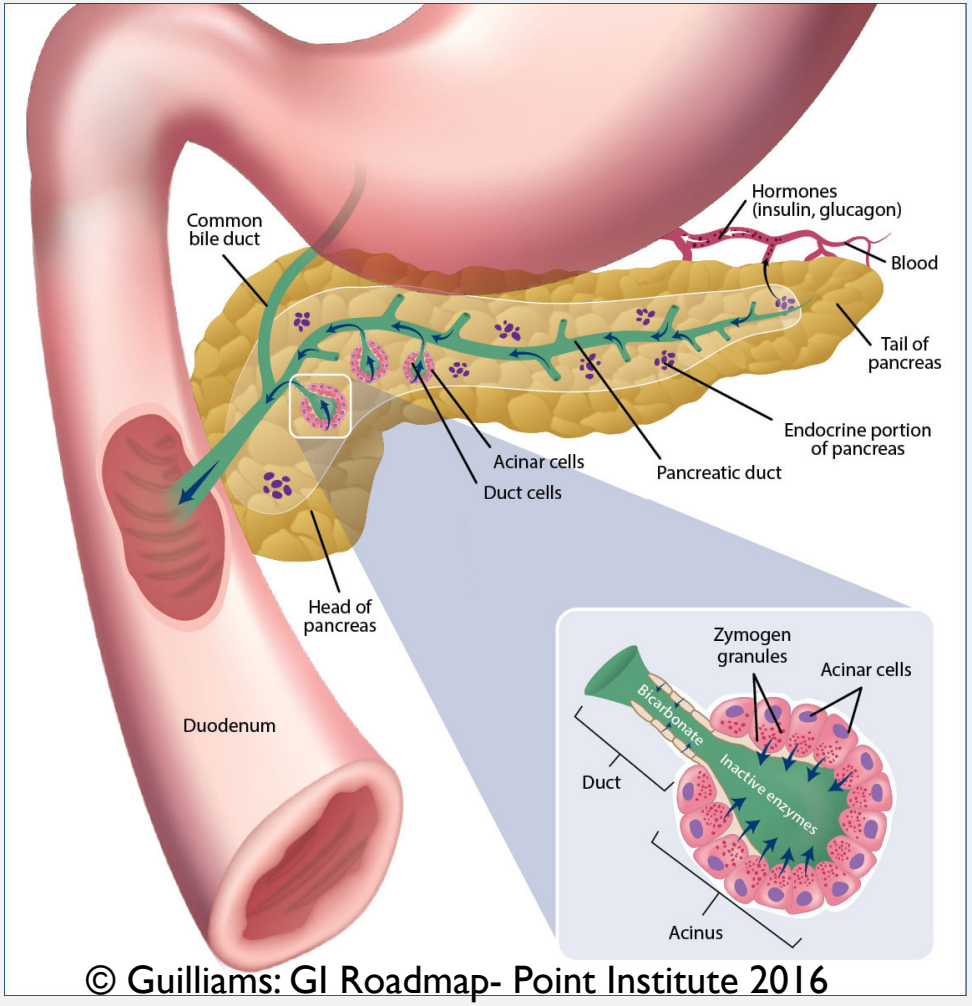
Table I. Comparison of Gastric pH Between Young and Elderly Subjects

Treatment phase	Young (N = 24)[a]	Elderly (N = 79)[b]	P value[c]
Fasted			
Median pH[d]	1.7 (1.4–2.0)	1.3 (1.1–1.6)	0.014
AUC (pH * hr)	2.0 (1.6–2.4)	1.4 (1.2–1.9)	
	(N = 24)	(N = 75)	0.006
During the meal			
Median pH	5.0 (4.4–5.6)	4.9 (3.9–5.5)	0.74
Peak pH	6.6 (6.3–7.0)	6.2 (5.8–6.7)	0.02
Postprandial			
Time to return to pH 5 (min)	8 (2–17)	23 (6–46)	0.015
Time to return to pH 4 (min)	14 (8–40)	52 (27–115)	0.0002
Time to return to pH 3 (min)	42 (26–83)	89 (44–167)	0.0026
Time to return to pH 2 (min)	100 (44–143)	154 (82–210)	0.026
AUC (pH * 4 hr)	10.8 (8.1–12.2)	12.3 (8.6–15.3)	
	(N = 24)	(N = 78)	0.0001





THE PANCREAS: MULTIPURPOSE GLAND



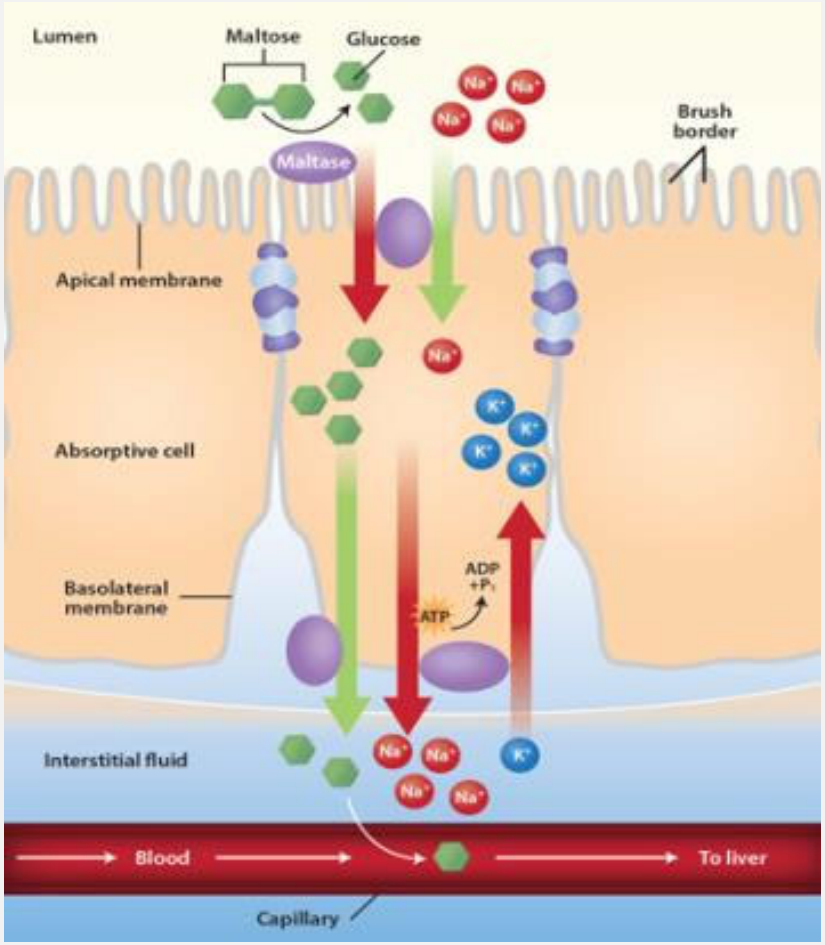
trypsin, chymotrypsin, elastase, pancreatic lipase, pancreatic amylase

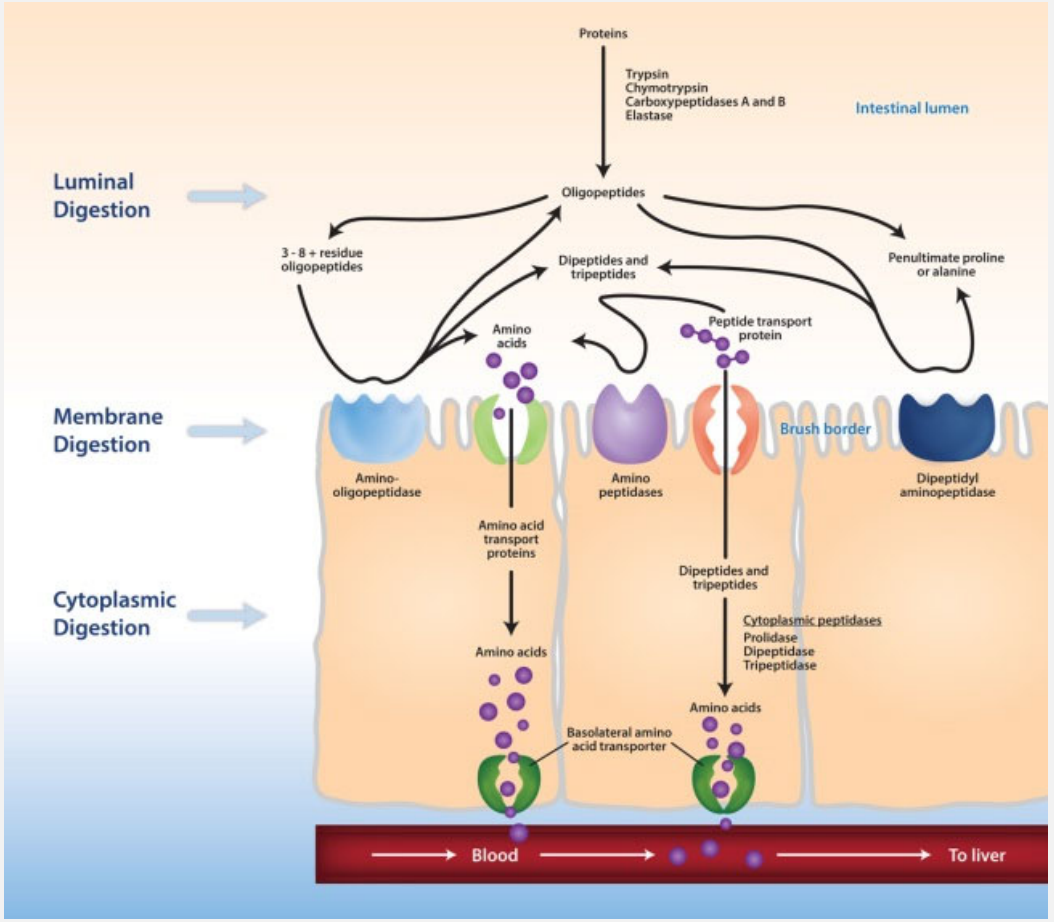
Enzymes of the Human Exocrine Pancreas

Enzyme	Proenzyme	Activator	Action
Trypsin	Trypsinogen	Enteropeptidase	Cleaves internal peptide bonds
Chymotrypsin	Chymotrypsinogen	Trypsin	Cleaves internal peptide bonds
Elastase	Proelastase	Trypsin	Cleaves internal peptide bonds
Carboxypeptidase	Procarboxypeptidase	Trypsin	Cleaves last amino acid from carboxyl-terminal end of polypeptide
Phospholipase	Prophospholipase	Trypsin	Cleaves fatty acids from phospholipids such as lecithin
Lipase	None	None	Cleaves fatty acids from glycerol
Amylase	None	None	Digests starch to maltose and short chains of glucose molecules
Cholesterolesterase	None	None	Releases cholesterol from its bonds with other molecules
Ribonuclease	None	None	Cleaves RNA to form short chains
Deoxyribonuclease	None	None	Cleaves DNA to form short chains

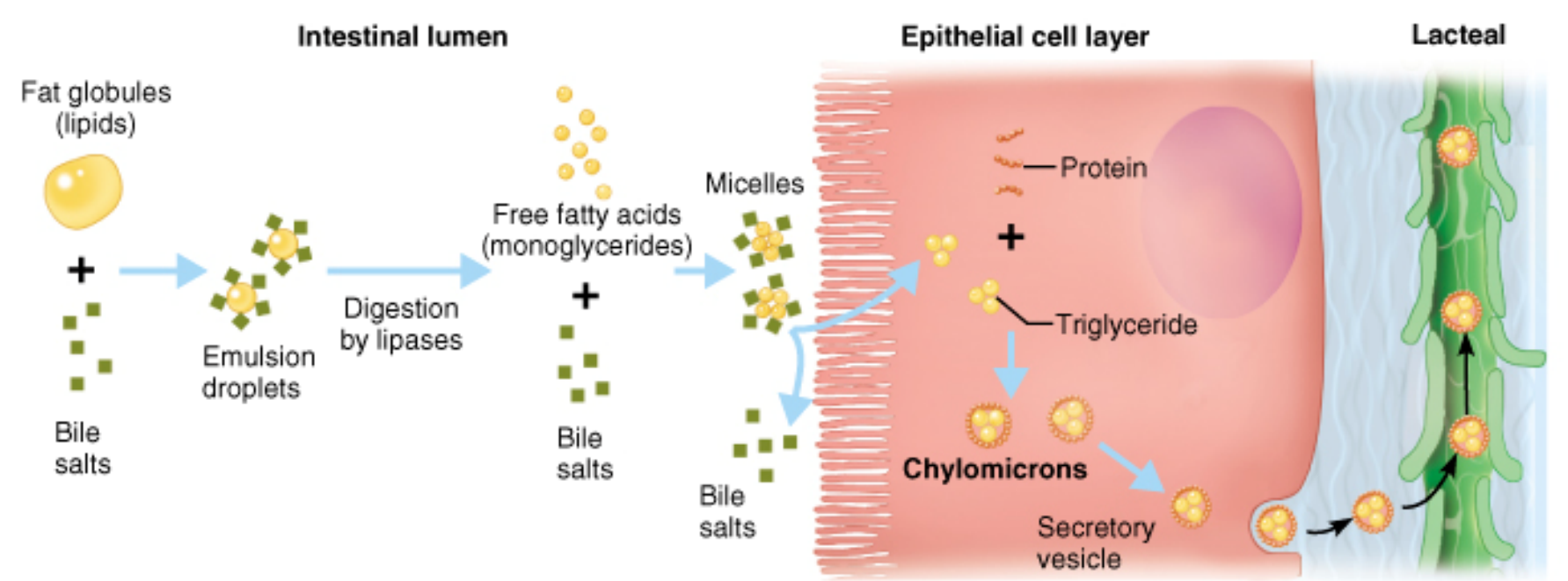
- Note the importance of trypsin in cleaving several precursor enzymes (zymogens) into their final active form
- Note also that trypsinogen is first cleaved by enteropeptidase (enterokinase)- an enzyme located on the surface of duodenal cells- requiring an intact brush border system

IMPORTANCE OF BRUSH BORDER ENZYMES





LIPID TRANSPORT INTO CIRCULATION



LOW PANCREATIC ENZYME OUTPUT

- Pancreatic Exocrine Insufficiency: defined as output below 10% of normal (i.e. 90% reduction).
  - Common in chronic pancreatitis, cystic fibrosis, pancreatic cancer
  - Also caused by: gastrectomy, gastric bypass or GI tract disorders like celiac disease…and aging!
- Measured by:
  - Fecal Fat Analysis
  - Radiolabeled TG test
  - Or Pancreatic Elastase 1 (a.k.a. fecal elastase)

Labs can measure this

1. Digestive Markers

		Result	Suspect	Consider
Analyte, Related Profiles	**Chymotrypsin** • CDSA/P • CDSA • Digestive Function • Optional add-on with › CDSA 2.0 › CDSA 2.0 without Parasitology	Low < 0.9 mcg/g	• Pancreatic insufficiency or hypochlorhdyria • Other factors include slow transit time	• Assess putrefactive SCFAs • Therapeutic Interventions: › Pancreatic enzyme supplementation and/or betaine HCL › Dietary fiber (insoluble) to improve transit time
		Normal 0.9-26.8 mcg/g 1 SD = 2.1-13.7	Adequate exocrine pancreatic function	1-2 SD = Results from 1-2 SD (yellow range) warrant clinical correlation even though within the "normal" reference range.
		Elevated > 26.8 mcg/g		Rule out false elevations from diarrhea (assess pancreatic elastase 1 levels) • Further Testing: › Comprehensive Parasitology Profile › Bacterial Overgrowth of the Small Intestine › Lactose Intolerance › Food Antibody Assessment › Celiac Testing
Analyte, Related Profiles	**Pancreatic Elastase 1 (PE1)** • CDSA 2.0 • CDSA 2.0 without Parasitology • Optional add-on with › CDSA/P › CDSA › Digestive Function stand alone test	Low 100-200 mcg/g	Mild to moderate pancreatic insufficiency	• Further Testing › Intestinal Permeability Assessment › Comprehensive Parasitology Profile › Celiac Testing • Therapeutic Intervention › Pancreatic enzyme supplementation
		Very Low < 100 mcg/g	Moderate to severe pancreatic insufficiency	• Further Testing › Bone Resorption Assessment › Glucose/Insulin Analysis* › Celiac Testing › Bacterial Overgrowth of the Small Intestine • Therapeutic Interventions › Pancreatic enzyme supplementation › Vitamin and mineral supplementation
		Normal > 200 mcg/g	Adequate exocrine pancreatic function	No further action necessary. Pancreatic supplementation may be of benefit in low normal (< 400 mcg/g) range

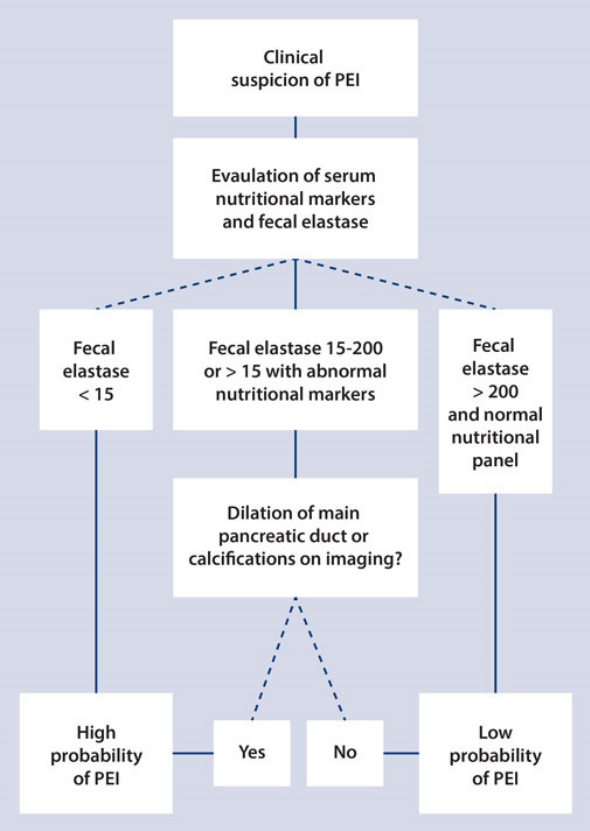
Note <400 Borderline Low

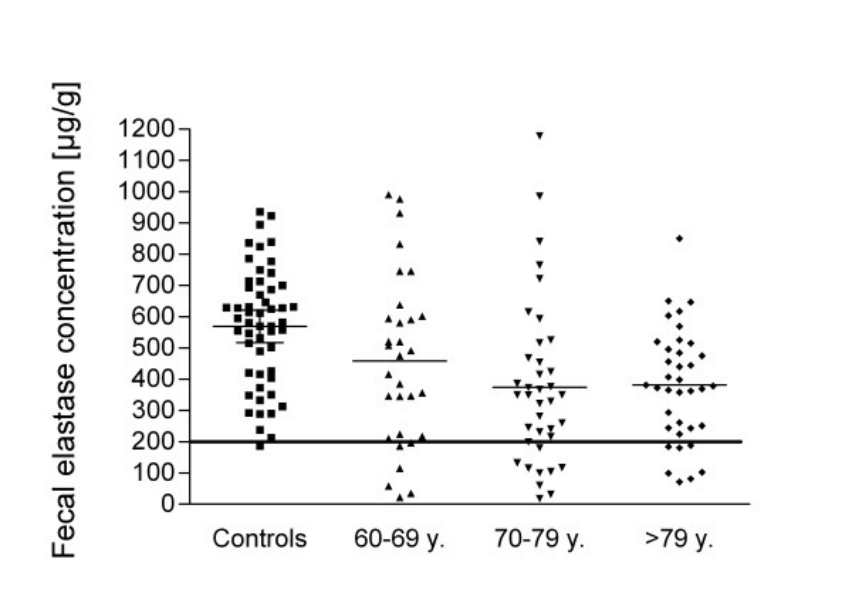
WHO ELSE MIGHT HAVE LOW PEI?

- Celiac disease
- Inflammatory Bowel Disease (IBD)
- HIV
- Diabetes (type 1 and 2)
- Obesity

- Leeds JS, Hopper AD, Hurlstone DP, et al. Is exocrine pancreatic insufficiency in adult coeliac disease a cause of persisting symptoms? *Aliment Pharmacol Ther*. 2007 Feb 1;25(3):265-71.
- Maconi G, Dominici R, Molteni M, et al. Prevalence of pancreatic insufficiency in inflammatory bowel diseases. Assessment by fecal elastase-1. *Dig Dis Sci*. 2008 Jan;53(1):262-70.
- Carroccio A, Di Prima L, Di Grigoli C, et al. Exocrine pancreatic function and fat malabsorption in human immunodeficiency virus-infected patients. *Scand J Gastroenterol*. 1999 Jul;34(7):729-34.
- Hardt PD, Hauenschild A, Nalop J, et al. High prevalence of exocrine pancreatic insufficiency in diabetes mellitus. A multicenter study screening fecal elastase 1 concentrations in 1,021 diabetic patients. *Pancreatology*. 2003;3(5):395-402.
- Griesche-Philippi J, Otto J, Schwörer H, et al. Exocrine pancreatic function in patients with end-stage renal disease. *Clin Nephrol*. 2010 Dec;74(6):457-64.

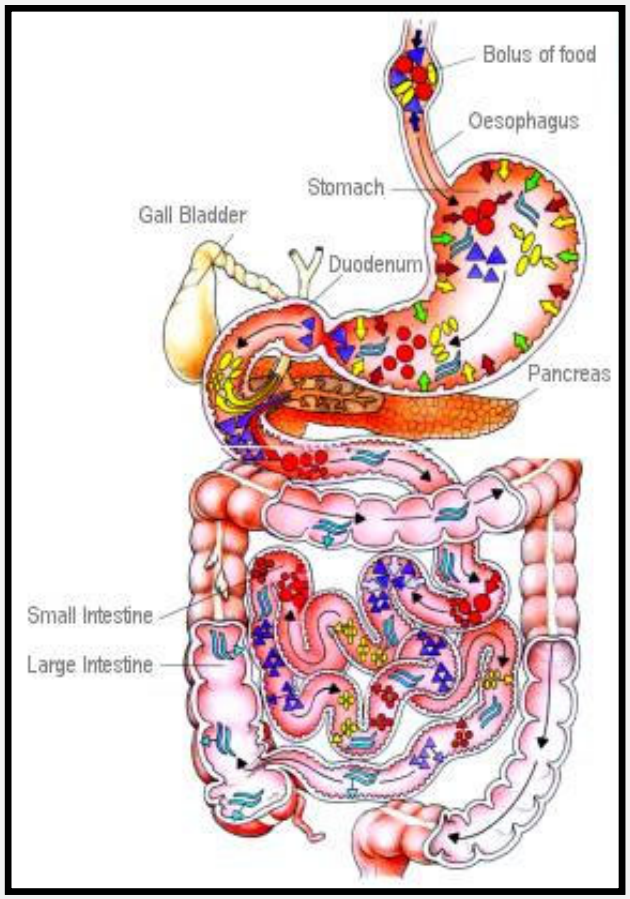
FECAL (PANCREATIC) ELASTASE 1 AND AGING





Fecal pancreatic elastase-1 levels in older individuals without known gastrointestinal diseases or diabetes mellitus *BMC Geriatr.* 2011 Jan 25;11:4.

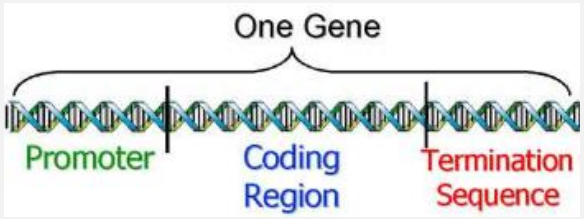
THE CHALLENGE OF DIGESTION & ABSORPTION



- Breakdown complex foods into basic constituents
- Divide Macronutrients into basic units
- Release Micronutrients from food matrix
- Selectively absorb nutrients
- Transform and/or activate nutrients and phytonutrients
- While maintaining a barrier against entry of unwanted particles

EVERYTHING HAPPENS AT THE INTERFACE!

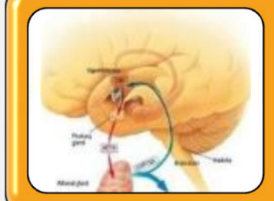
- Biological systems are designed to create discrete functional units
  - Tissues
  - Cells
  - Organelles
  - Genes



- All of which are equipped to modulate each other by signals at their interfaces

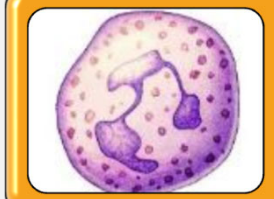
Functional Interfaces require Intact Barriers!

COORDINATED SURVEILLANCE SYSTEMS: PROTECTING "SELF" AT THE INTERFACES



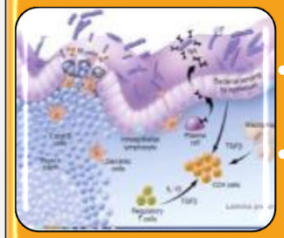
HPA Axis (Stress Response)

- Assessing threats from outside (interface with outside world)
- Compensating for internal imbalances



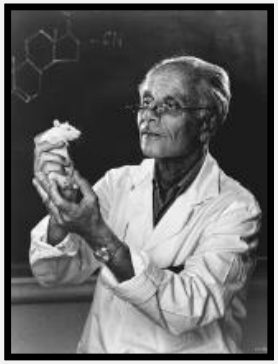
Immune System

- Surveillance of Self vs. Non-Self
- Highly coordinated by GC signals, highly concentrated in the Gut



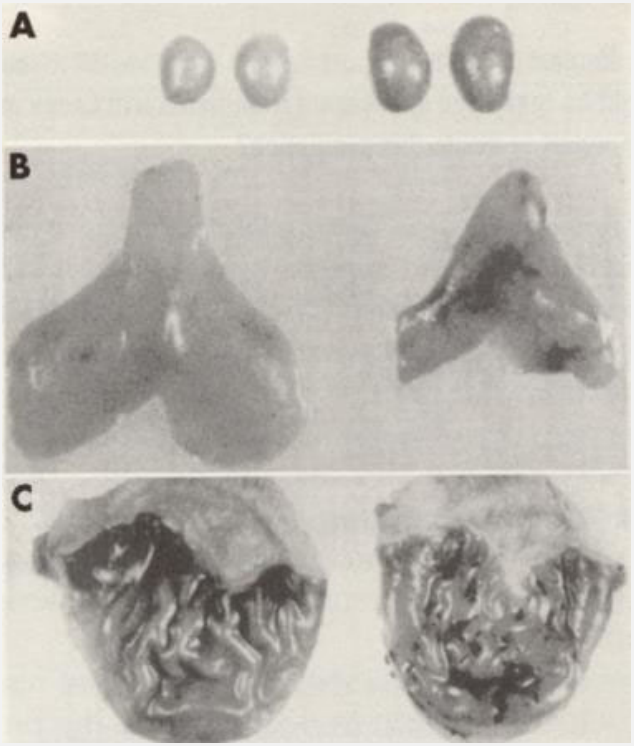
Gastrointestinal Tract- GALT

- Maintaining Barrier Function (interface with outside world)
- Signal coordination to brain using direct and immune facilitated signals.



SELYE AND SURVEILLANCE SYSTEM STRESS

Control "Stress"



Hypertrophy of Adrenal Gland (HPA)

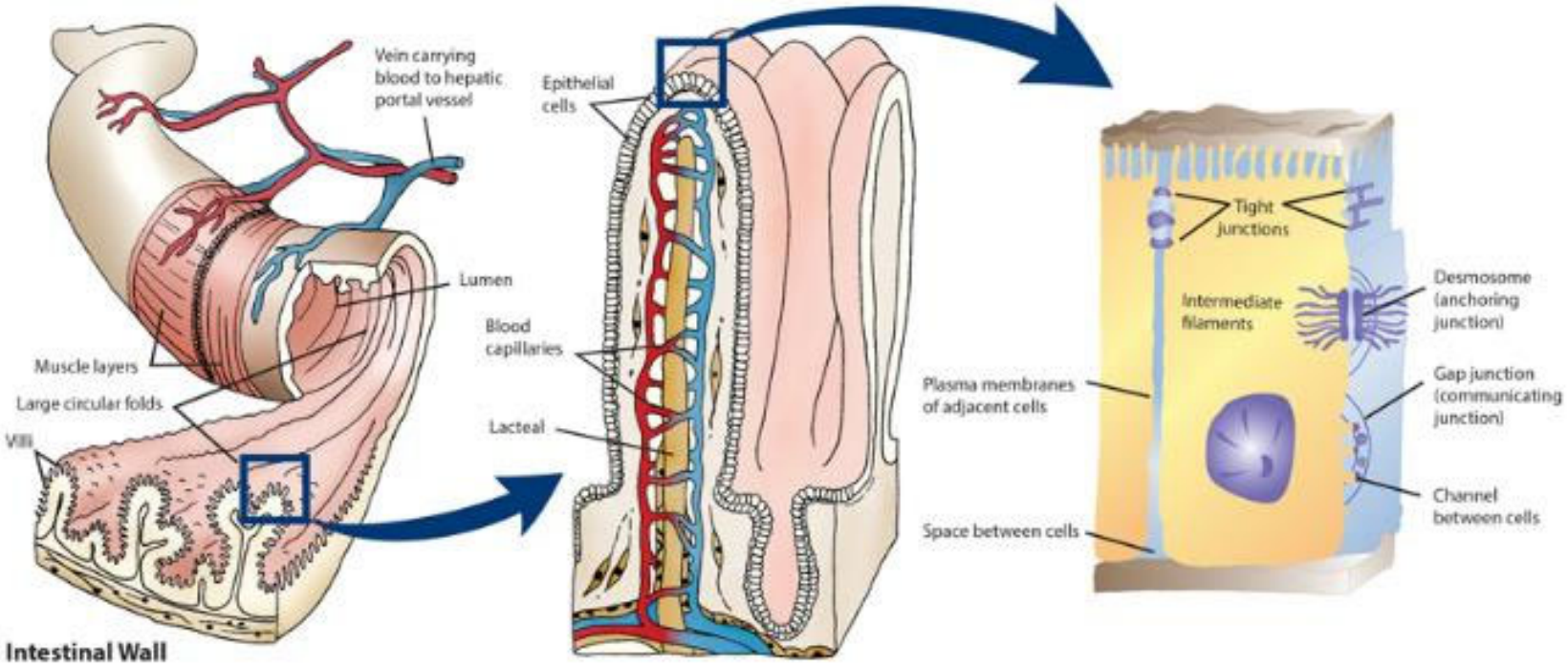
Atrophy of the thymus and other lymphatic glands (Immune system)

Erosions and ulcers in the duodenum (GI-system)

INTERFACE OR BARRIER?

- *"The barrier/permeability functions of the gut represent one of the most important interfaces between a person and the external environment. However, we should not imagine this barrier function as simply a means to keep things out, but as a sophisticated system to communicate with, and allow selective entry of, certain contents from the gut lumen into the body. This requires a tightly controlled, but thin barrier of tissues and secretions* ***intentionally designed for close proximity to the gut lumen****. This proximity permits the absorption of available nutrients and physiological interaction with trillions of non-human microbes and their metabolites and signals, but also creates a vulnerability to those same microbes, toxins and immunologically reactive components from the gut lumen."*

EXPANDING THE SURFACE AREA
MORE INTERFACE: MORE VULNERABILITY



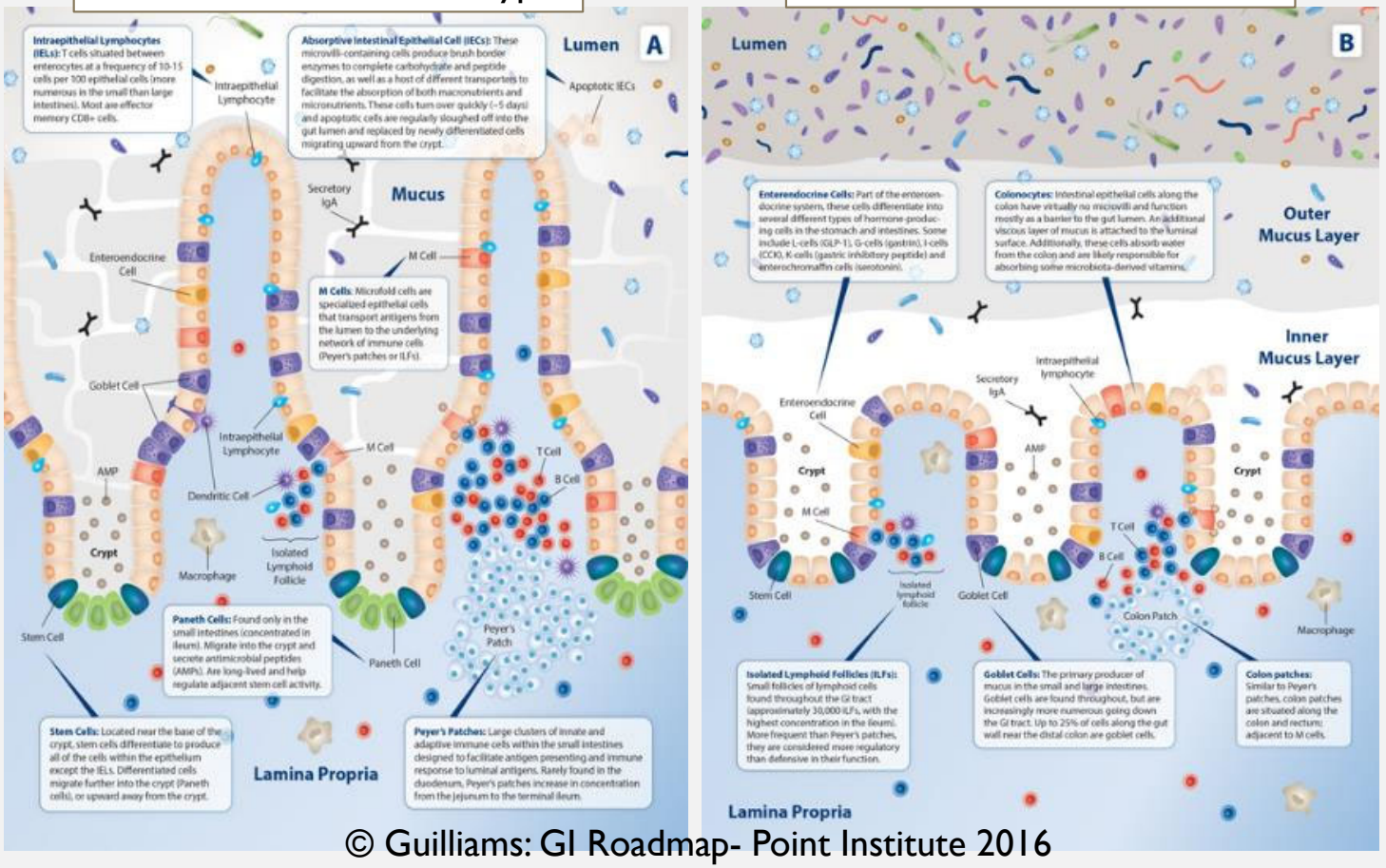
THE FUNCTIONAL COMPONENTS OF THE GUT BARRIER

- Human GI cells that create the interface (Enterocytes, Colonocyte etc.)
- Human Immune cells that line the inside or penetrate the interface
- Human Neuroendocrine cells and neurons with synapses nearby.
- Luminal Excretions from human cells (Mucus, sIGA, anti-microbial peptides, enzymes, acid, neurotransmitters etc.)
- Non-Human microbes in the lumen and mucus lining
  - Commensal, Pathobiont, Pathogenic Bacteria
  - Viruses (free and bacteriophages)
  - Fungi
  - Non-human eukaryotic organisms (are any of these commensals?)

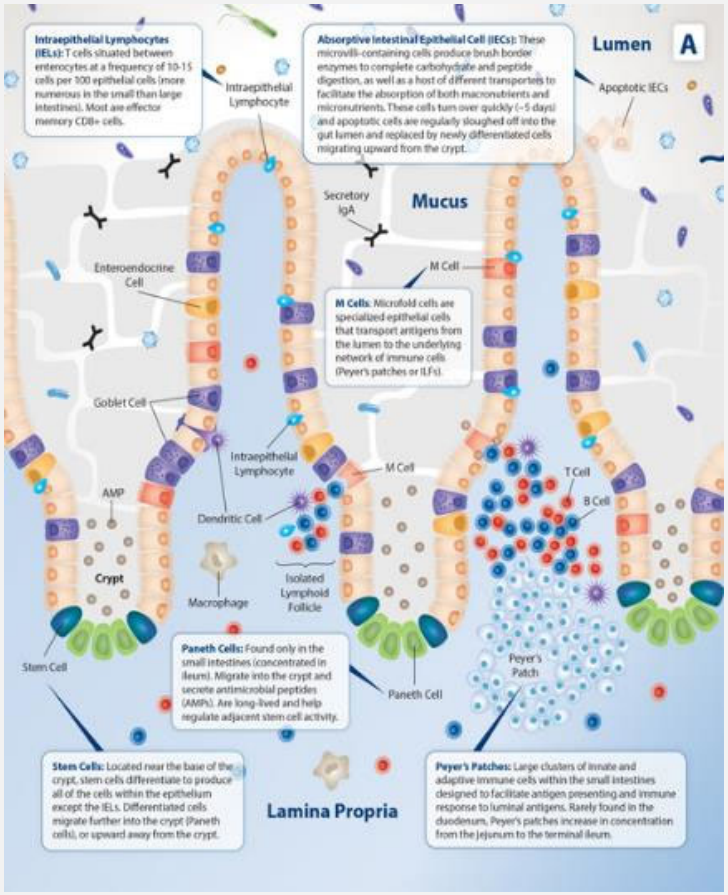
BASIC FEATURES OF THE GUT BARRIER

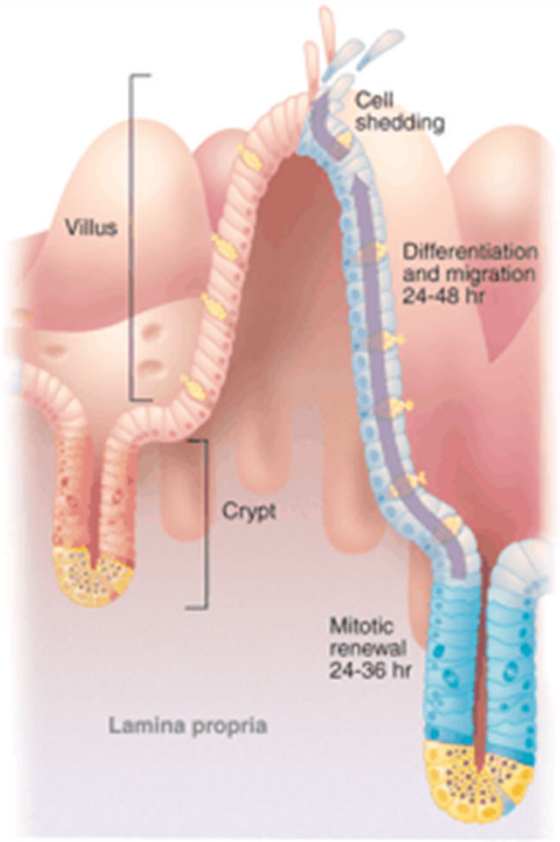
Small Intestine- Villi and Crypt

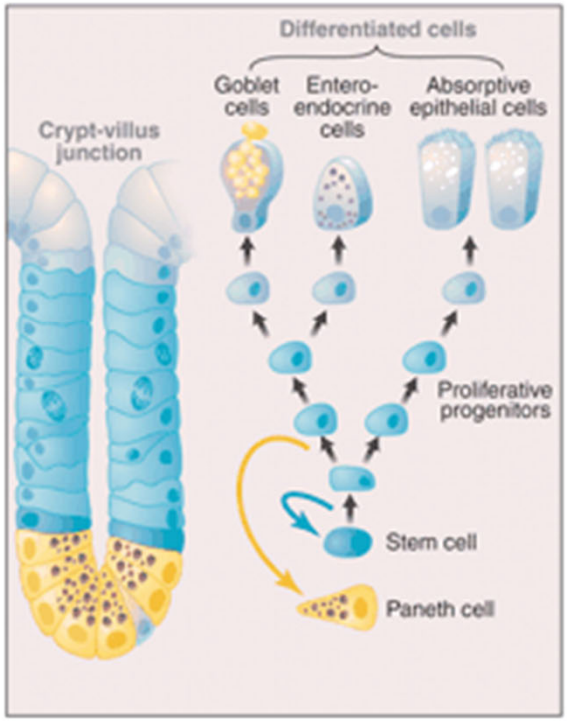
Colon- 2 mucus layers, crypt



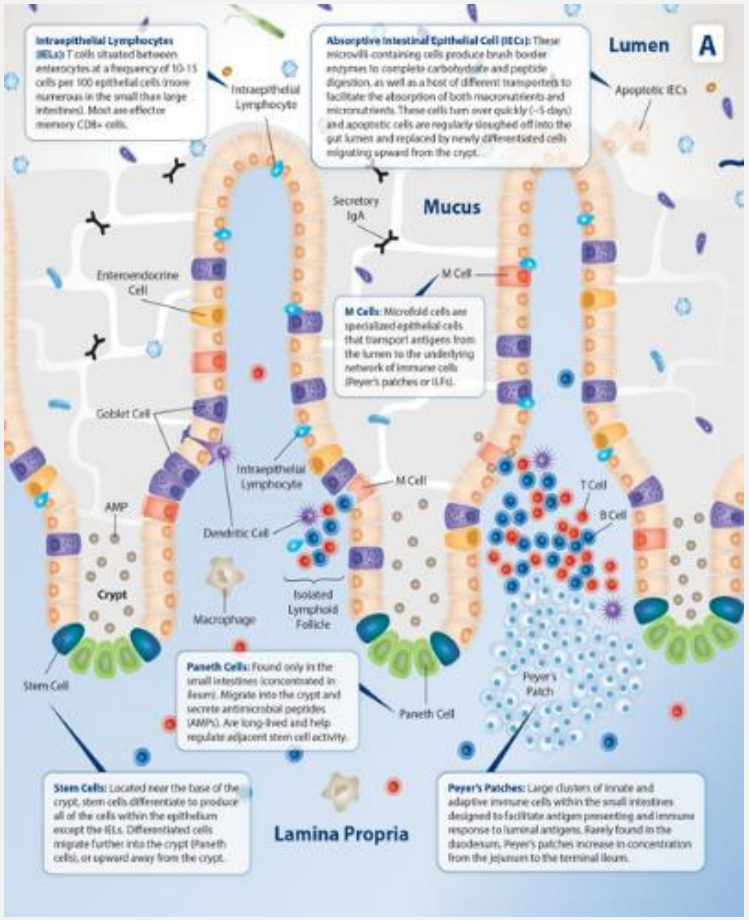
STEM CELLS: CONSTANT TURNOVER

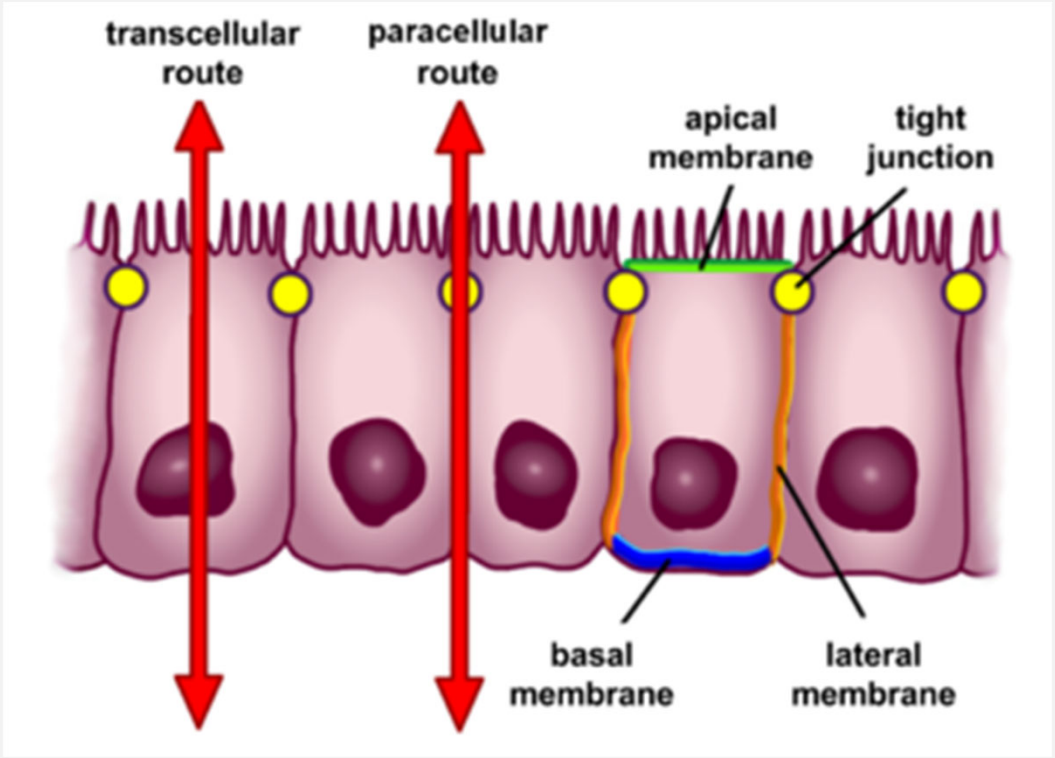




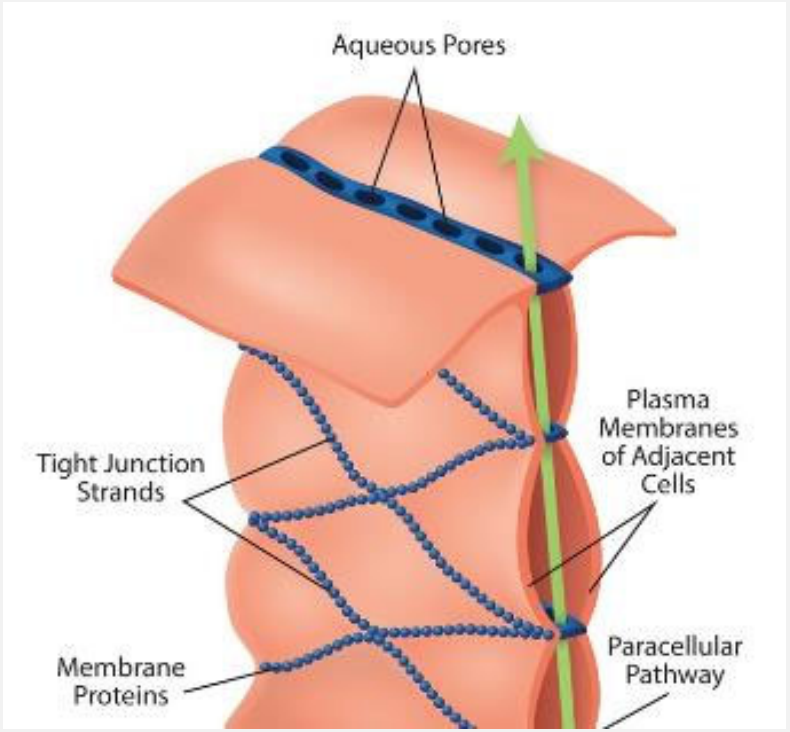


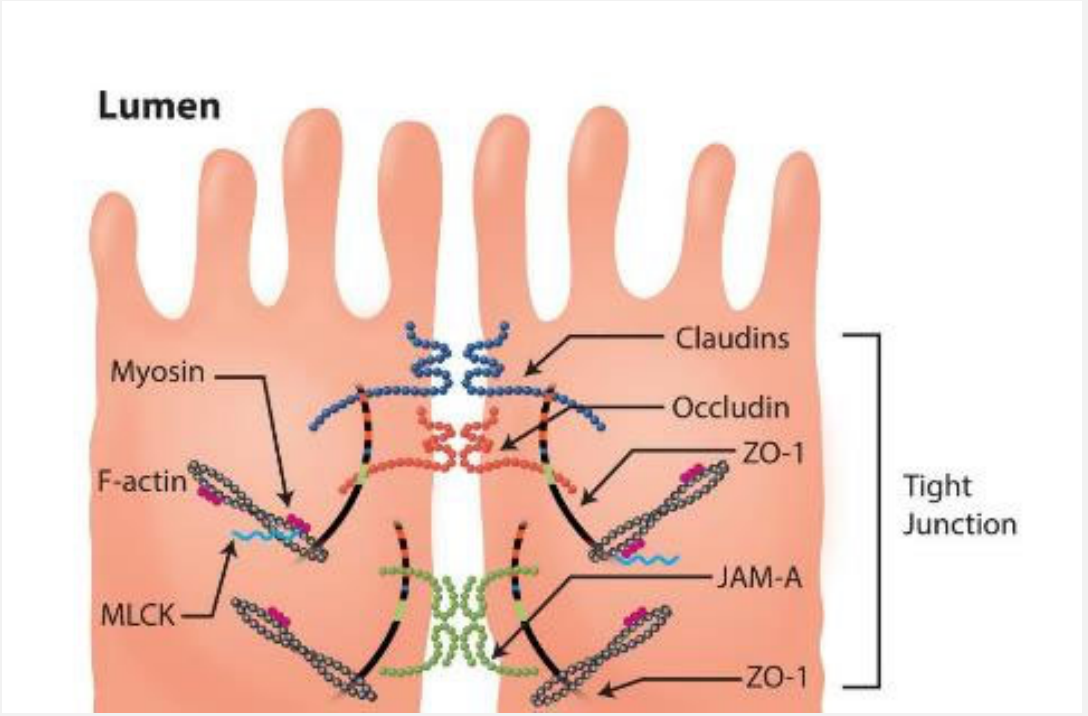
ABSORPTIVE EPITHELIAL CELLS





TIGHT JUNCTIONS

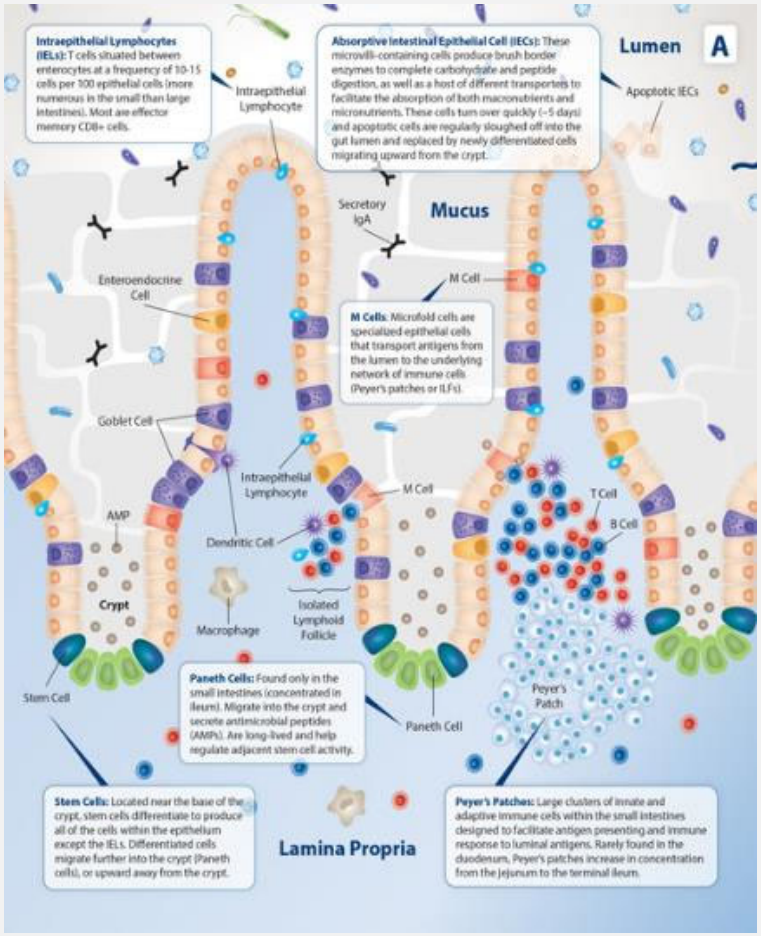




MLCK- Myosin Light Chain Kinase

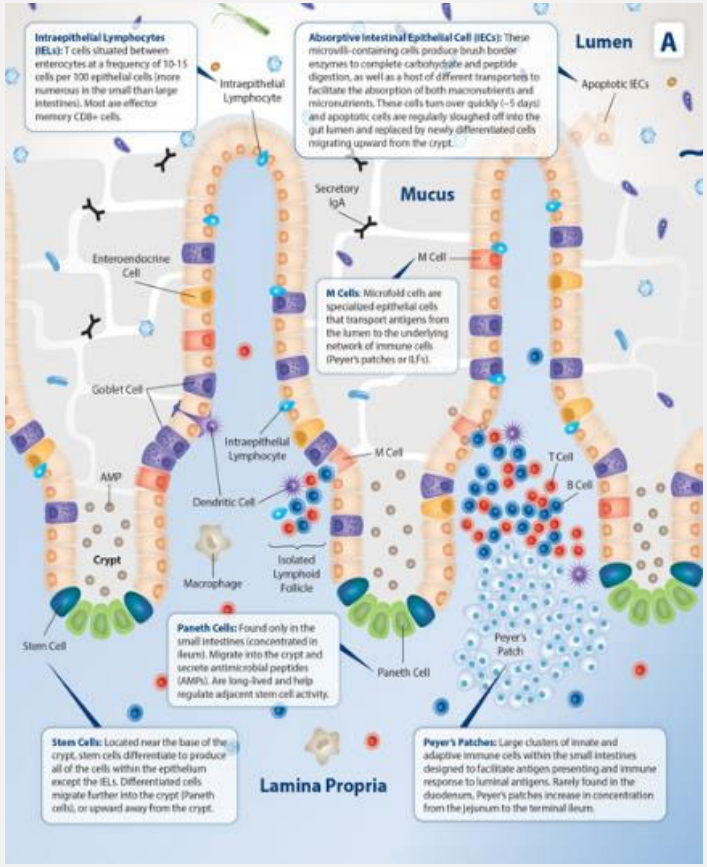
ZO-zonula occludens

PANETH CELLS: MANAGING DYSBIOSIS



- Found only in Small Intestines (primarily Ilium)
- Migrate into crypt after differentiation from stem cells
- Secrete antimicrobial peptides (AMPs) into gut lumen
- Are long-lived (months) compared to absorptive cells.
- Help regulate stem cell activity

IMMUNE SYSTEM-TIGHTLY BOUND



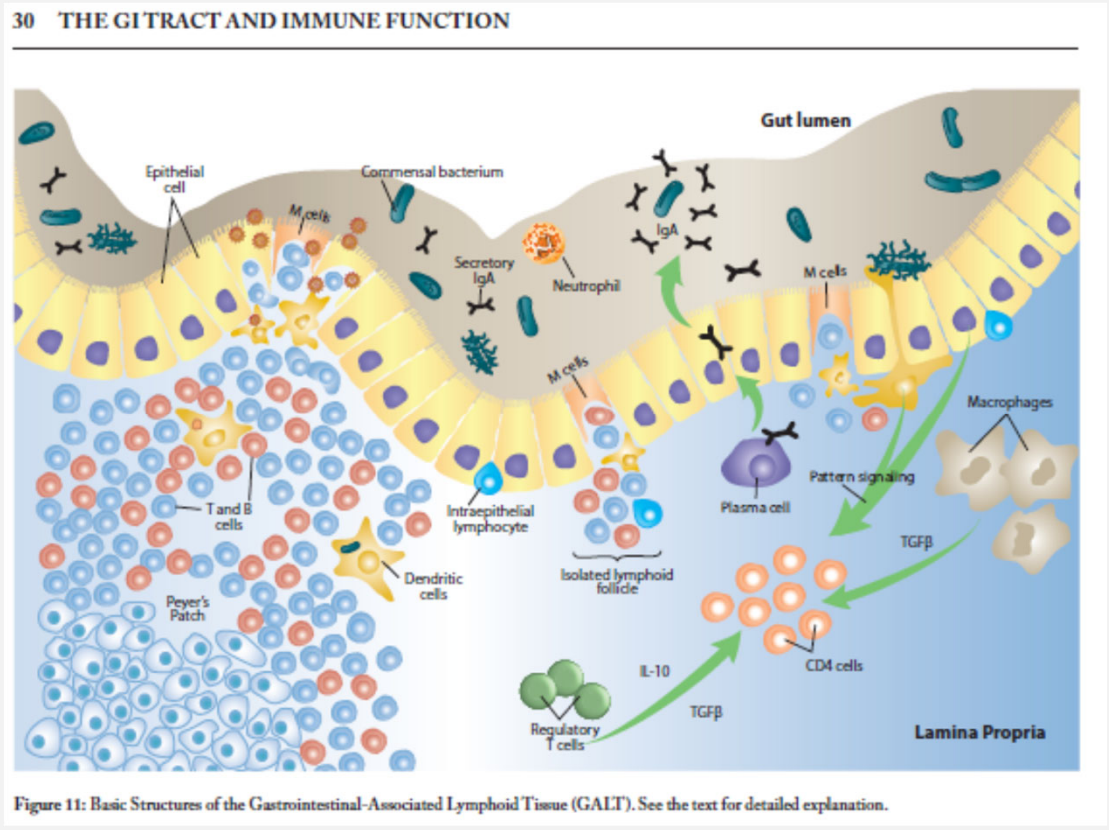


Figure 11: Basic Structures of the Gastrointestinal-Associated Lymphoid Tissue (GALT). See the text for detailed explanation.

TRAINING: FRIEND OF FOE?

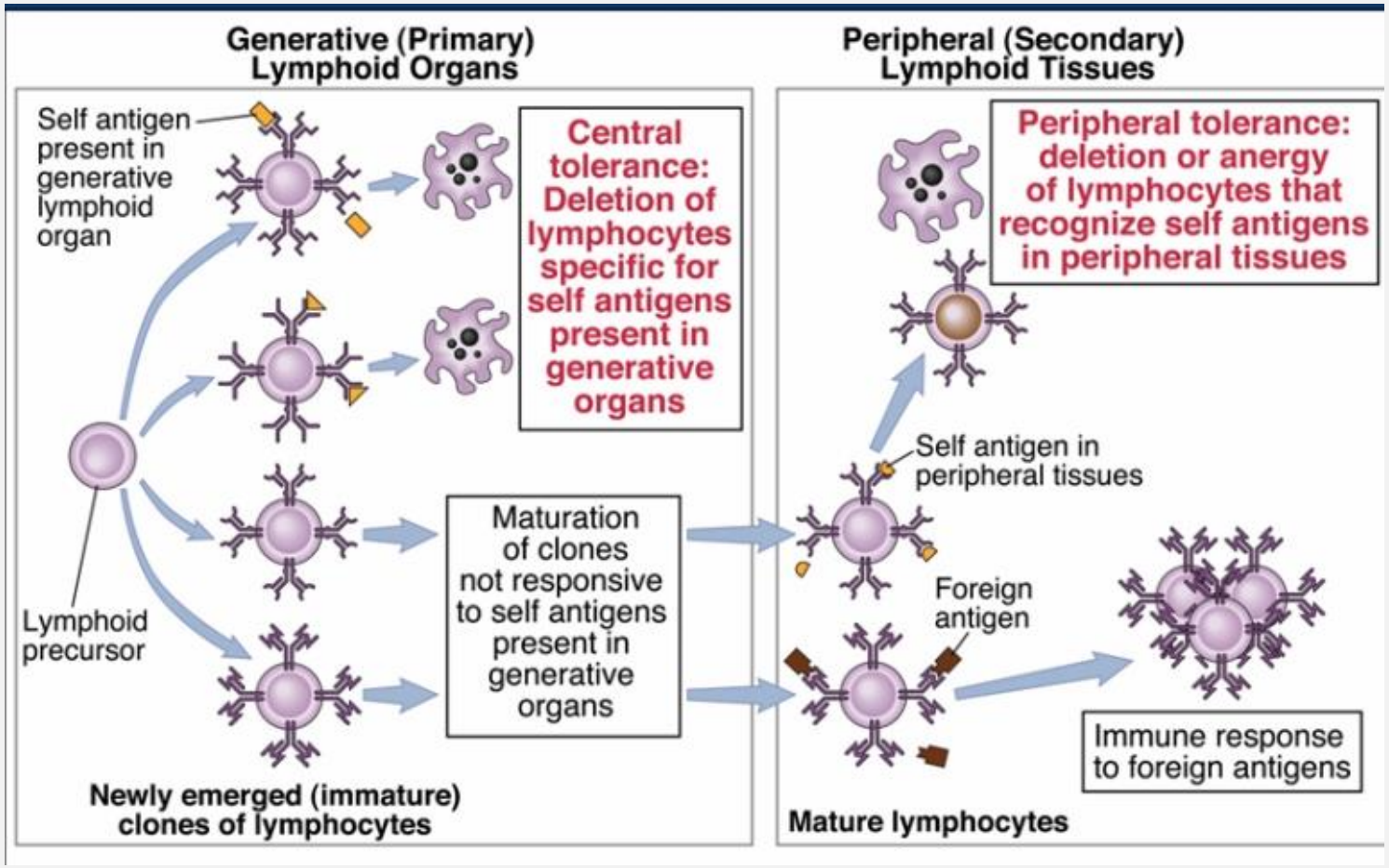
Most people say "75% of the immune system is in the gut"

This is sort of true, if you only calculate the location of cells.

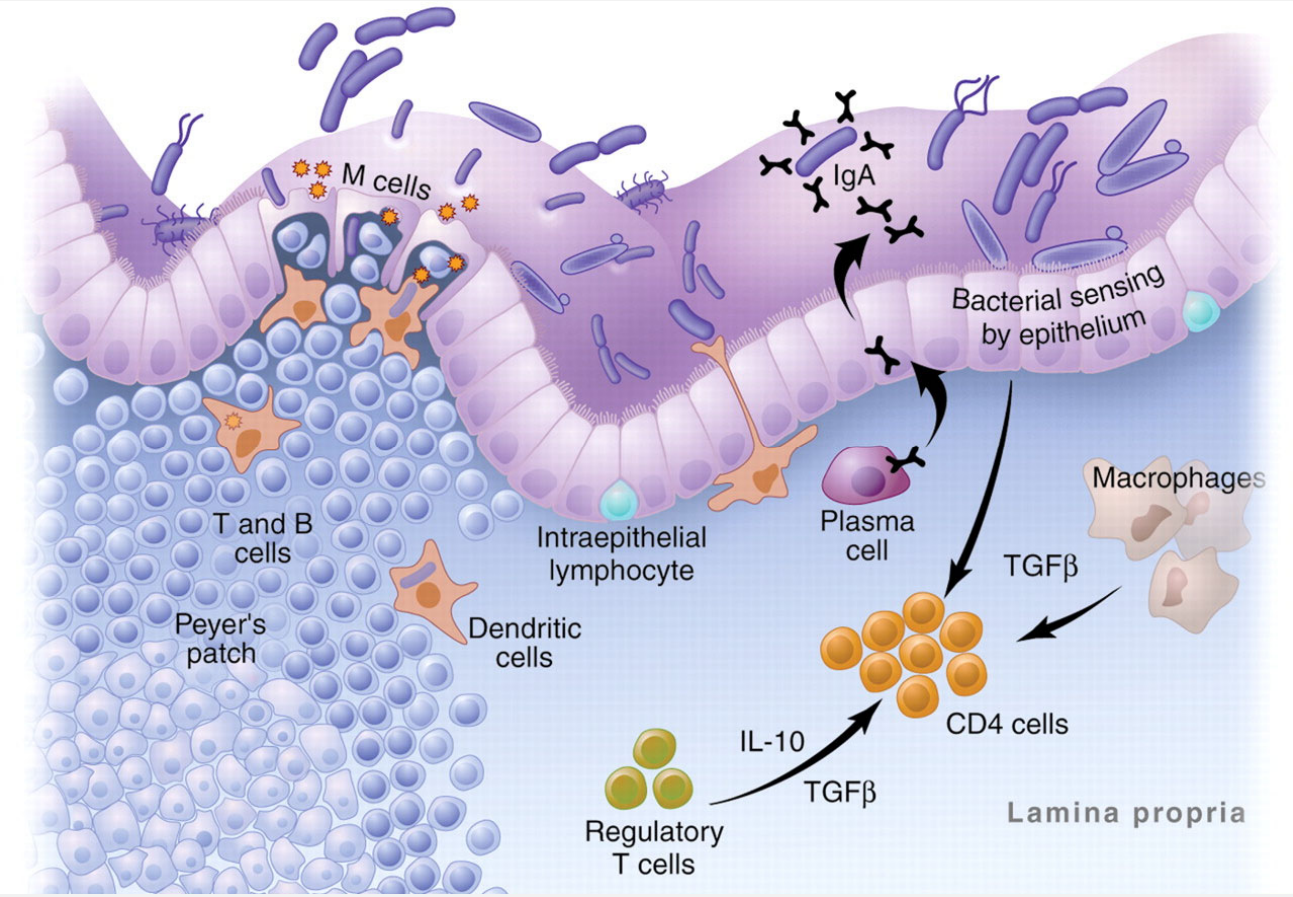
The immune system is the gathering place of naïve B and T cells, and the primary location for peripheral tolerance.

This also requires many innate immune cells, including antigen presenting cells (in the gut: dendritic cells are in control)

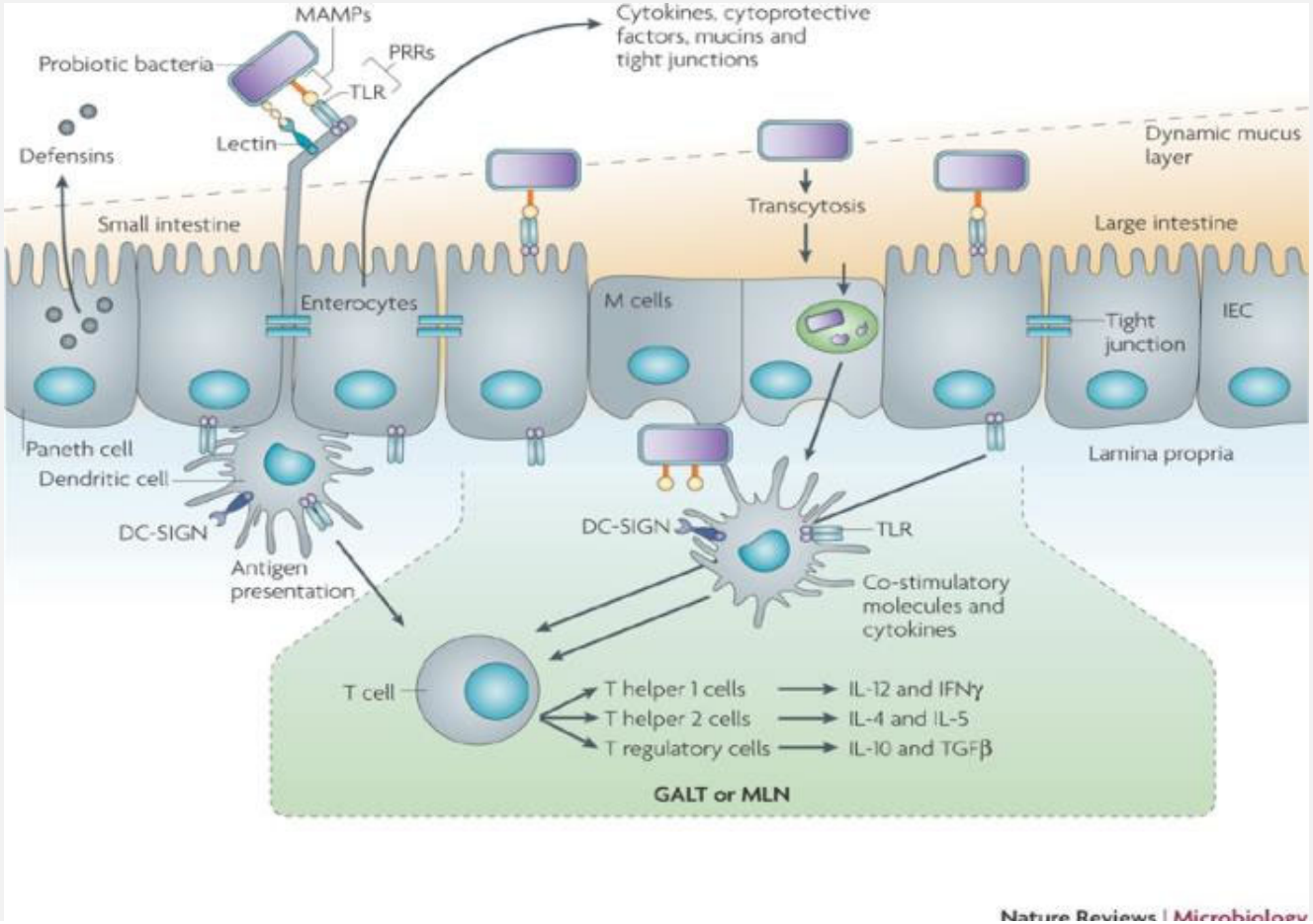
SELF-TOLERANCE IS KEY!



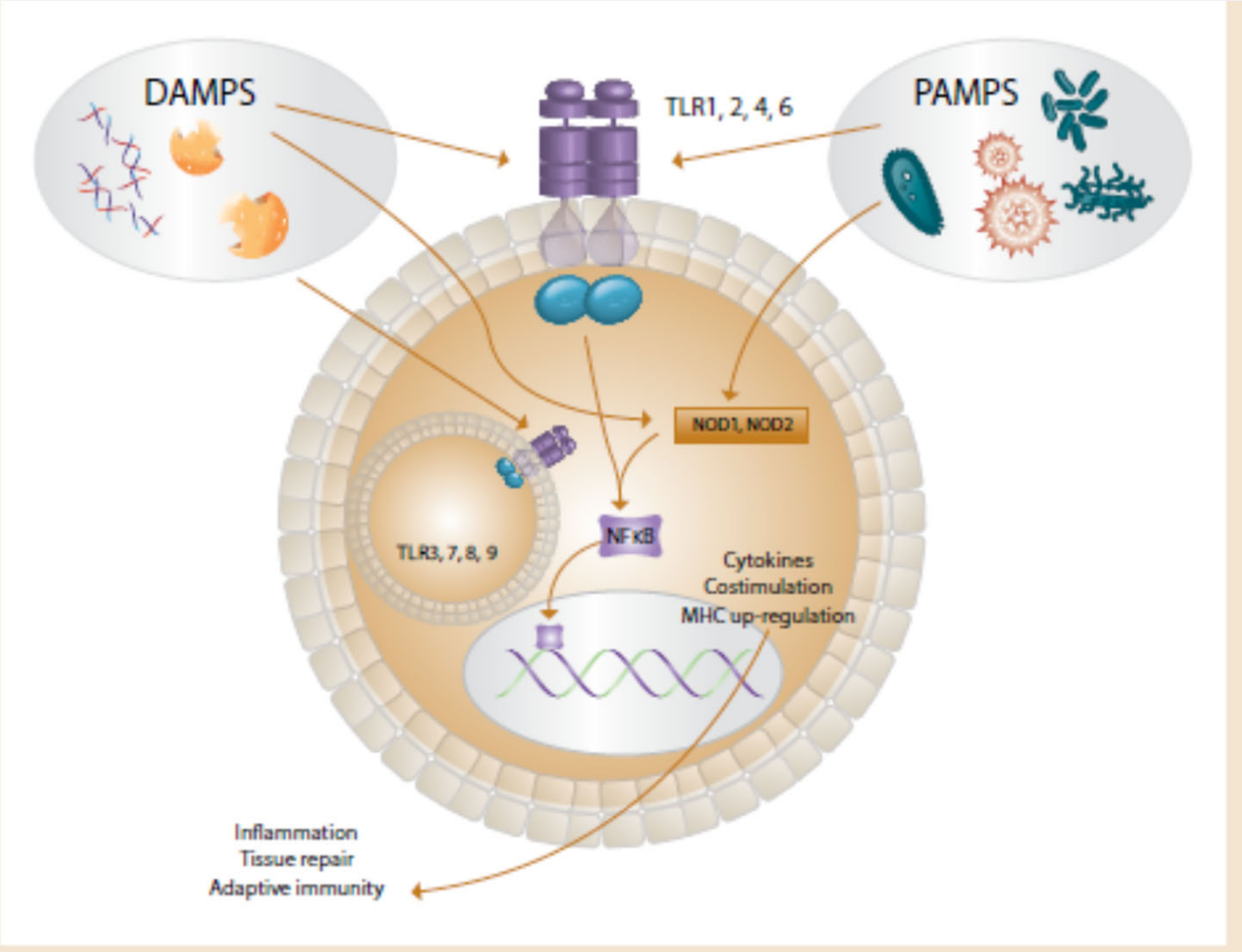
EDUCATION THROUGH SAMPLING NON-SELF FRIEND OR FOE?



DENDRITIC CELLS



PATTERN RECOGNITION RECEPTORS



TOLL-LIKE RECEPTOR FAMILY (TLRS)

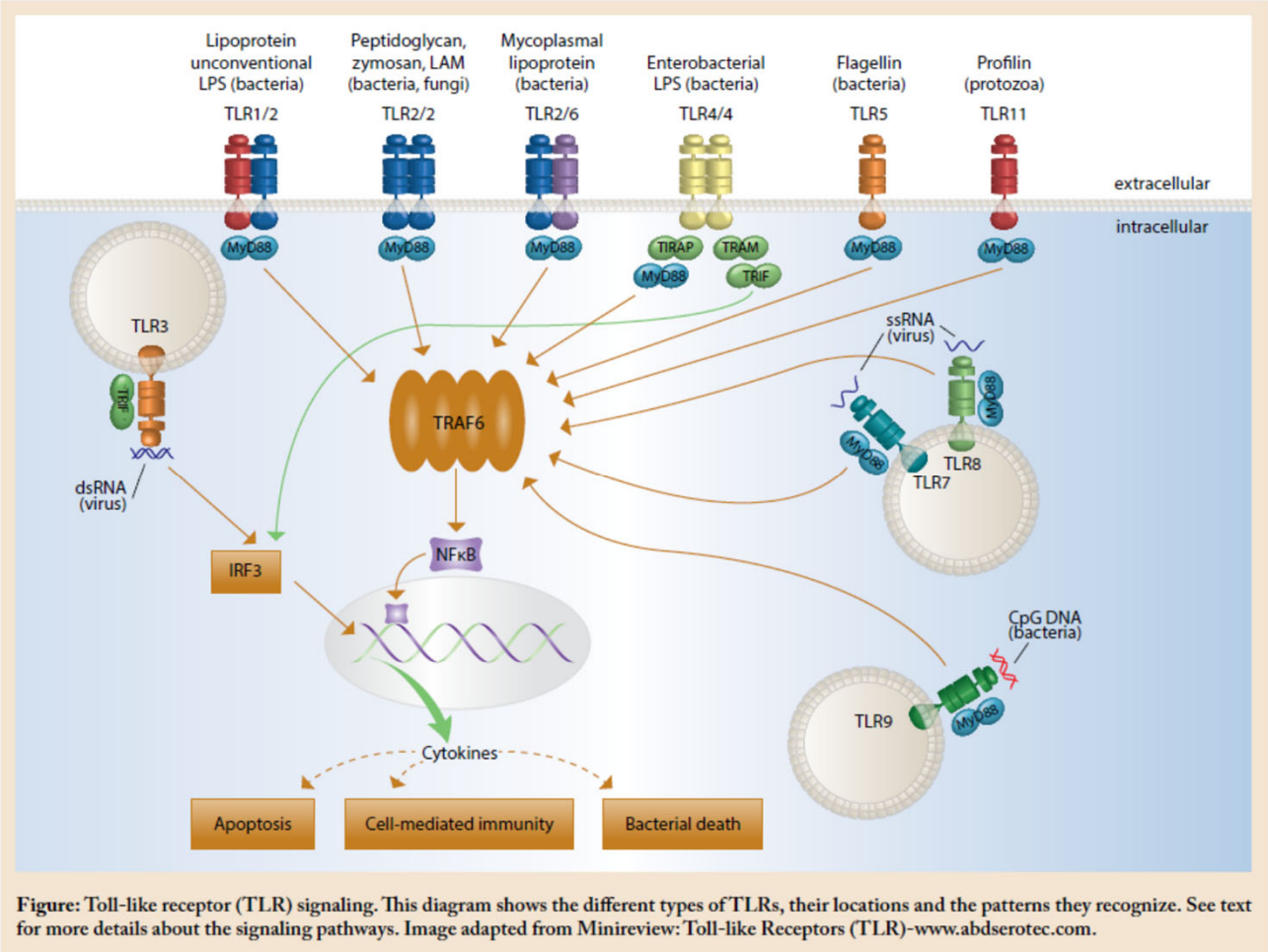
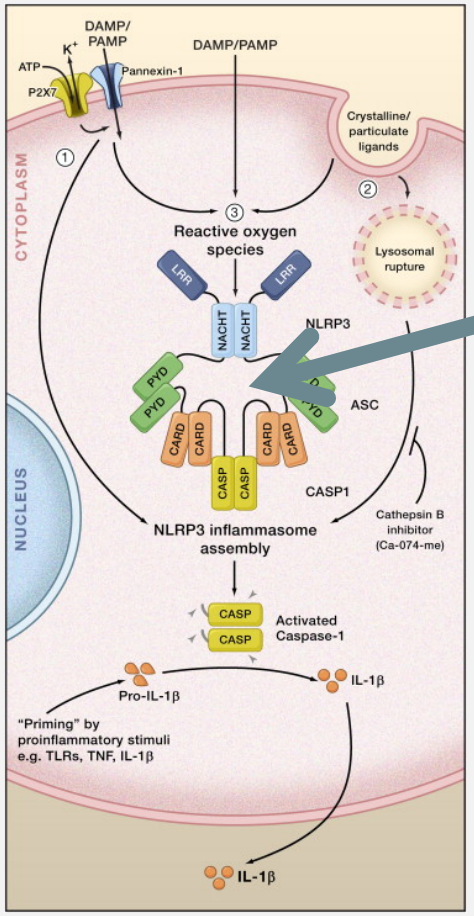
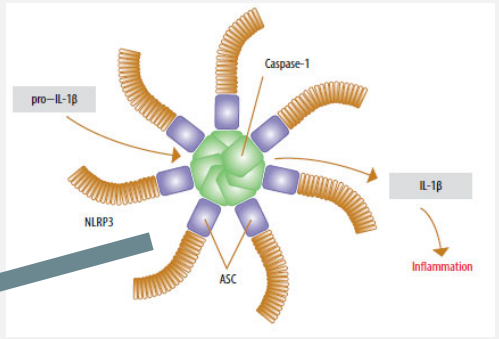


Figure: Toll-like receptor (TLR) signaling. This diagram shows the different types of TLRs, their locations and the patterns they recognize. See text for more details about the signaling pathways. Image adapted from Minireview: Toll-like Receptors (TLR)-www.abdserotec.com.

INFLAMMASOME (NLRP3-TYPE)

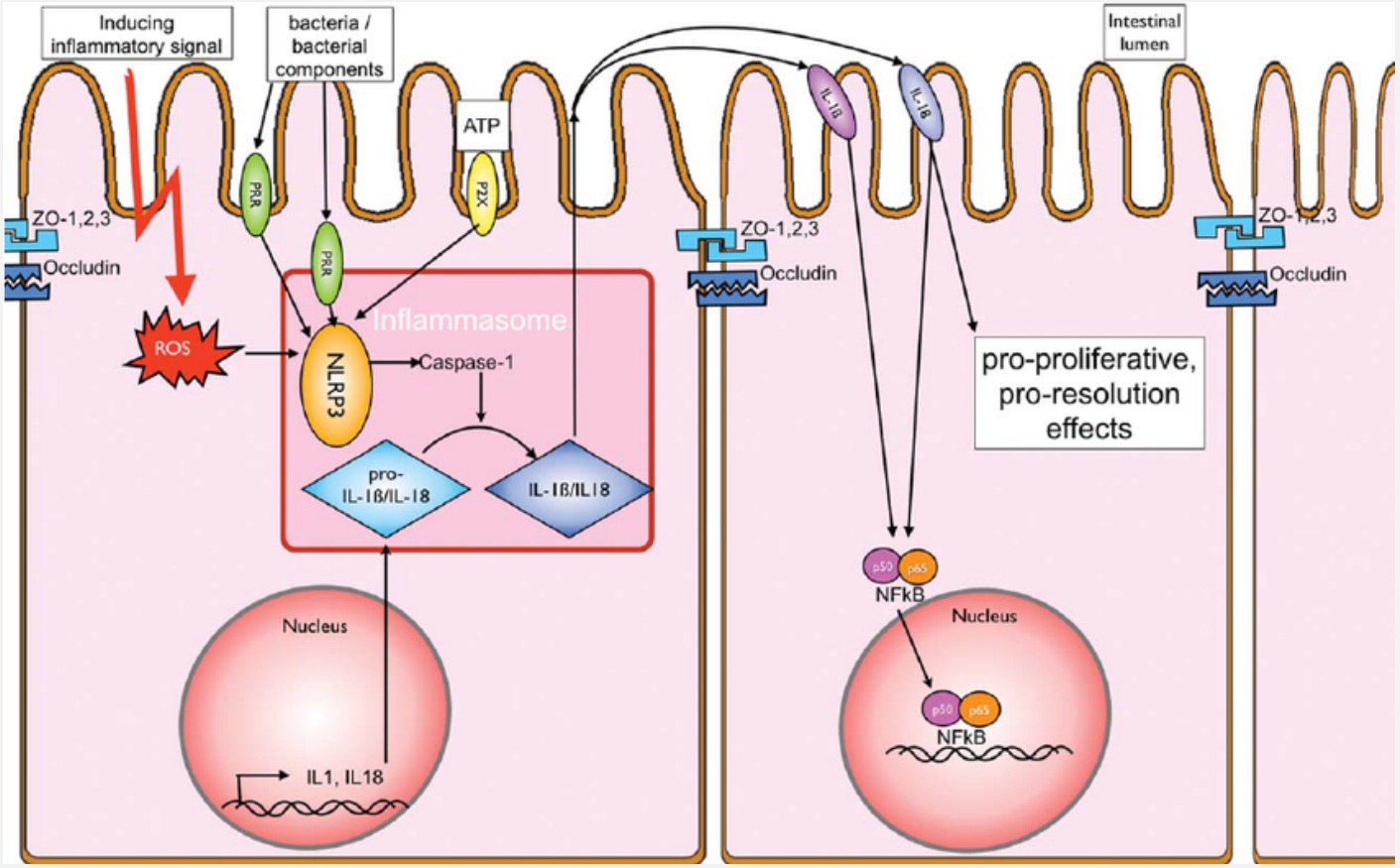




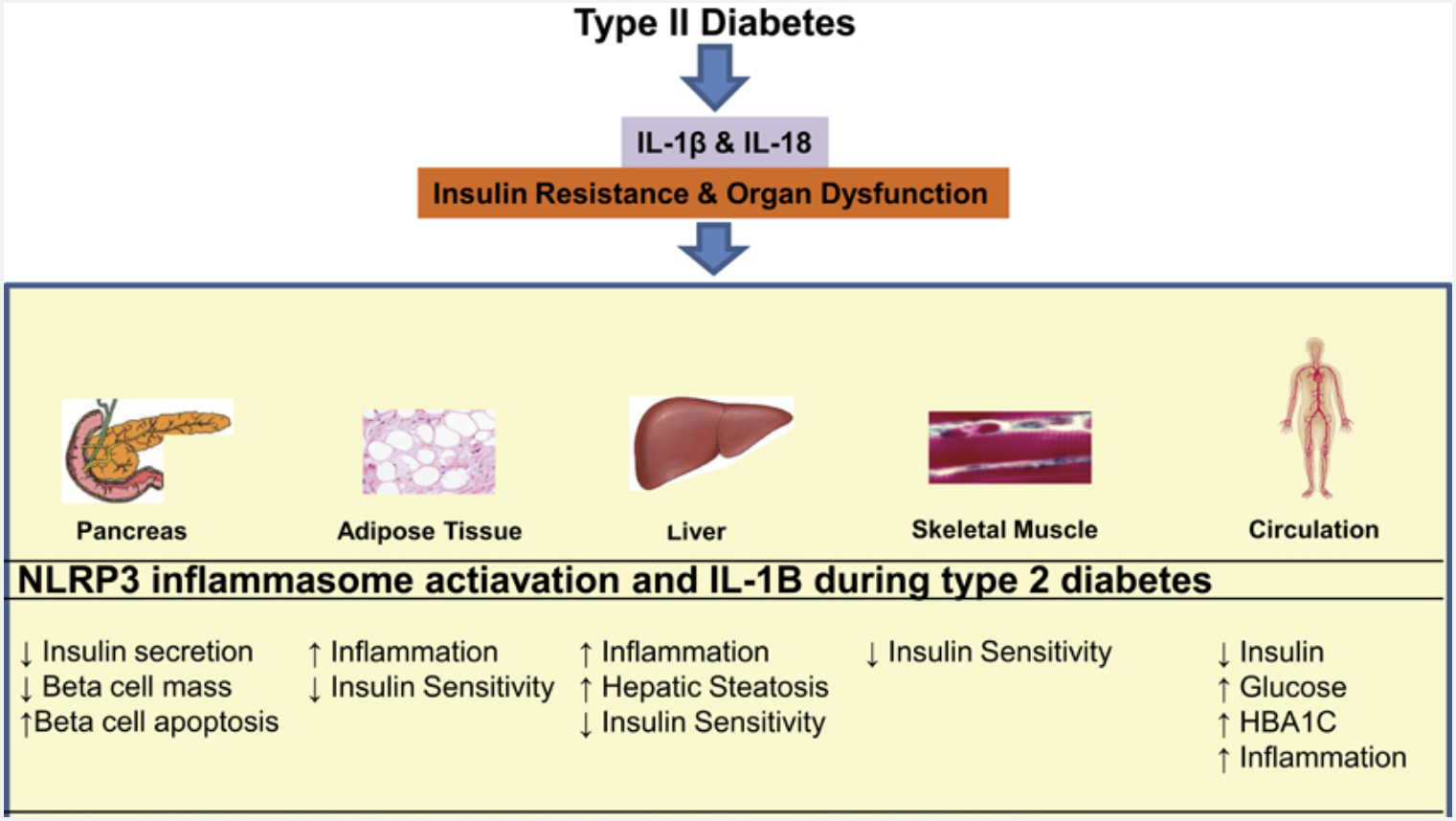
- Heptamer complex between caspase and NRLP3
- Caspase activates the release of IL-1b, furthering inflammatory cascade
- 3 potential triggers

Cell: Volume 140, Issue 6, 19 March 2010, Pages 821–832

"PARACRINE" FUNCTION OF INFLAMMASOMES IN IBD

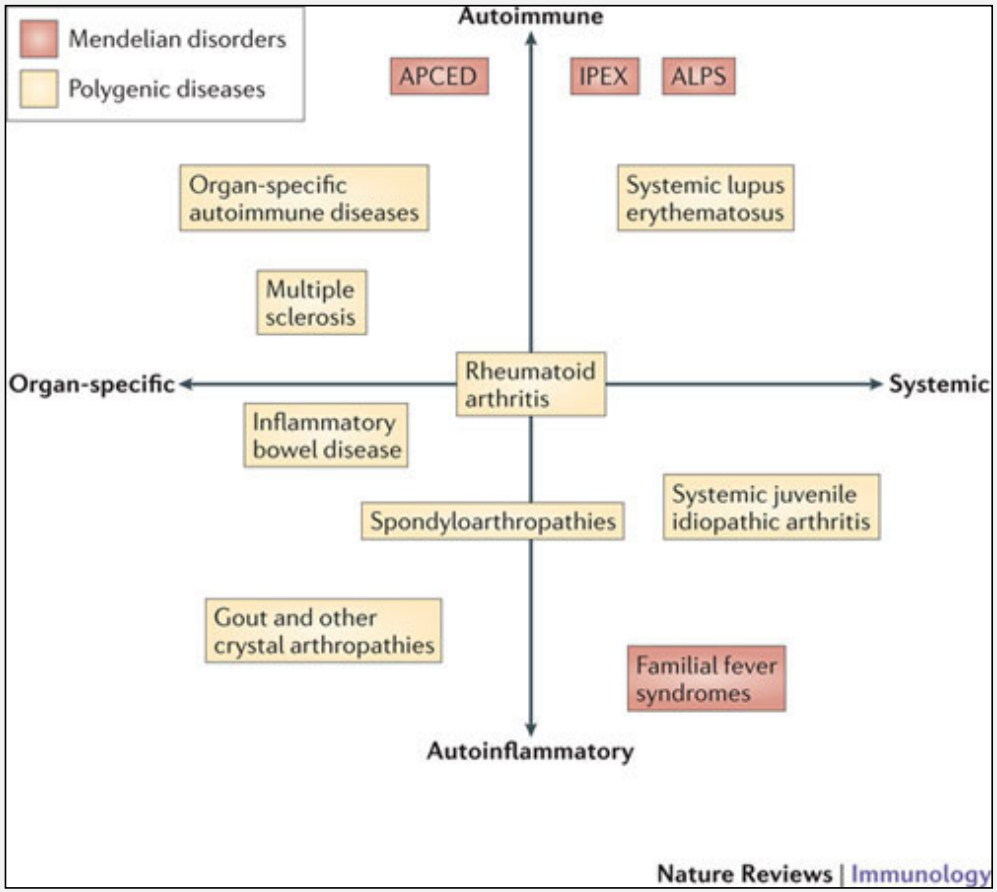


INFLAMMASOME AND CHRONIC DISEASE

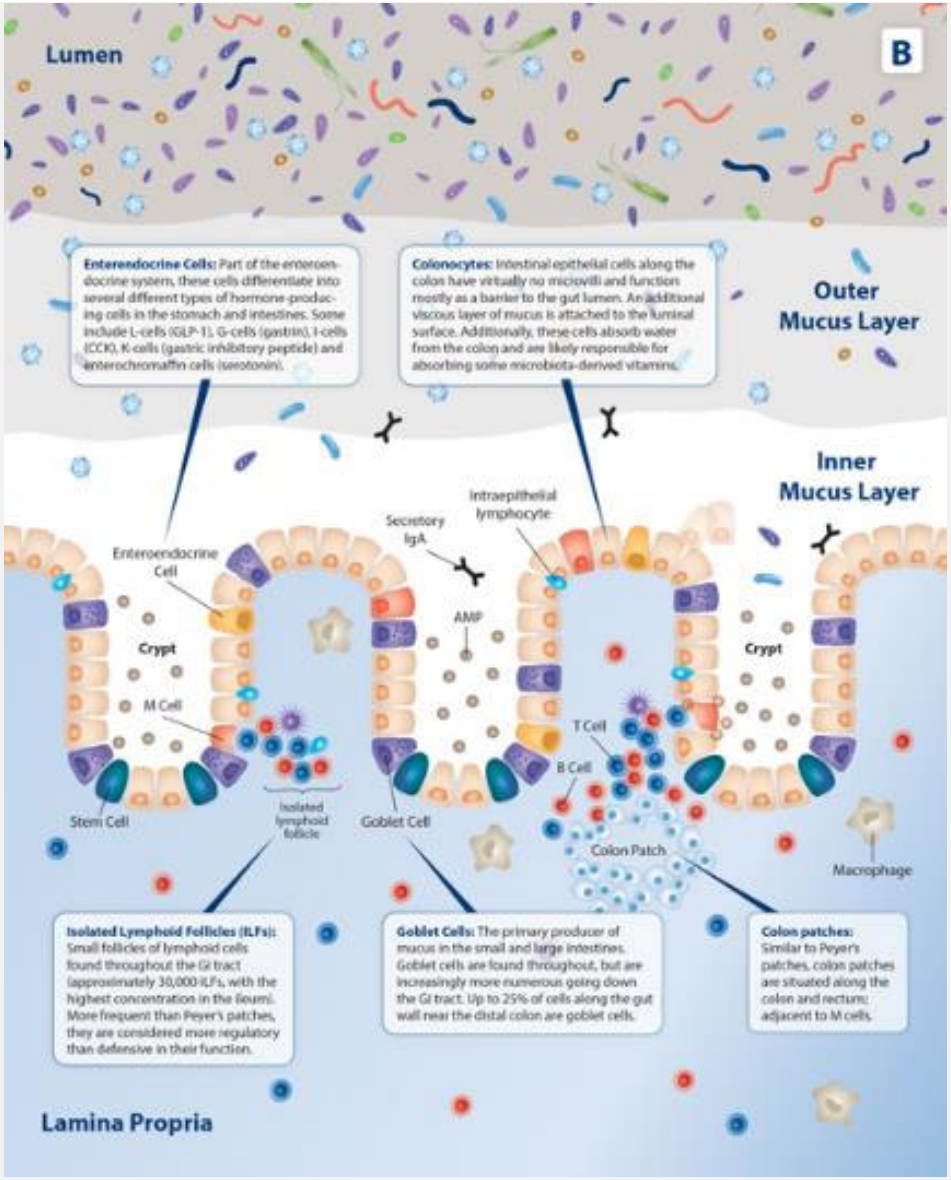


Front. Immunol., 08 March 2013

AUTOINFLAMMATORY DISEASES



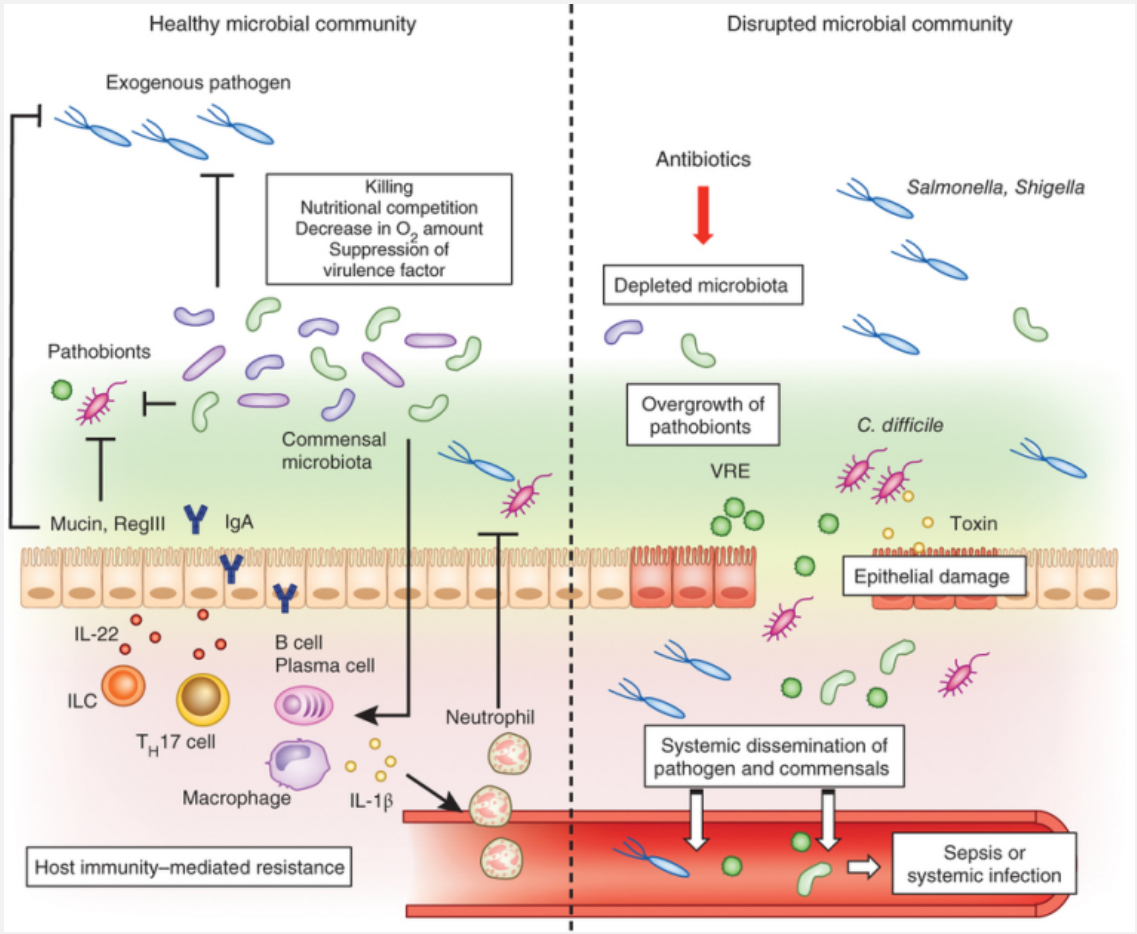
Nature Reviews Immunology 12, 570-580 (August 2012)



BASIC FEATURES OF THE COLON BARRIER

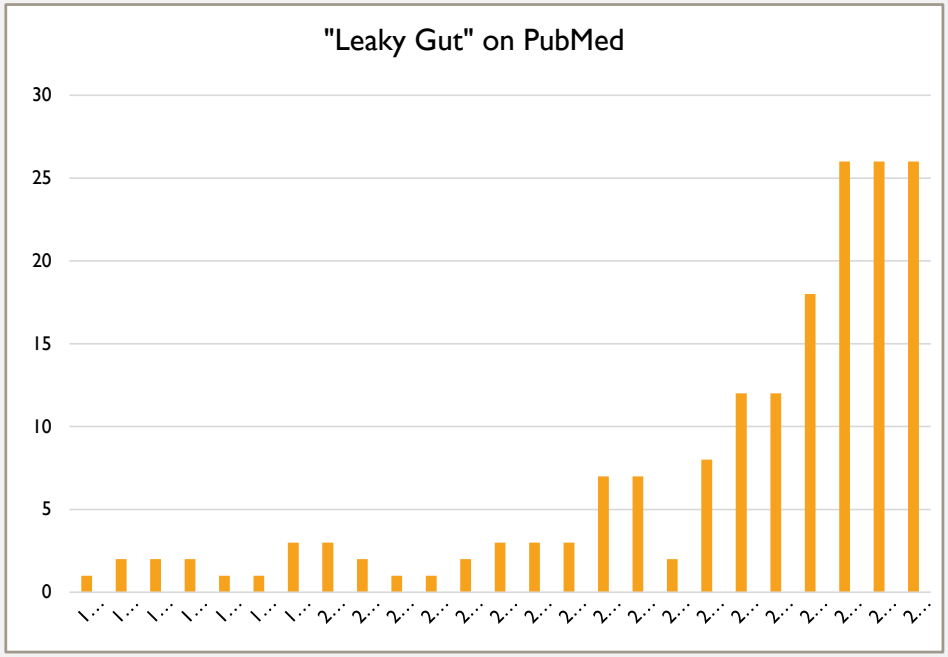
- Two layers of Mucus
- Increased number of Goblet Cells
- Less interface, more barrier
- Lower concentration of immune cells
- Fewer Enteroendocrine Cells
- Lumen acts as large fermenting vessel

LEAKY GUT: MANY DIFFERENT VIEWS



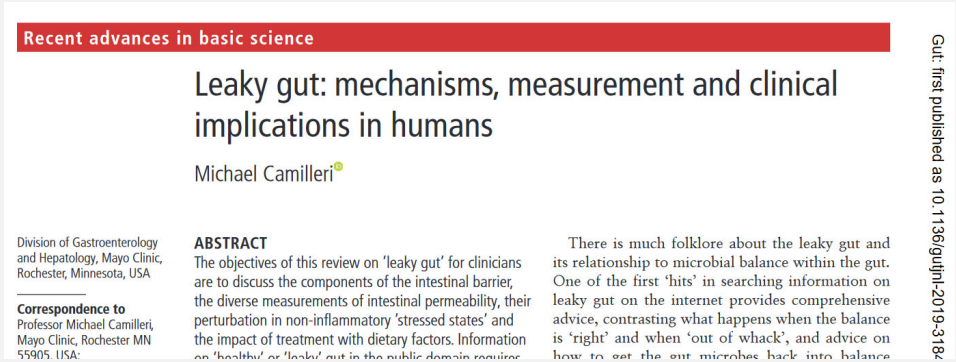
Nature Immunology 14, 685–690 (2013

IS "LEAKY GUT" A LEGITIMATE TERM?



- "From an MD's standpoint, it's a very gray area," says gastroenterologist Donald Kirby, MD, director of the Center for Human Nutrition at the Cleveland Clinic. "Physicians don't know enough about the gut, which is our biggest immune system organ."
- "Leaky gut syndrome" isn't a diagnosis taught in medical school. Instead, "leaky gut really means you've got a diagnosis that still needs to be made," Kirby says. "You hope that your doctor is a good-enough Sherlock Holmes, but sometimes it is very hard to make a diagnosis."
- "We don't know a lot but we know that it exists," says Linda A. Lee, MD, a gastroenterologist and director of the Johns Hopkins Integrative Medicine and Digestive Center. "In the absence of evidence, we don't know what it means or what therapies can directly address it."
- From WebMD (page hasn't been updated since 2013!)

“LEAKY GUT”- MORE COMMONLY USED



Recent advances in basic science

Leaky gut: mechanisms, measurement and clinical implications in humans

Michael Camilleri

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence to
Professor Michael Camilleri, Mayo Clinic, Rochester MN 55905, USA;

ABSTRACT
The objectives of this review on ‘leaky gut’ for clinicians are to discuss the components of the intestinal barrier, the diverse measurements of intestinal permeability, their perturbation in non-inflammatory ‘stressed states’ and the impact of treatment with dietary factors. Information on ‘healthy’ or ‘leaky’ gut in the public domain requires

There is much folklore about the leaky gut and its relationship to microbial balance within the gut. One of the first ‘hits’ in searching information on leaky gut on the internet provides comprehensive advice, contrasting what happens when the balance is ‘right’ and when ‘out of whack’, and advice on how to get the gut microbes back into balance

Gut: first published as 10.1136/gutjnl-2019-318

F1000Research

F1000Research 2020, 9(F1000 Faculty Rev):69 Last updated: 31 JAN 2020

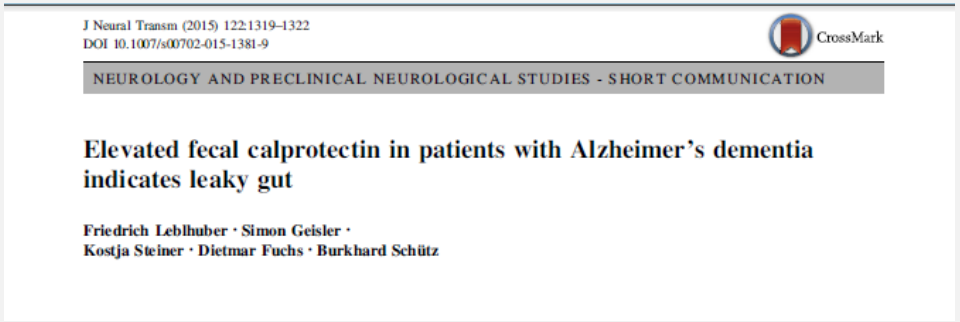
Check for updates

REVIEW

All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases [version 1; peer review: 3 approved]

Alessio Fasano [1,2]

[1]Mucosal Immunology and Biology Research Center, Center for Celiac Research and Treatment and Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital for Children, Boston, Massachusetts, USA
[2]European Biomedical Research Institute of Salerno, Salerno, Italy



J Neural Transm (2015) 122:1319–1322
DOI 10.1007/s00702-015-1381-9

CrossMark

NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - SHORT COMMUNICATION

Elevated fecal calprotectin in patients with Alzheimer’s dementia indicates leaky gut

Friedrich Leblhuber · Simon Geisler · Kostja Steiner · Dietmar Fuchs · Burkhard Schütz

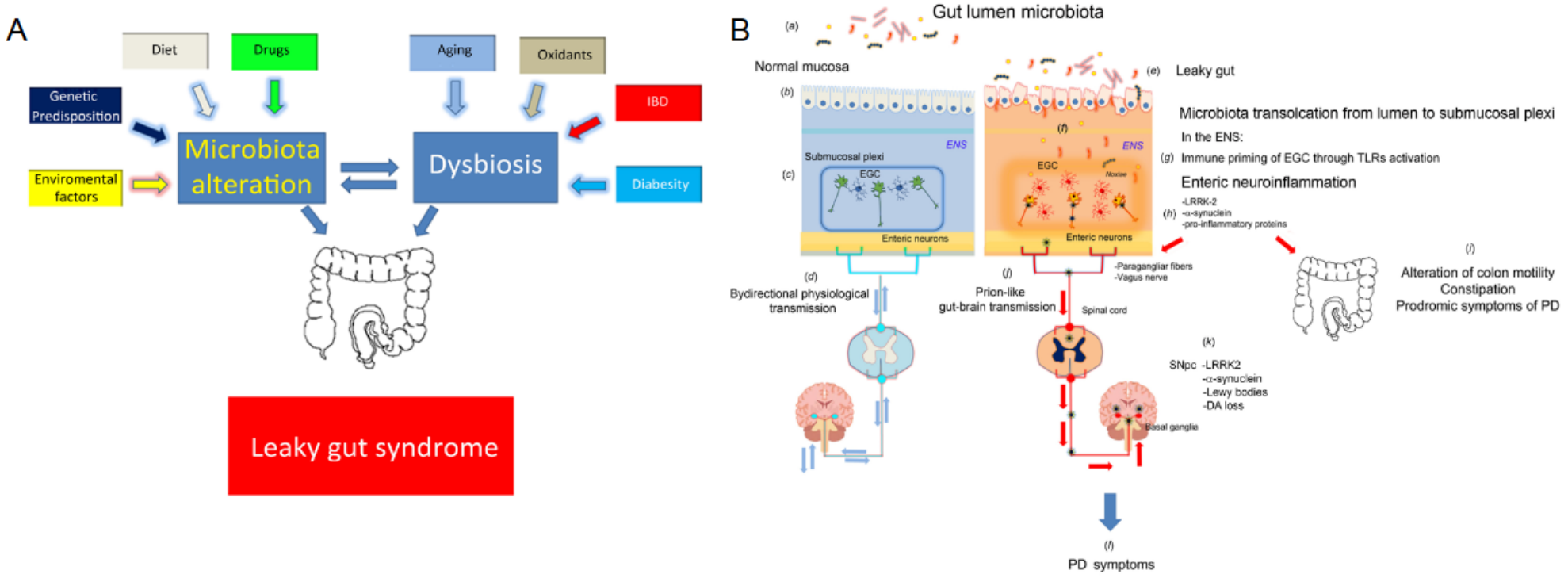
frontiers in Immunology

REVIEW

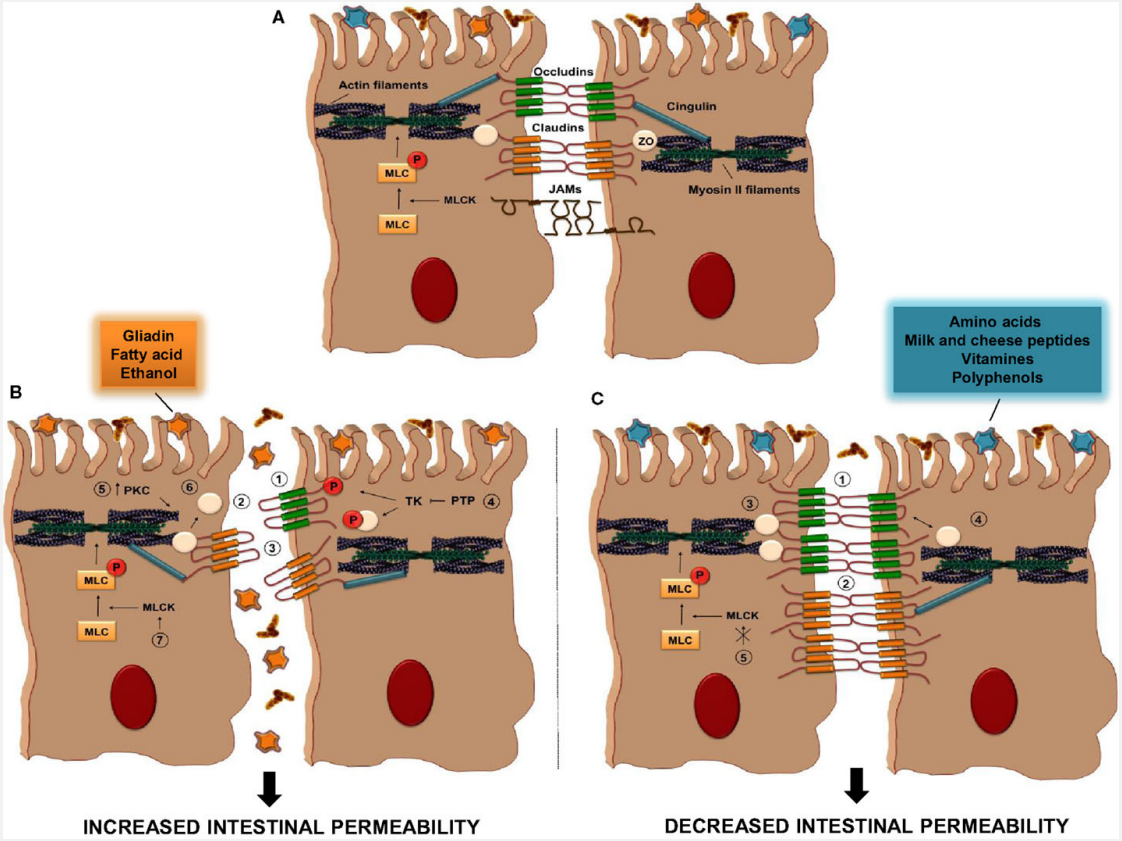
Leaky Gut As a Danger Signal for Autoimmune Diseases

Qinghui Mu[1], Jay Kirby[1], Christopher M. Reilly[2] and Jin M. Luo[1]*

Seguella L, Sarnelli G, Esposito G (2020) Leaky gut, dysbiosis, and enteric glia activation: the trilogy behind the intestinal origin of Parkinson's disease. Neural Regen Res 15(6):1037-1038. doi:10.4103/1673-5374.270308

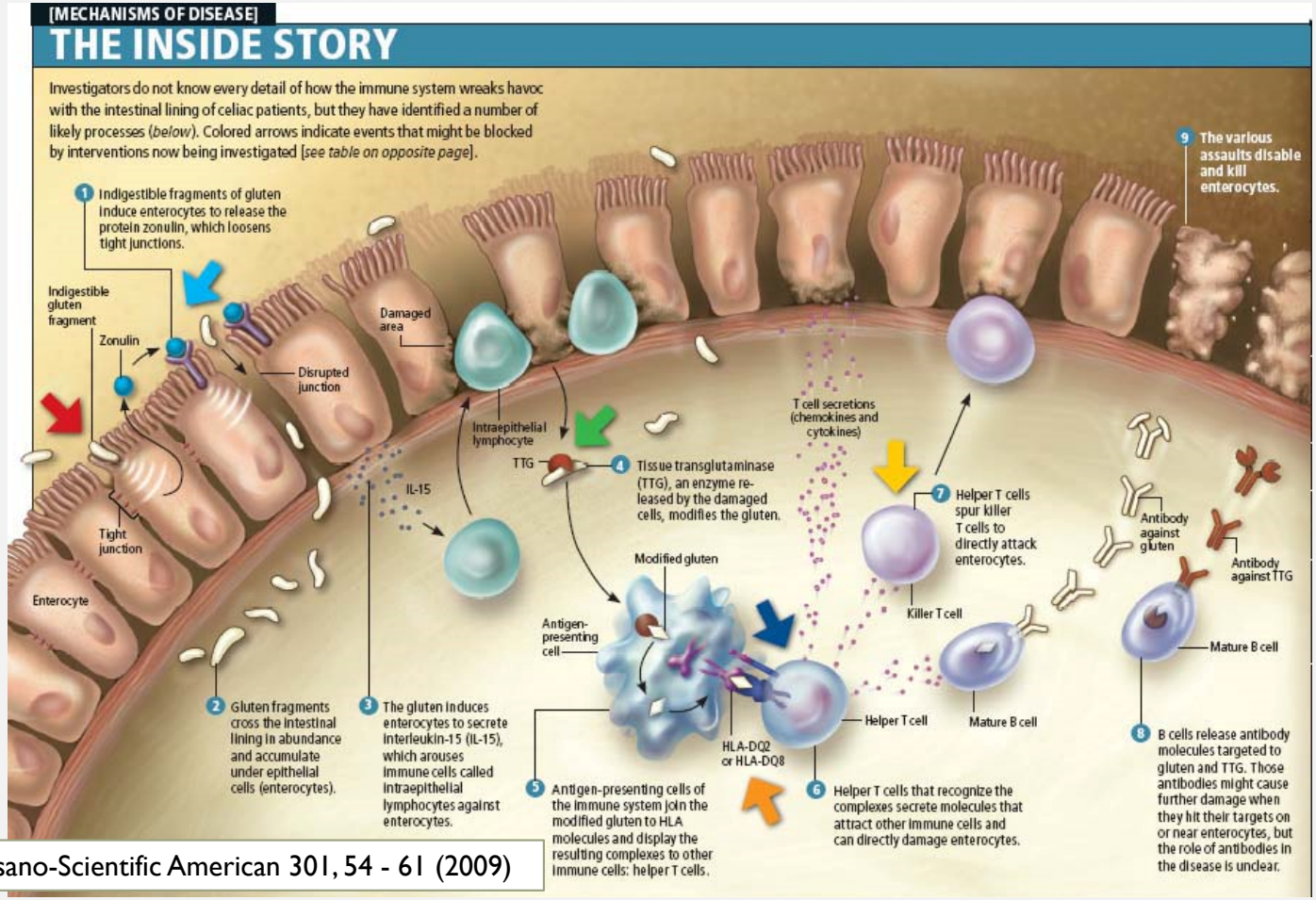


MORE COMMON SCENARIO



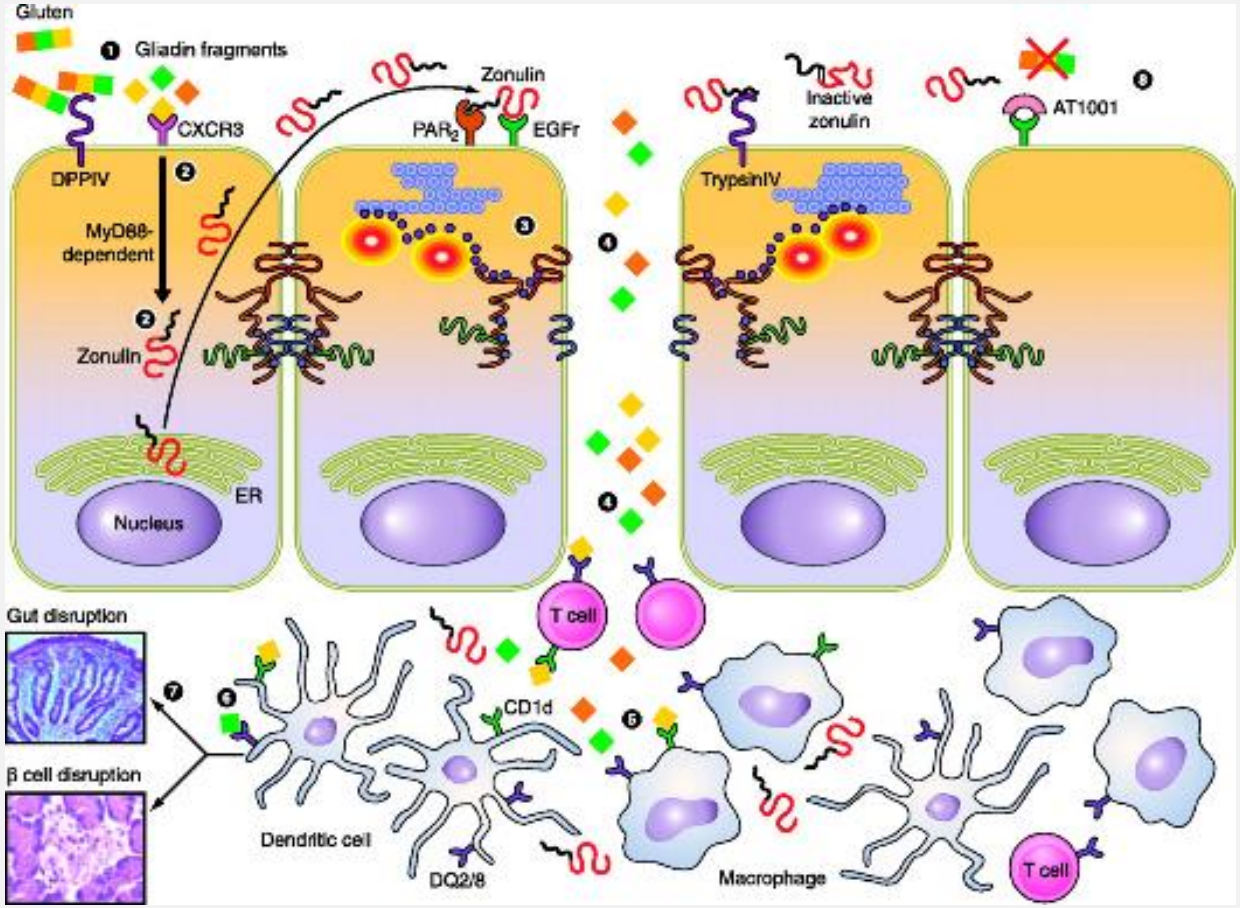
Front. Immunol., 07 December 2015 | https://doi.org/10.3389/fimmu.2015.00612

WHAT WE CAN LEARN FROM CELIAC DISEASE



A. Fasano-Scientific American 301, 54 - 61 (2009)

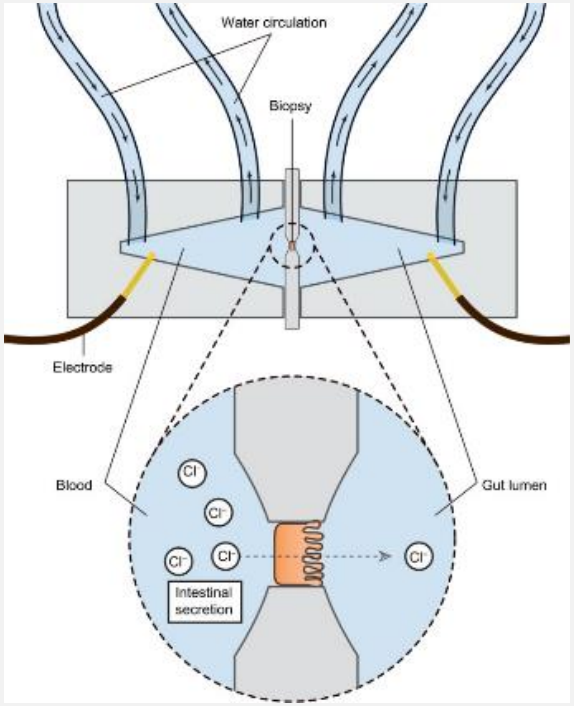
Mechanisms of gliadin-induced zonulin release, increased intestinal permeability, and onset of autoimmunity.



Zonulin= pre-haptoglobulin 2

Alessio Fasano Physiol Rev 2011;91:151-175

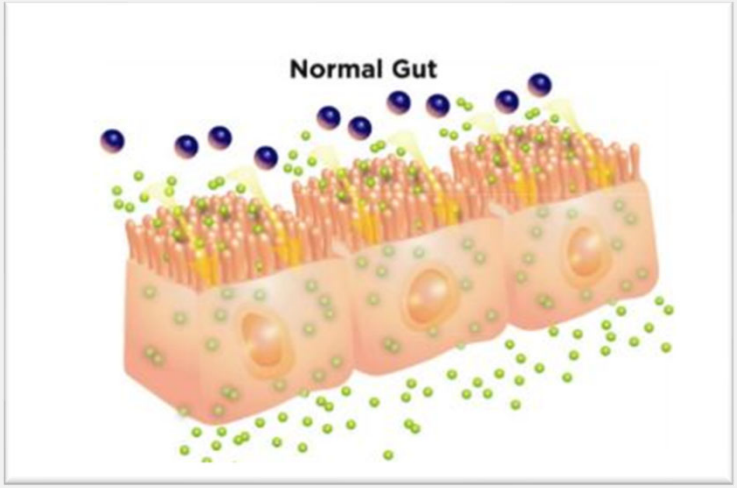
MEASURING GUT BARRIER FUNCTION

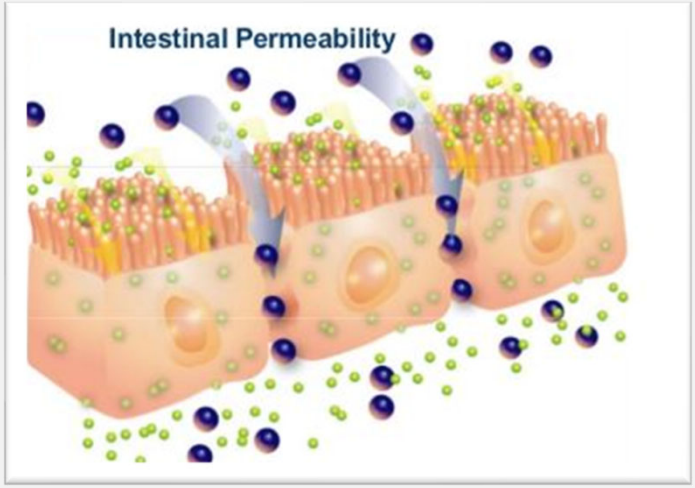


- Gold Standard: Ex-VIVO Ussing Chamber
- Biopsied Tissue (Or experimental monolayer) oriented across membrane
- Can measure Transepithelial electrical resistance (TEER)
- Model system for measuring insults to Gut epithelium
- No support cell structures, no microbiome etc.

MEASURING GUT BARRIER FUNCTION

- In Vivo: size Exclusion Test (Urine Analysis)





- Lactulose/Mannitol Test most common
- Mannitol is general measure of gut area, denominator can be altered (low) during atrophy (Celiac, Inflammation etc.)- Ratio can rise even when lactulose levels do not increase due to low mannitol absorption
- Other test reagents: rhamnose, different size PEG molecules etc.
- Be careful to follow dietary and timing instructions to prevent false interpretations

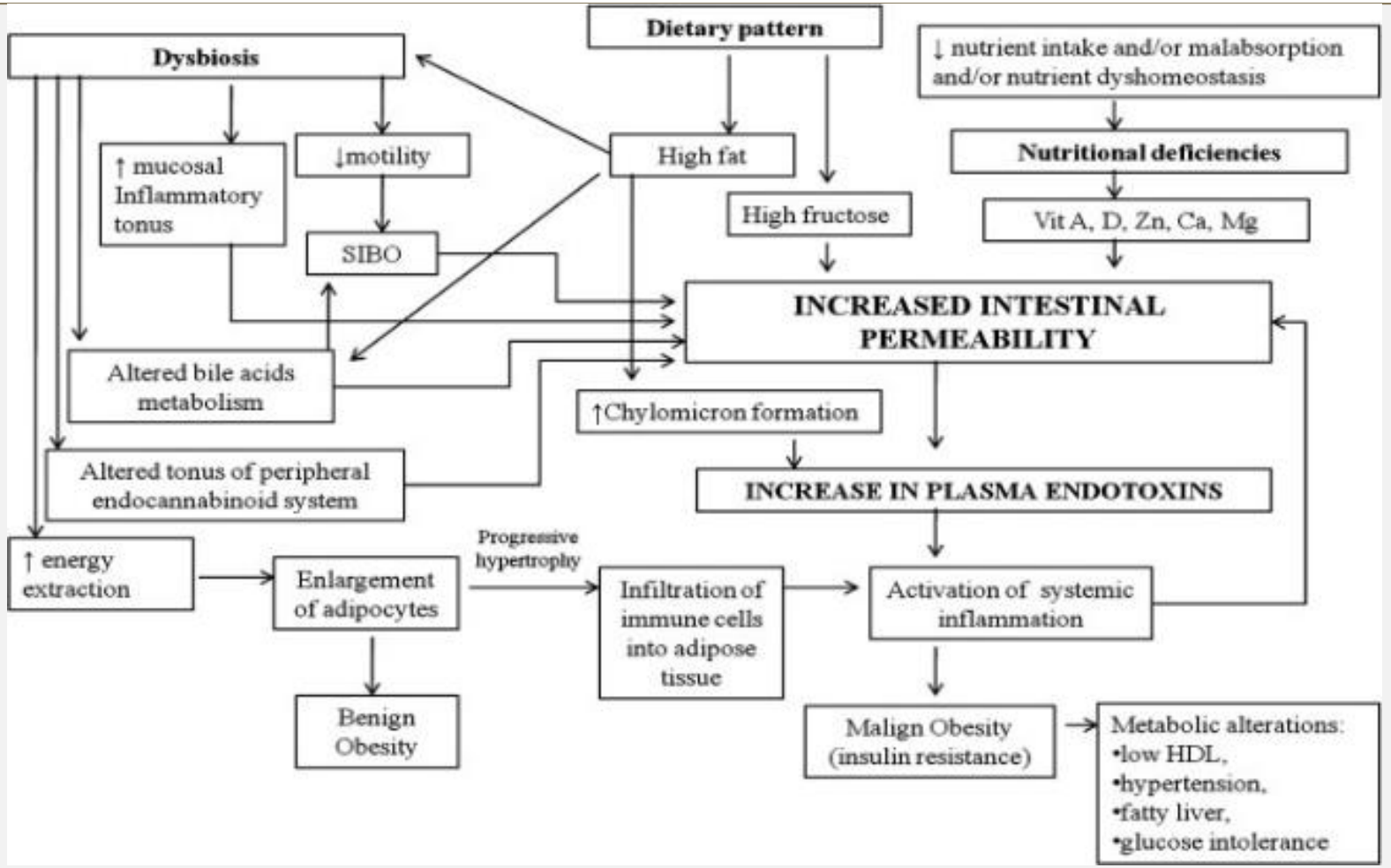
OTHER (POTENTIAL) MEASURES OF GUT PERMEABILITY

- Urine/Serum levels of microbial metabolites: D-lactate, endotoxin etc.
- Increased level of bacteria in dense mucus (biopsy)
- Reduced plasma citrulline (biomarker of Glutamine)
- Fecal Calprotectin (Inflammation)
- Measures of TJ proteins [ZO, Claudins, Occludin etc.)
- Serum [or FECAL?] zonulin

GI CONDITIONS FOR WHICH BARRIER FUNCTION IS OFTEN COMPROMISED

- GI Infections (V. Cholera, EH E.coli, C. diff, H. pylori)
- Gut inflammation of any kind likely triggers some gut permeability
- Celiac Disease and 30% of asymptomatic relatives.
- Inflammatory Bowel Disease (both UC and Crohn's)
- IBS-D (though not stat. sig. in all studies)
- SIBO?

Gut Permeability connected to Obesity, Insulin Resistance and the Western dietary Pattern



Nutrition Research, 2012-09-01, Volume 32, Issue 9, Pages 637-647

ZONULIN LEVELS ARE OFTEN INCREASED IN OBESE SUBJECTS AND TYPE 2 DIABETICS

J Nutr. 2016 Sep;146(9):1694-700. doi: 10.3945/jn.116.235358. Epub 2016 Jul 27.

Gut Microbiota Richness and Composition and Dietary Intake of Overweight Pregnant Women Are Related to Serum Zonulin Concentration, a Marker for Intestinal Permeability.

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Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects.

PLoS One. 2012;7(5):e37160. doi: 10.1371/journal.pone.0037160. Epub 2012 May 18.

Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance.

Eur J Endocrinol. 2015 Jan;172(1):29-36. doi: 10.1530/EJE-14-0589. Epub 2014 Oct 21.

Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation.

THE FUNCTIONAL COMPONENTS OF THE GUT BARRIER

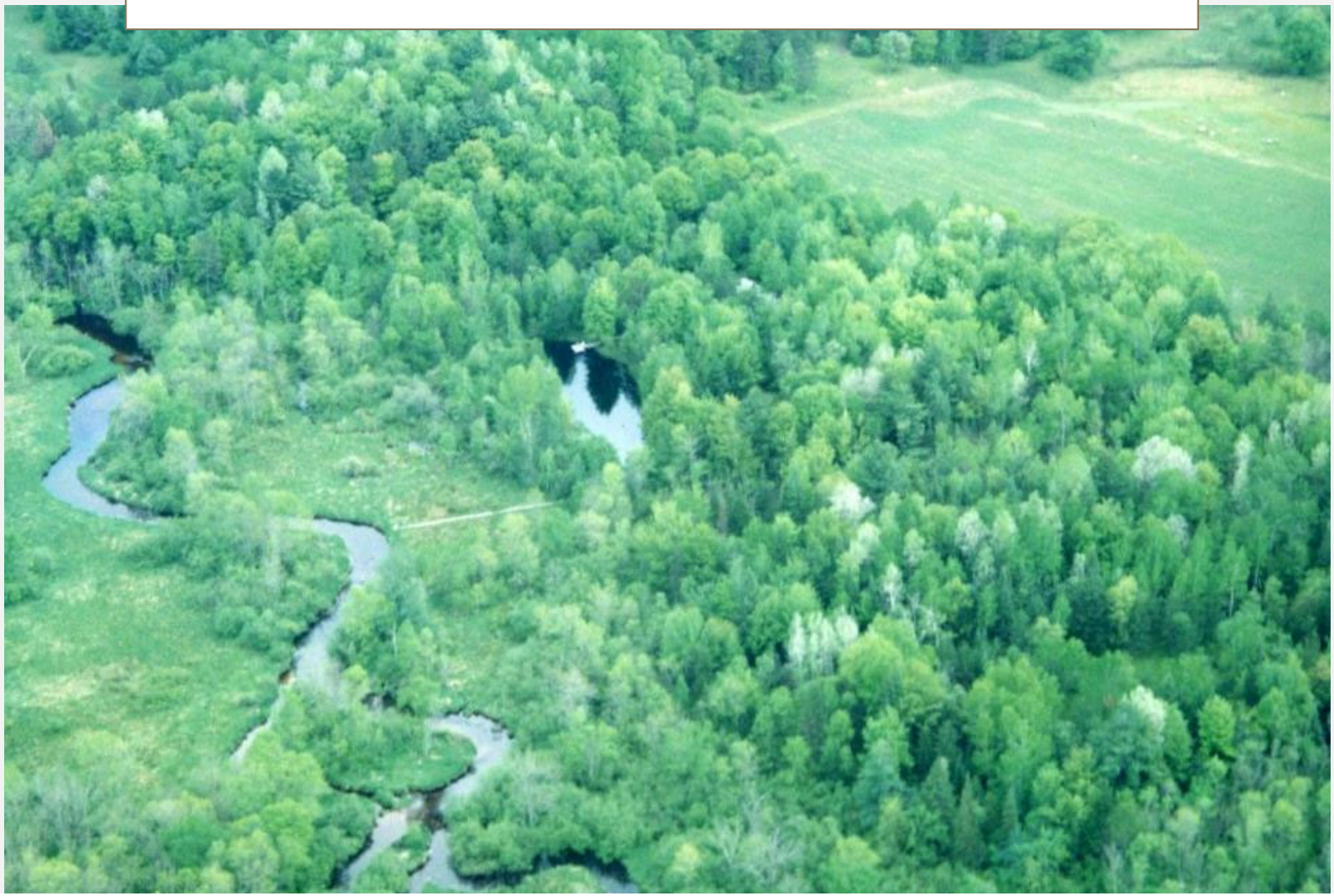
- Human GI cells that create the interface (Enterocytes, Colonocyte etc.)
- Human Immune cells that line the inside or penetrate the interface
- Human Neuroendocrine cells and neurons with synapses nearby.
- Luminal Excretions from human cells (Mucus, sIGA, anti-microbial peptides, enzymes, acid, neurotransmitters etc.)
- Non-Human microbes in the lumen and mucus lining
  - Commensal, Pathobiont, Pathogenic Bacteria
  - Viruses (free and bacteriophages)
  - Fungi
  - Non-human eukaryotic organisms (are any of these commensals?)

IS THIS STILL THE CURRENT STATE OF MICROBIOTA KNOWLEDGE?

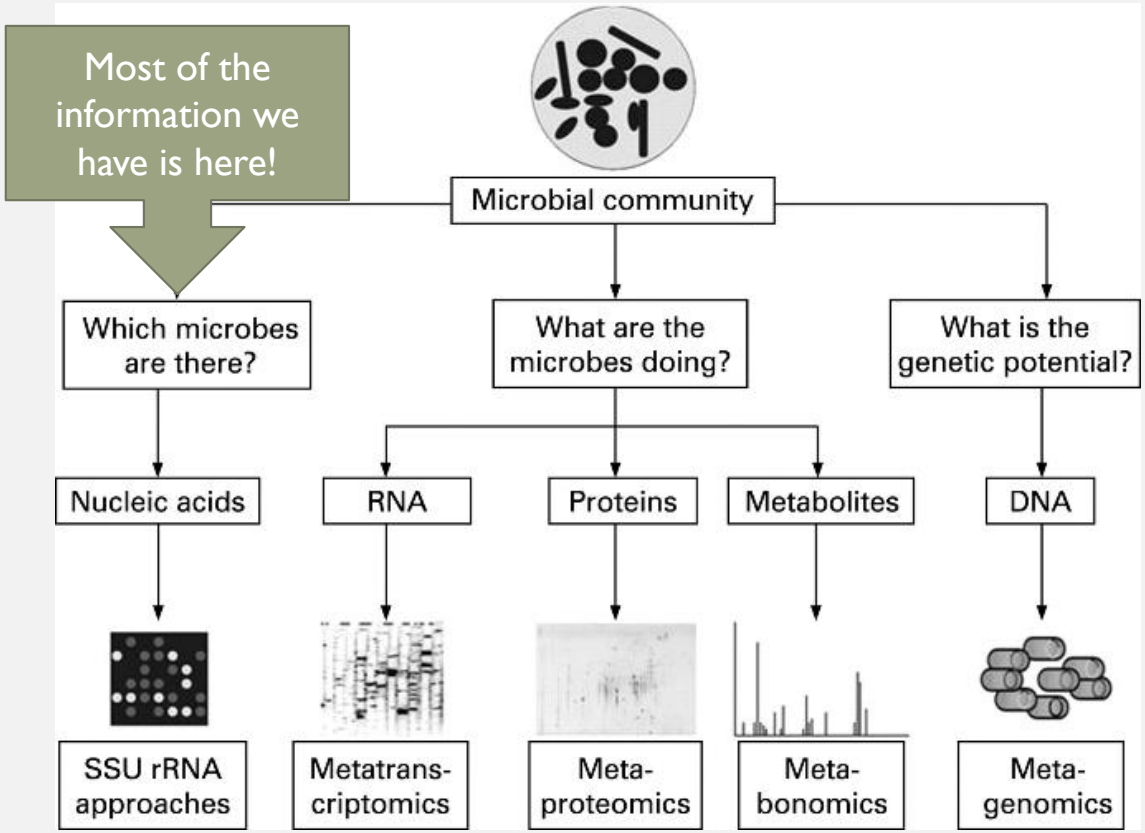
NOMENCLATURE ISSUES

- Commensal Organisms:
  - the totality of nonpathogenic Organisms that are "natural" residents in or on the host (supplied through the environment or diet).
  - This term can also be used to distinguish these "natural" organisms from "supplemented" organisms that are generally incapable of long-term GI residence(e.g., probiotics).
- Pathobiont:
- This is a commensal organism with the potential for pathogenic activity that, in some circumstances, can trigger negative outcomes for the host (e.g., antibiotics and *C. diff.*). These might require the presence of other microorganisms, host immune system dysfunctions or other unknown factors to become pathogenic.

WHAT'S IN YOUR ECOSYSTEM?

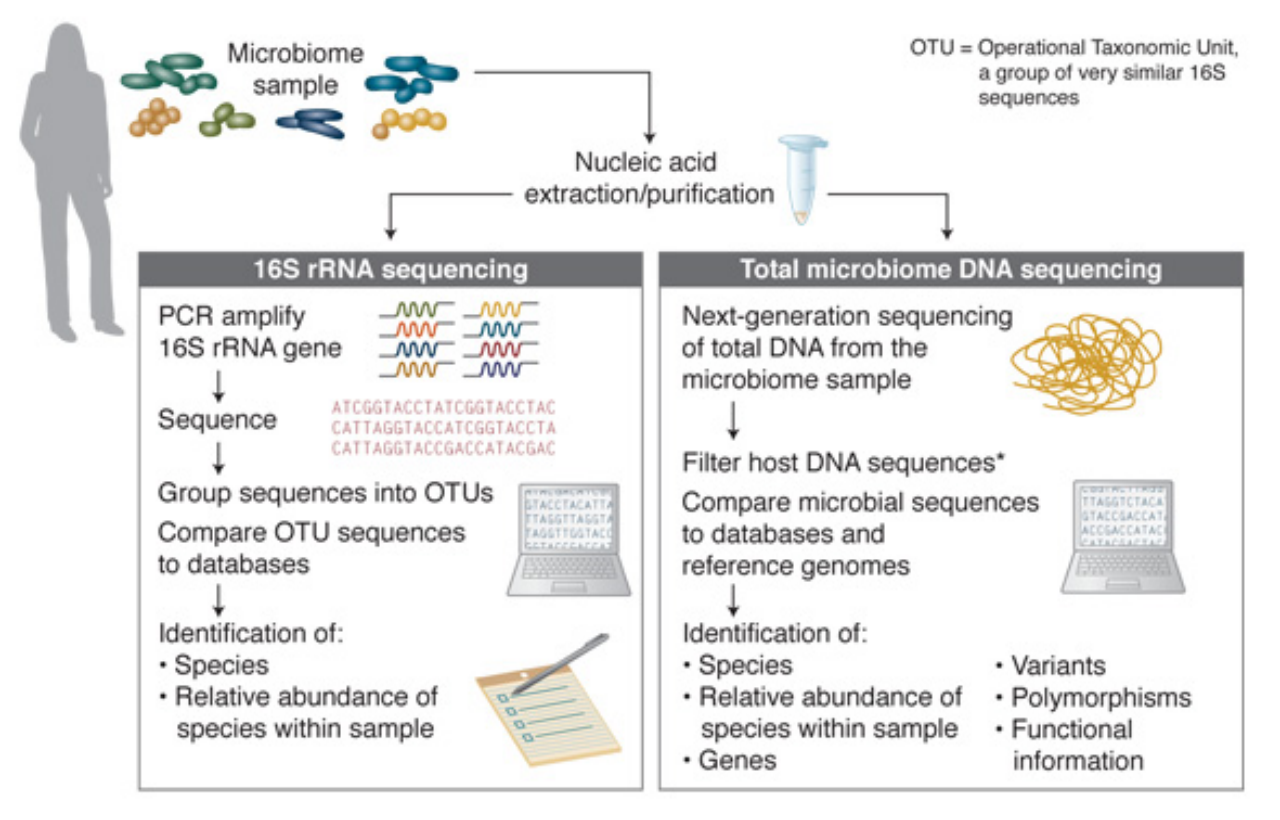


WHAT IS THE MOST IMPORTANT TO KNOW?



E G Zoetendal et al. Gut 2008;57:1605-1615

MICROBIOME DNA ANALYSIS



MORE DEFINITIONS

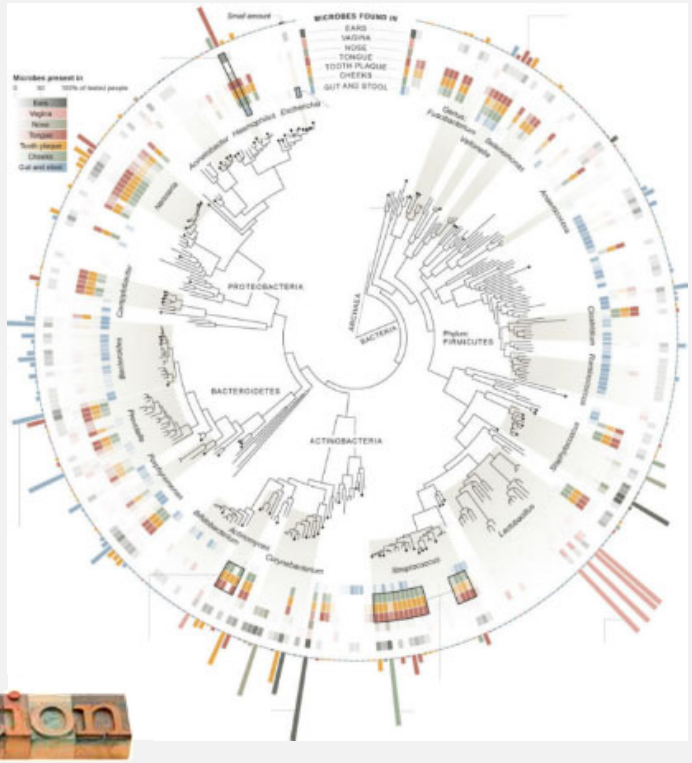
- Operational Taxonomic Unit (OTU):

- This operational definition of species is used when only genetic material (mostly 16S rRNA) is analyzed to distinguish one species from another. Since many bacteria within the gut microbiome cannot be isolated, grown and investigated in a laboratory setting, they are identified by their genetic sequences and classified into OTUs. Diversity is often described as the number of OTUs. **For the clinician, this is functionally identical to the number of species.**

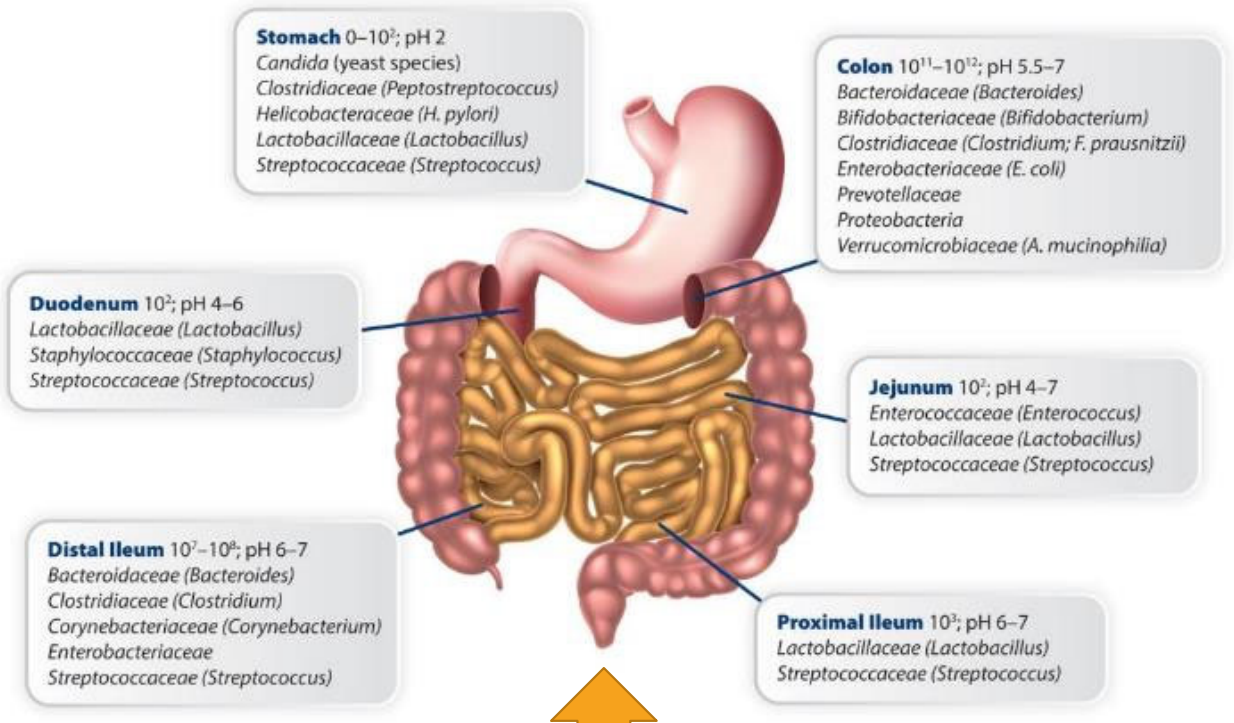
DNA TESTING CONUNDRUM

- There is a debate amongst researchers as to the most appropriate measures of metagenetic information to define a person's or a population's microbial species.

- Clinically-speaking- we have extremely limited knowledge as to what to do with this information, how to define an ideal microbiome (if such exists) or how to predictably manipulate the microbial environment in a given subject.



MICROBIOME(S) IN THE GUT

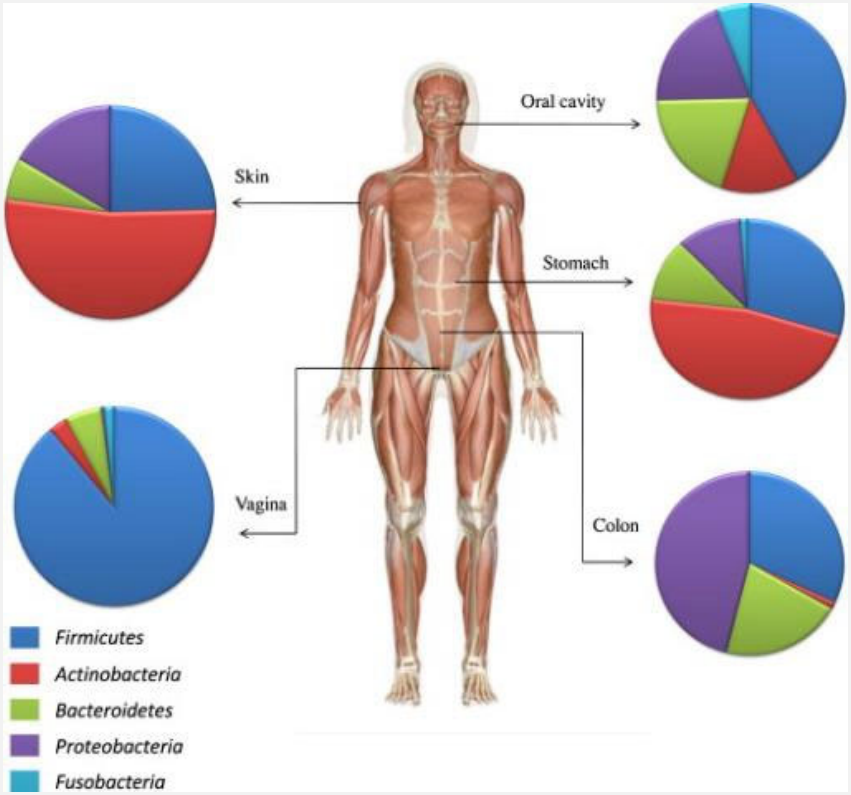


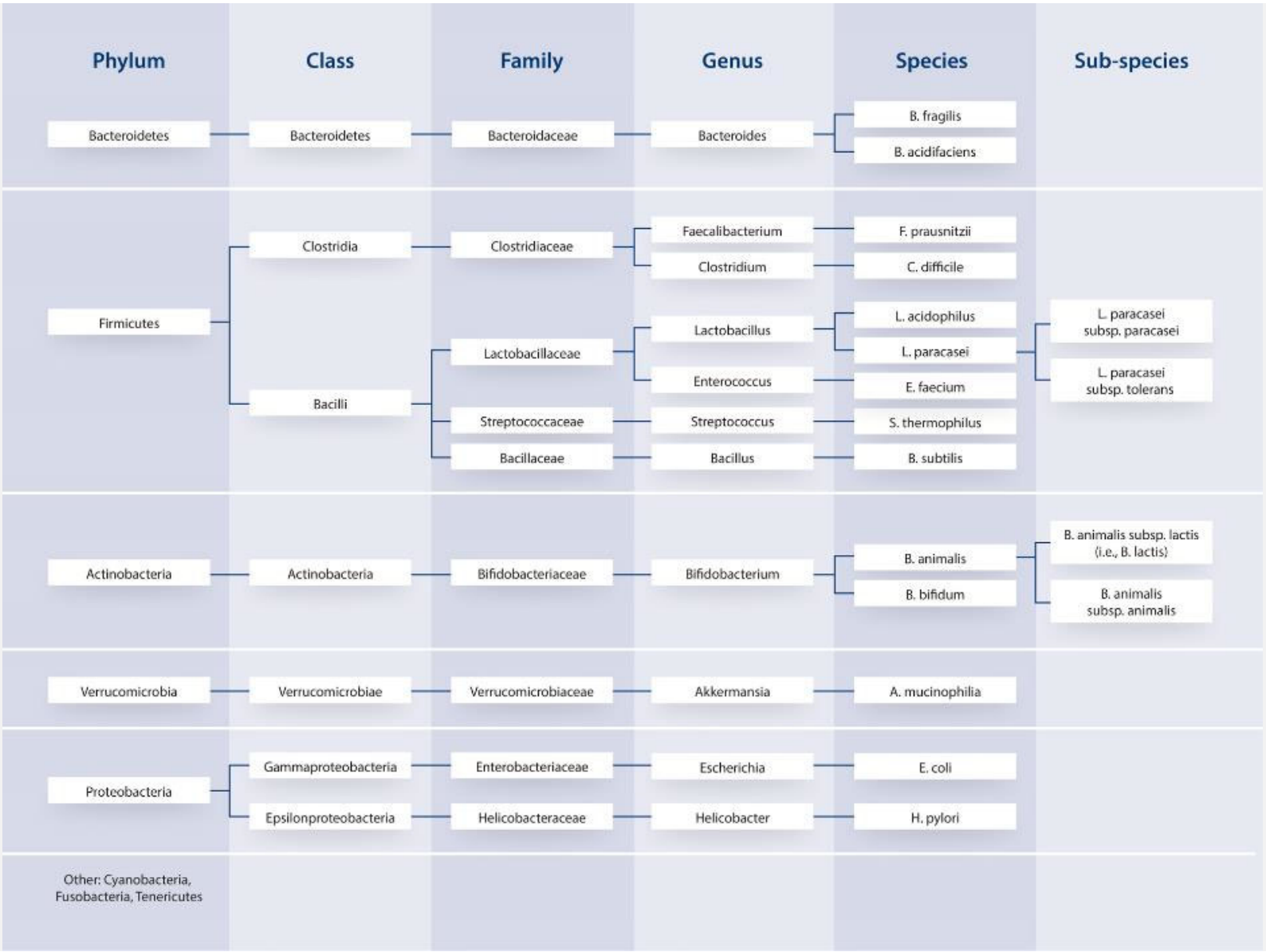
Fecal Microbiota Analysis (by any means) is only a biomarker of these microbiomes (heavily skewed to the distal colon)

WHAT IS THE BEST WAY TO DEFINE AN INDIVIDUAL'S GUT MICROBIOME?

- Phylum level Difference?
- Enterotypes?
- Specific OTUs?
- Overall Diversity?
- Presence or absence of specific species?

PHYLUM LEVEL MEASUREMENTS




Phylum
Class
Family
Genus
Species
Sub-species
Bacteroidetes
Bacteroidetes
Bacteroidaceae
Bacteroides
B. fragilis
B. acidifaciens
Firmicutes
Clostridia
Clostridiaceae
Faecalibacterium
F. prausnitzii
Clostridium
C. difficile
Bacilli
Lactobacillaceae
Lactobacillus
L. acidophilus
L. paracasei
L. paracasei subsp. paracasei
L. paracasei subsp. tolerans
Enterococcus
E. faecium
Streptococcaceae
Streptococcus
S. thermophilus
Bacillaceae
Bacillus
B. subtilis
Actinobacteria
Actinobacteria
Bifidobacteriaceae
Bifidobacterium
B. animalis
B. animalis subsp. lactis (i.e., B. lactis)
B. bifidum
B. animalis subsp. animalis
Verrucomicrobia
Verrucomicrobiae
Verrucomicrobiaceae
Akkermansia
A. mucinophilia
Proteobacteria
Gammaproteobacteria
Enterobacteriaceae
Escherichia
E. coli
Epsilonproteobacteria
Helicobacteraceae
Helicobacter
H. pylori
Other: Cyanobacteria, Fusobacteria, Tenericutes

PNAS

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo[a], Duccio Cavalieri[a], Monica Di Paola[b], Matteo Ramazzotti[c], Jean Baptiste Poullet[d], Sebastien Massart[d], Silvia Collini[b], Giuseppe Pieraccini[a], and Paolo Lionetti[b,1]

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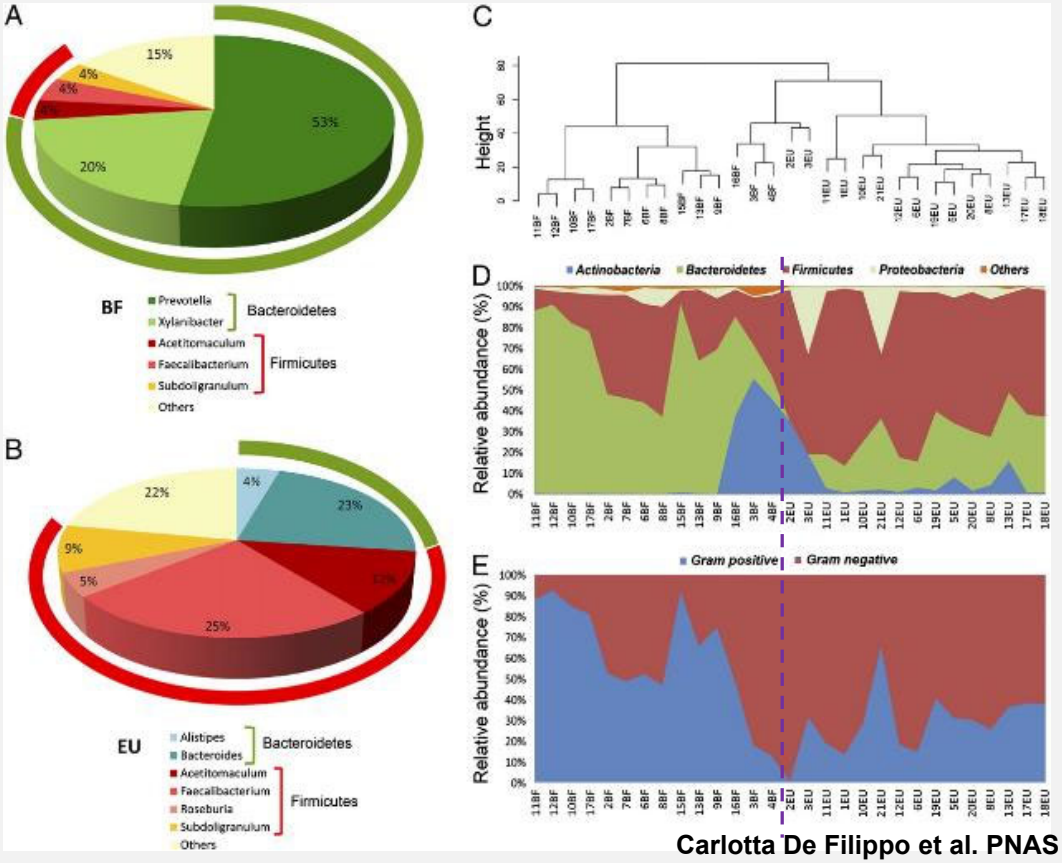


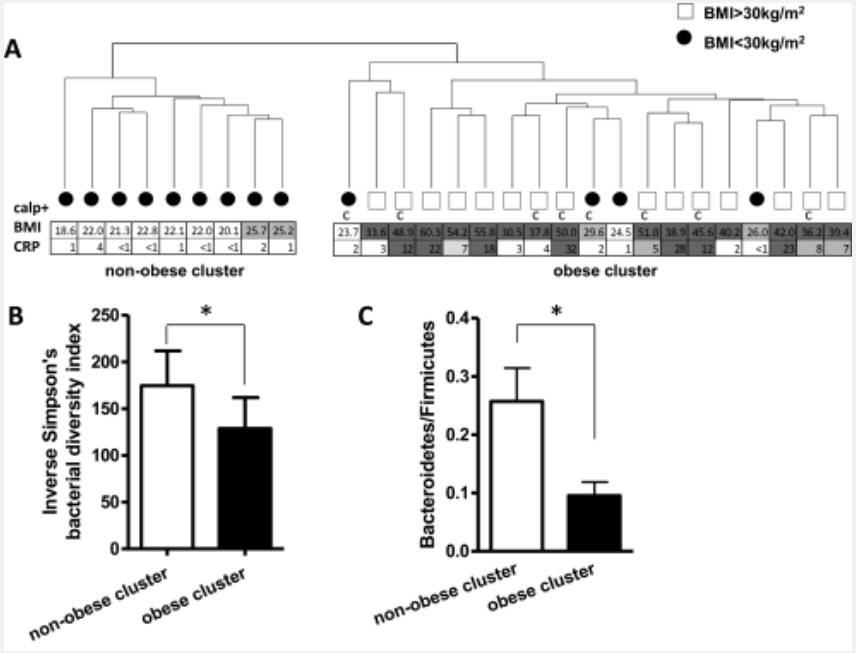
Carlotta De Filippo et al. PNAS 2010;107:14691-14696

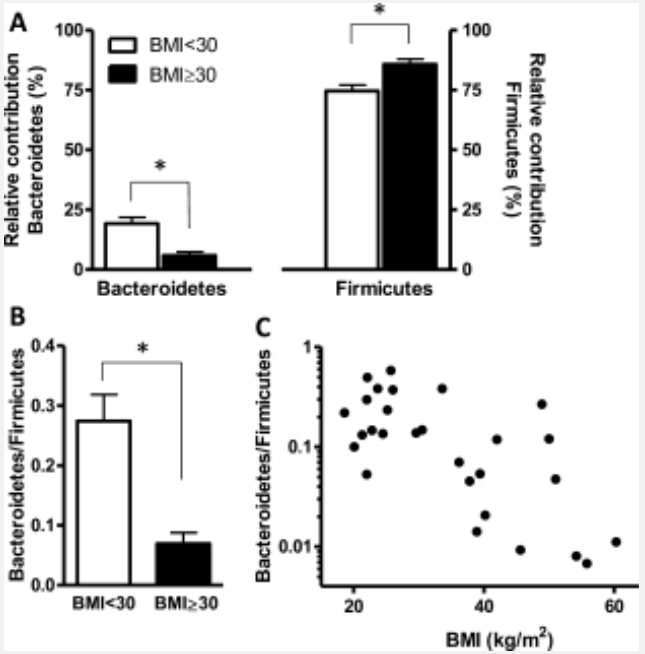
Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Human Intestinal Microbiota Composition Is Associated with Local and Systemic Inflammation in Obesity

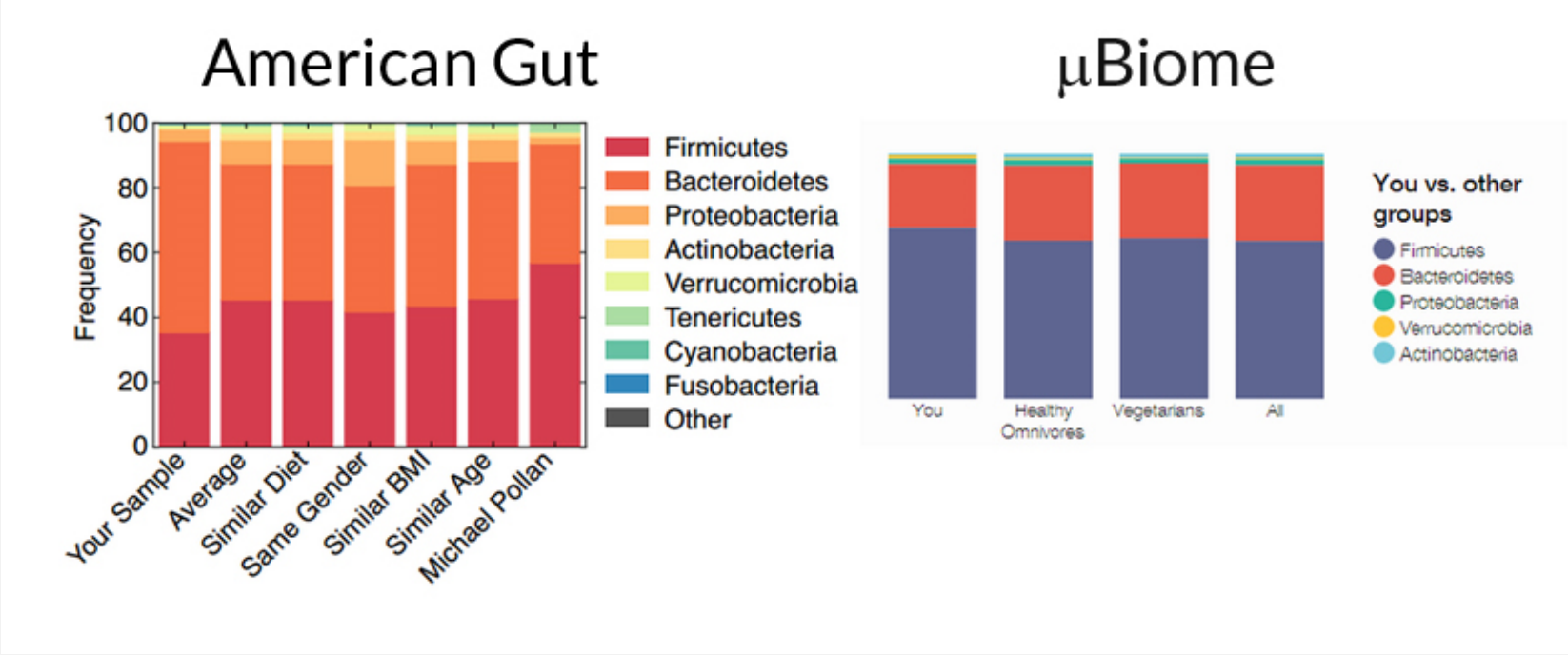
*Froukje J. Verdam,[1,2] Susana Fuentes,[3] Charlotte de Jonge,[1,2] Erwin G. Zoetendal,[3] Runi Erbil,[1] Jan Willem Greve,[1,2] Wim A. Buurman,[1] Willem M. de Vos[3] and Sander S. Rensen[1]**





COMMERCIAL LABS AND PHYLUM REPORTING

(DOES THIS TELL THE PATIENT ANYTHING?)



ENTEROTYPES

ARTICLE

doi:10.1038/nature09944

Enterotypes of the human gut microbiome

Manimozhiyan Arumugam[1]*, Jeroen Raes[1,2]*, Eric Pelletier[3,4,5], Denis Le Paslier[3,4,5], Takuji Yamada[1], Daniel R. Mende[1], Gabriel R. Fernandes[1,6], Julien Tap[1,7], Thomas Bruls[3,4,5], Jean-Michel Batto[7], Marcelo Bertalan[8], Natalia Borruel[9], Francesc Casellas[9], Leyden Fernandez[10], Laurent Gautier[8], Torben Hansen[11,12], Masahira Hattori[13], Tetsuya Hayashi[14], Michiel Kleerebezem[15], Ken Kurokawa[16], Marion Leclerc[7], Florence Levenez[7], Chaysavanh Manichanh[9], H. Bjørn Nielsen[8], Trine Nielsen[11], Nicolas Pons[7], Julie Poulain[3], Junjie Qin[17], Thomas Sicheritz-Ponten[8,18], Sebastian Tims[15], David Torrents[10,19], Edgardo Ugarte[3], Erwin G. Zoetendal[15], Jun Wang[17,20], Francisco Guarner[9], Oluf Pedersen[11,21,22,23], Willem M. de Vos[15,24], Søren Brunak[8], Joel Doré[7], MetaHIT Consortium†, Jean Weissenbach[3,4,5], S. Dusko Ehrlich[7] & Peer Bork[1,25]

Our knowledge of species and functional composition of the human gut microbiome is rapidly increasing, but it is still based on very few cohorts and little is known about variation across the world. By combining 22 newly sequenced faecal metagenomes of individuals from four countries with previously published data sets, here we identify three robust clusters (referred to as enterotypes hereafter) that are not nation or continent specific. We also confirmed the enterotypes in two published, larger cohorts, indicating that intestinal microbiota variation is generally stratified, not continuous. This indicates further the existence of a limited number of well-balanced host–microbial symbiotic states that might respond differently to diet and drug intake. The enterotypes are mostly driven by species composition, but abundant molecular functions are not necessarily provided by abundant species, highlighting the importance of a functional analysis to understand microbial communities. Although individual host properties such as body mass index, age, or gender cannot explain the observed enterotypes, data-driven marker genes or functional modules can be identified for each of these host properties. For example, twelve genes significantly correlate with age and three functional modules with the body mass index, hinting at a diagnostic potential of microbial markers.

Various studies of the human intestinal tract microbiome based on the 16S ribosomal-RNA-encoding gene reported species diversity within and between individuals[1–3], and the first metagenomics studies characterized the functional repertoire of the microbiomes of several American[4,5] and Japanese[6] individuals. Although a general consensus about the phylum level composition in the human gut is emerging[1,4,7], the variation in species composition[1,2] and gene pools[5,8] within the human population is less clear. Furthermore, it is unknown whether [illegible] continuum of different [illegible] microbiota con[illegible]munity composi[illegible]is complicated by [illegible]ocessing, sequen[illegible]ysiological, nutri[illegible] the feasibility of [illegible]ross cohorts and [illegible]alities and differ[illegible] populations, we [illegible] Danish, French, [illegible]or diversity (Sup[illegible]th existing Sanger (13 Japanese[6], 2 American[5]) and pyrosequencing (2 American[5]) gut data sets—totalling 39 individuals.

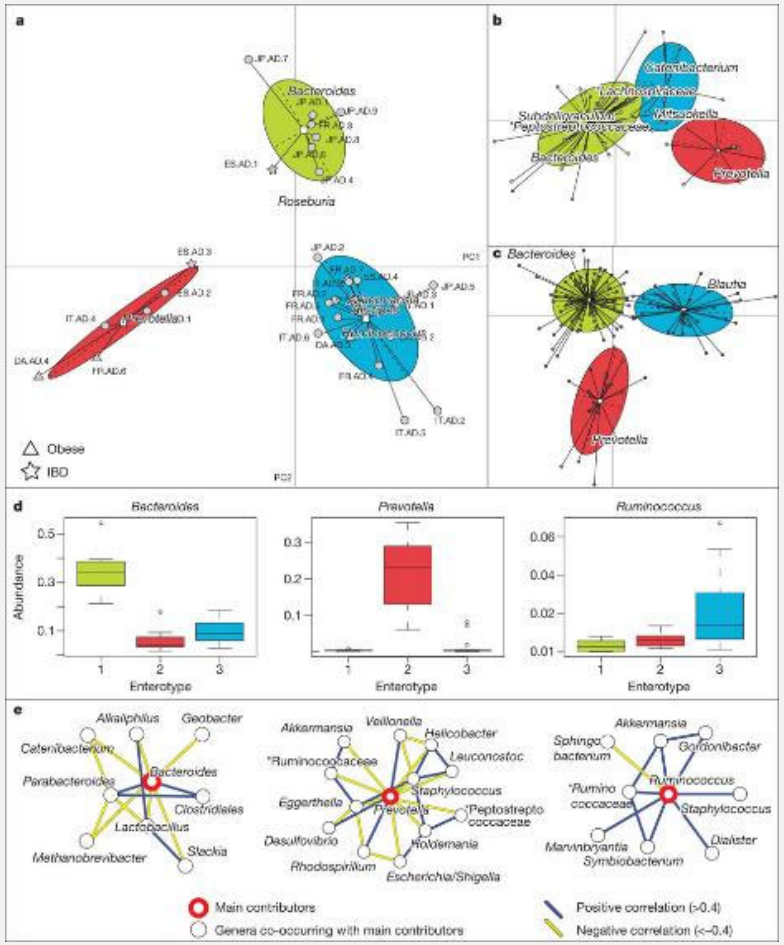
Global variation of human gut metagenomes

The vast majority of sequences in the newly sequenced 22 European samples belong to bacteria—only 0.14% of the reads could be classified as human contamination, all other eukaryotes together only comprised 0.5%, archaea 0.8% and viruses up to 5.8% (see Supplementary Notes section 2.1 for details).

To investigate the phylogenetic composition of the 39 samples from 6 nationalities, we mapped metagenomic reads, using DNA sequence homology, to 1,511 reference genomes (Supplementary Table 3) including 379 publicly available human microbiome genomes generated through the National Institutes of Health (NIH) Human Microbiome Project[10] and the European MetaHIT consortium[11] (Supplementary Methods section 4.1). To consistently estimate the functional composition of the samples, we annotated the predicted genes from the metagenomes using eggNOG[13] orthologous groups (Supplementary Methods section 6.2). We ensured that comparative analysis using these procedures was not biased by data-set origin, sample preparation, sequencing technology and quality filtering (see Supplementary Notes section 1).

[illegible]lberg, Germany. [2]VIB—Vrije Universiteit Brussel, 1050 Brussels, Belgium. [3]Commissariat à l'Energie Atomique, Genoscope, [illegible]8030, 91 000 Evry, France. [4]Université d'Evry Val d'Essone, 91 000 Evry, France. [5]Department of Biochemistry and Immunology, [illegible]01 Belo Horizonte, Minas Gerais, Brazil. [7]Institut National de la Recherche Agronomique, 78350 Jouy en Josas, France. [8]Center for [illegible]0 Lyngby, Denmark. [9]Digestive System Research Unit, University Hospital Vall d'Hebron, Ciberehd, 08035 Barcelona, Spain. [illegible]Spain. [11]Marie Krogh Center for Metabolic Research, Section of Metabolic Genetics, Faculty of Health Sciences, University of [illegible]es, University of Southern Denmark, DK-5000 Odense, Denmark. [13]Computational Biology Laboratory Bld. The University of Tokyo [illegible]. [15]Division of Bioenvironmental Science, Frontier Science Research Center, University of Miyazaki, 5200 Kyotake, Miyazaki 889-[illegible]08A Ede, The Netherlands. [19]Tokyo Institute of Technology, Graduate School of Bioscience and Biotechnology, Department of [illegible]anagawa Pref. 226-8501, Japan. [17]BGI-Shenzhen, Shenzhen 518083, China. [18]Novo Nordisk Foundation Center for [illegible]rm, Denmark. [19]Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg Lluís Companys 23, 08010 Barcelona, Spain. [20]Department [illegible]. [21]Institute of Biomedical Science, Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark. [illegible]of Health Sciences, University of Aarhus, DK-8000 Aarhus, Denmark. [24]University of Helsinki, FI-00014 Helsinki, Finland. [25]Max

*These authors contributed equally to this work.
†Lists of authors and affiliations appear at the end of the paper.



Cell Host & Microbe
Forum

Rethinking "Enterotypes"

Dan Knights,[1,2] Tonya L. Ward,[2] Christopher E. McKinlay,[1,2] Hannah Miller,[1] Antonio Gonzalez,[3] Daniel McDonald,[3] and Rob Knight[3,4,5,6,*]
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University of Minnesota, St. Paul, MN 55108, USA
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*Correspondence: rob.knight@colorado.edu
http://dx.doi.org/10.1016/j.chom.2014.09.013

Classification of the human gut microbiome into distinct types, or "enterotypes," provides an attractive framework for understanding microbial variation in health and disease. However, as discussed here, several different methods of collapsing enterotype variation into a few discrete clusters suggest that enterotype distribution is continuous and can vary widely within an individual.

"In light of our findings, we believe that previous analyses produced overconfidence in the claim of discrete enterotypes and that continuous variation is the simpler and therefore better-supported conclusion. …….Consequently, although discrete clusters may be significantly correlated with a disease state, they may not be appropriate for predicting that disease state due to masking of important within-cluster variation in critical taxa. Finally, in a meta-analysis including both dense single-individual time series data and cross-sectional multiple-individual data, we demonstrated that a healthy adult human's microbiome can traverse much of the total variation space of healthy human gut microbiomes throughout the course of a year, providing evidence that enterotypes are fluid and continuous."

HOW STABLE IS AN ADULT'S MICROBIOME?


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RESEARCH ARTICLE

The Long-Term Stability of the Human Gut Microbiota

Jeremiah J. Faith[1,*], Janaki L. Guruge[1], Mark Charbonneau[1], Sathish Subramanian[1], Henning Seedorf[1], Andrew L. Goodman[1,†], Jose C. Clemente[3,*], Rob Knight[2,4,5], Andrew C. Heath[2], Rudolph L. Leibel[6], Michael Rosenbaum[6], Jeffrey I. Gordon[1,‡]

+ Author Affiliations

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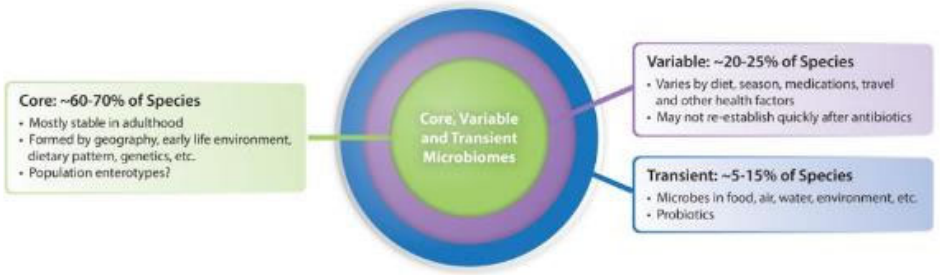
‖† Present address: Department of Microbial Pathogenesis and Microbial Diversity Institute, Yale University School of Medicine, West Haven, CT 06516, USA.

Science 05 Jul 2013:
Vol. 341, Issue 6141,
DOI: 10.1126/science.1237439

"Nonetheless, overall the set of microbial strains was remarkably stable, with over 70% of the same strains remaining after one year and few additional changes occurring over the following four years…. Finally, the stability we document highlights the impact of early colonization events on our microbiota in later life; earlier colonizers, such as those acquired from our parents and siblings, have the potential to provide their metabolic products and exert their immunologic effects for our entire lives."



From: Guilliams TG. Functional Strategies for the Management of Gastrointestinal Disorders (Point Institute, 2016)

PERSPECTIVES

OPINION

Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet?

Eamonn M. M. Quigley

Abstract | Spurred on by ever-evolving developments in analytical methodology, the microbiome, and the gut microbiome in particular, has become the hot topic in biomedical research. Ingenious experiments in animal models have revealed the extent to which the gut microbiota sustains health and how its disruption might contribute to disease pathogenesis. Not surprisingly, associations between the microbiota and disease states in humans have been the subject of considerable interest and many links proposed. However, with rare exceptions, the incrimination of an altered microbiota in disease pathogenesis seems premature at this time given our incomplete understanding of the composition of the gut microbiota in health and the effect of many confounding factors in the interpretation of supposedly abnormal microbial signatures. Future studies must account for these variables and the bidirectionality of host–microorganism interactions in health and disease. In this Perspectives, the status of microbiota signatures in the clinical arena (for facilitating diagnosis or refining prognosis) will be critically assessed and guidance toward future progress provided.

Few areas of biomedical science have witnessed such a rapid explosion in knowledge as that relating to the gut microbiome — the microbiome revolution[1]. Over the past two decades, our eyes have been opened to the various parts that our commensal bacterial populations play in keeping us healthy; not surprisingly, clinical and laboratory researchers have rushed to examine associations between the gut microbiome and various disease states. Initially, and for obvious reasons, the focus was on gastrointestinal diseases whereby examples of the effect of a disturbed gut microbiota were already present: enteric infections, *Helicobacter pylori*-related diseases and antibiotic-associated diarrhoea[2]. Over the past decade, and facilitated by rapid and ever-evolving progress in techniques that enable us to enumerate intestinal bacteria, their genes and metabolic products[3], we have witnessed claims for associations between the gut microbiota and a broad spectrum of neuropsychiatric, immunological and allergic disorders[4]. An altered microbiota has, for example, been implicated in a host of apparently diverse disorders ranging from Parkinson disease[5] and autism[6] to diabetes[7], asthma[8] and coeliac disease[9].

In a very short space of time, therefore, microbiome research has moved from the laboratory into the realms of clinical practice, for which its potential in facilitating diagnosis, predicting prognosis and guiding treatment has generated considerable interest among investigators and the biomedical industry alike. Three assumptions underlie a belief in the clinical applicability of microbiome research: first, that we know what is normal; second, that we can accurately and reproducibly define what is abnormal; and, third, and perhaps most important, that we can establish a biologically plausible and clinically meaningful relationship between a certain microbiota or microbiome profile and a given disease state.

For the purposes of this Perspectives, I will use the following definitions for clarification. The microbiota refers to the assemblage of microorganisms (and not just bacteria) present in a defined environment; by contrast, the microbiome comprises the full complement of microorganisms (bacteria, viruses, fungi and protozoa), their genes and genomes in a given locus (for example, the gut). It must be conceded that these terms are often used interchangeably in the literature to refer to microbial communities.

What is normal?

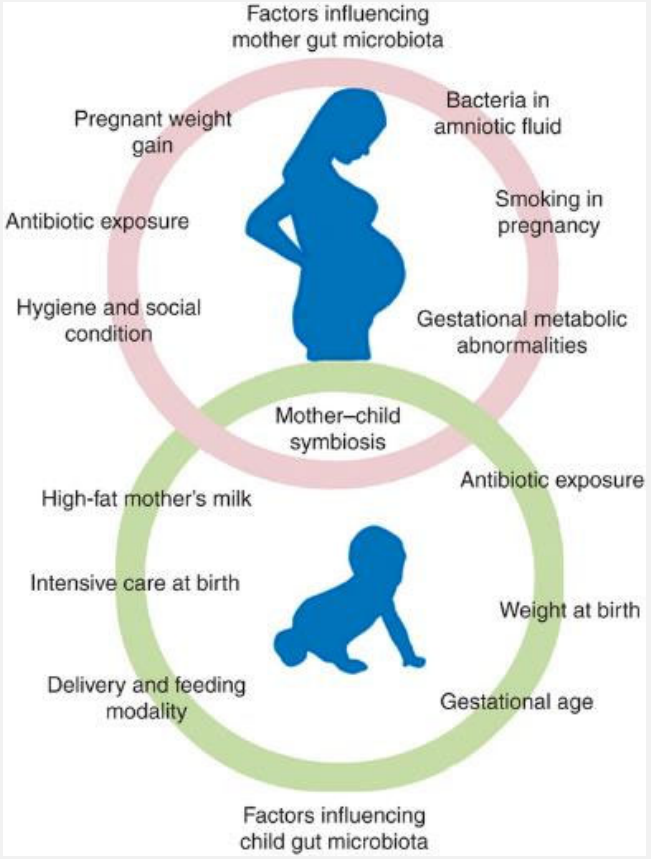
Despite advances in analytical techniques and their interpretation, our understanding of the composition and function of all of the bacterial populations, not to mention other microorganisms, such as viruses and protozoa, that inhabit various parts of the gastrointestinal tract remains incomplete[3]. Even the oft-quoted assumption of a 10:1 ratio between bacterial and human cells has been questioned[10]. Although much has been learned of the contributions of the microbiota to sustaining health[11], this progress does not mean that we can accurately define normality. Although fairly large population studies (ranging from the low hundreds to over one thousand) have demonstrated some commonality between healthy individuals at genus level, interindividual variation remains the order of the day at the level of species and strain[12–16]. As the factors that contribute to that variability are identified, one can begin to appreciate the extent to which factors — such as age[16,17], birth mode[18], breast-feeding or formula-feeding[18], diet[19], geography[18], exercise[21], other lifestyle factors, such as alcohol consumption[22], and exposure to antibiotics[23] — can affect any definition of 'normal' (FIG. 1). Diet might well be the foremost confounder of many human microbiome studies to date. Not only does the overall nature of a particular dietary pattern (for example, vegan versus vegetarian versus carnivore, or highly processed Western diet versus rural African diet) influence the microbiota but the relative amounts of specific components (carbohydrate, protein, fat, fibre) are also important[24]. Dietary habit

SOME PRECAUTIONS

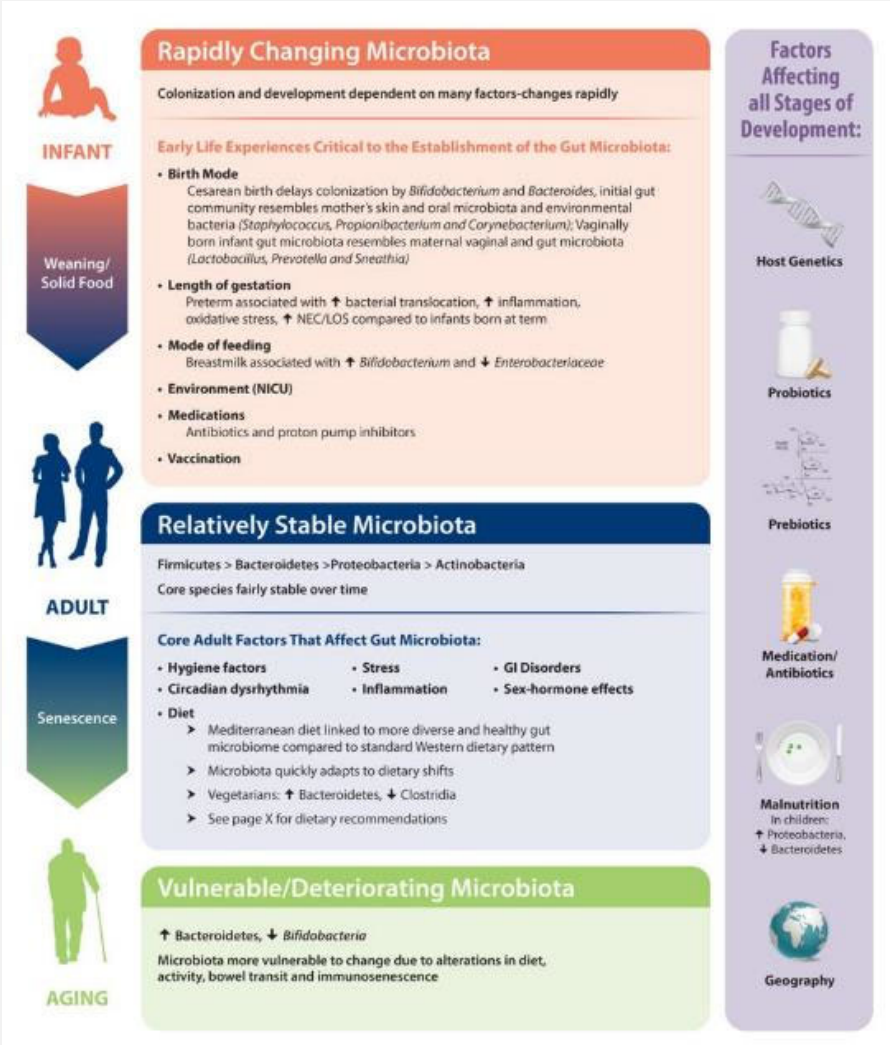
- We don't yet know what is "Normal"
- Most commercial Lab Data is not Reproducible enough to adequately deciphering Stool samples for diversity
- Stool samples may be representative of some portions, but perhaps not the most important features of the microbiome

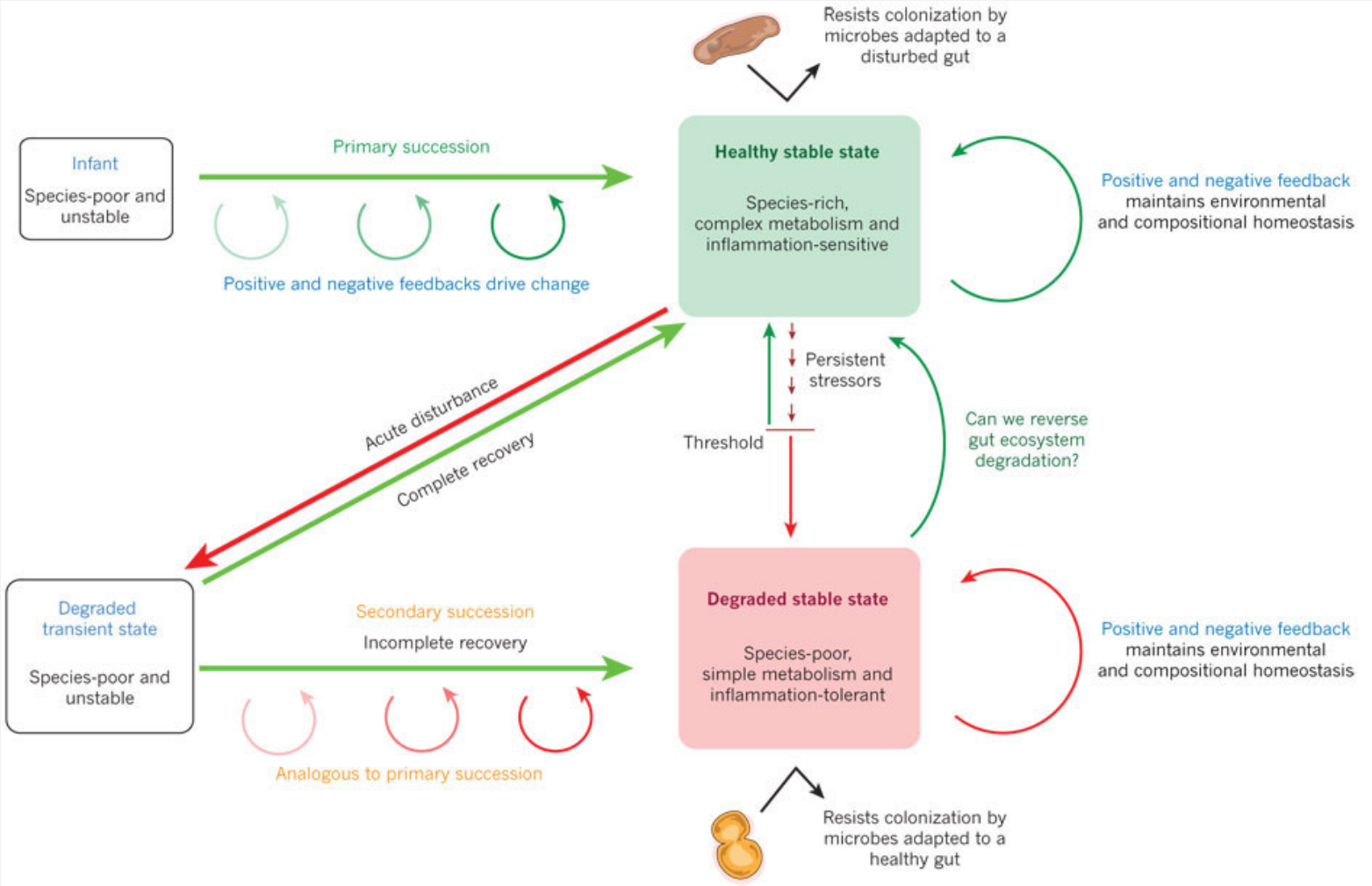
FUNCTION MATTERS

- Altered patterns within the microbiota in early life may influence health long after those patterns are discernable through microbiome testing (Know the patient's History!)



Putignani L, Del Chierico F, et al. The human gut microbiota: a dynamic interplay with the host from birth to senescence settled during childhood. *Pediatr Res*. 2014 Jul;76(1):2-10.

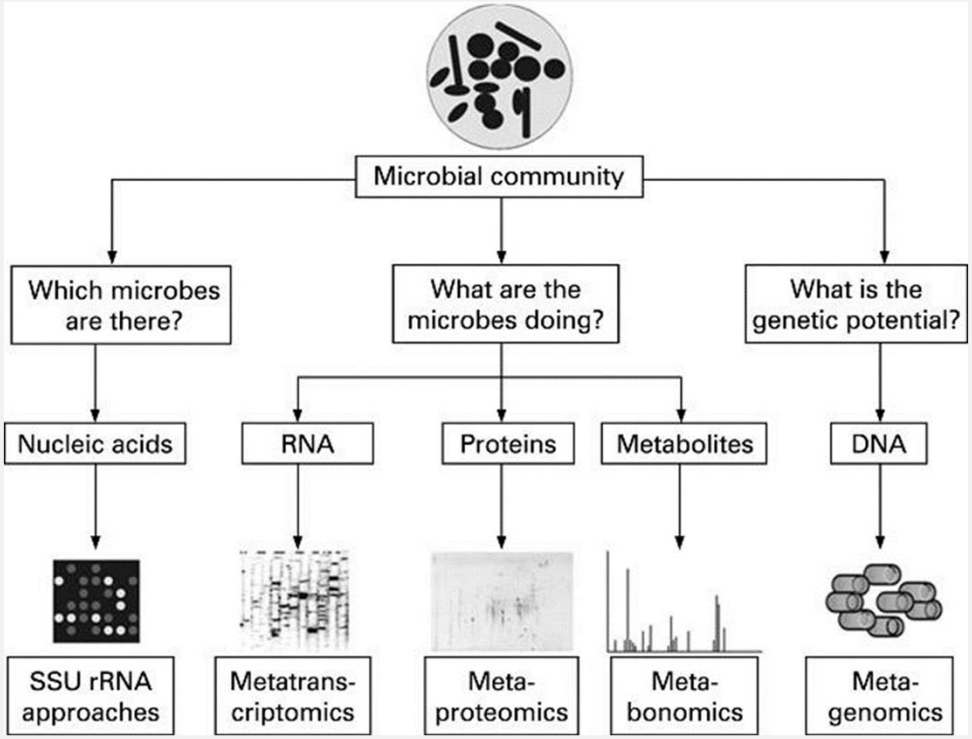




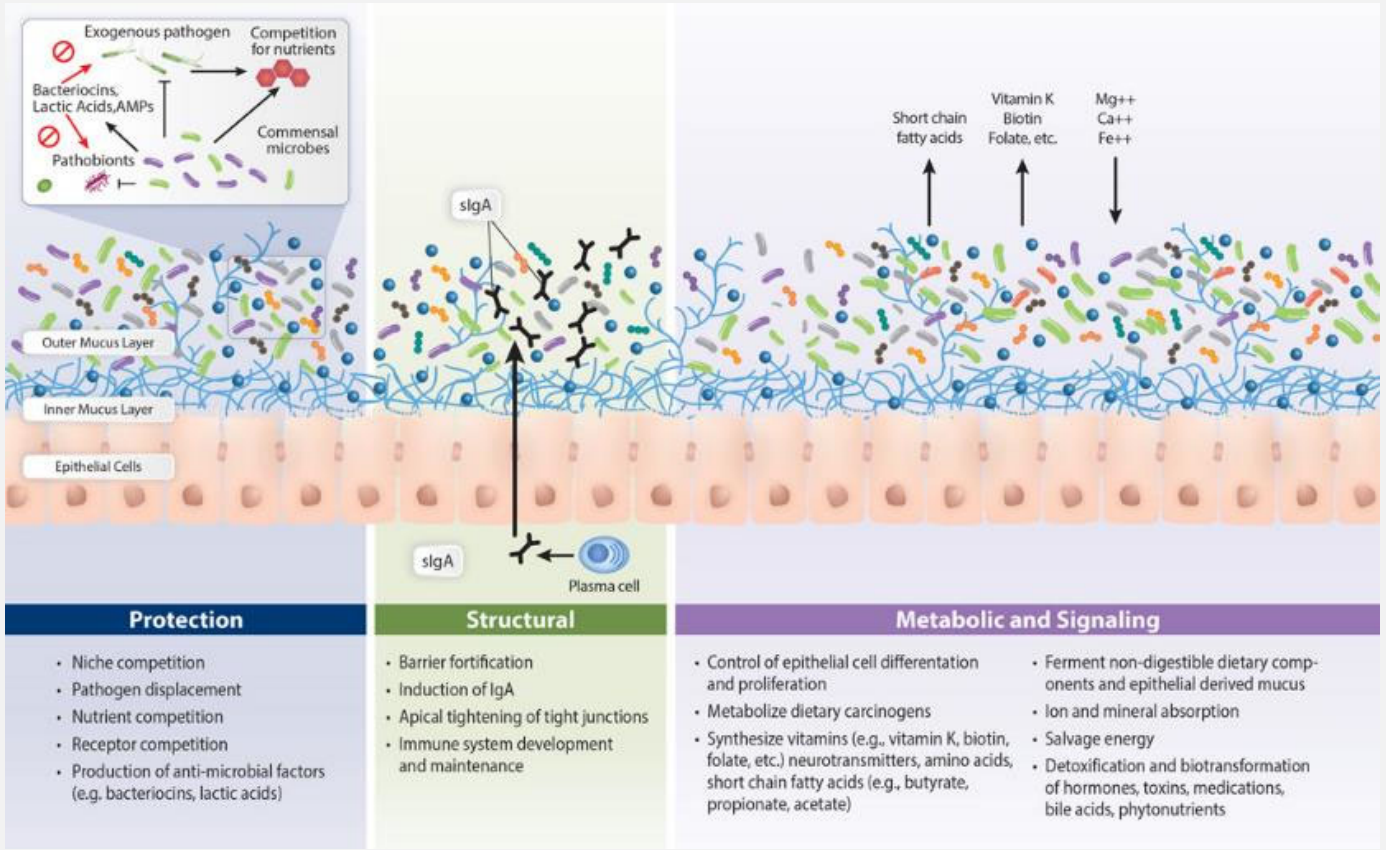
Nature 489, 220–230 (13 September 2012) doi:10.1038/nature11550

FUNCTION MATTERS

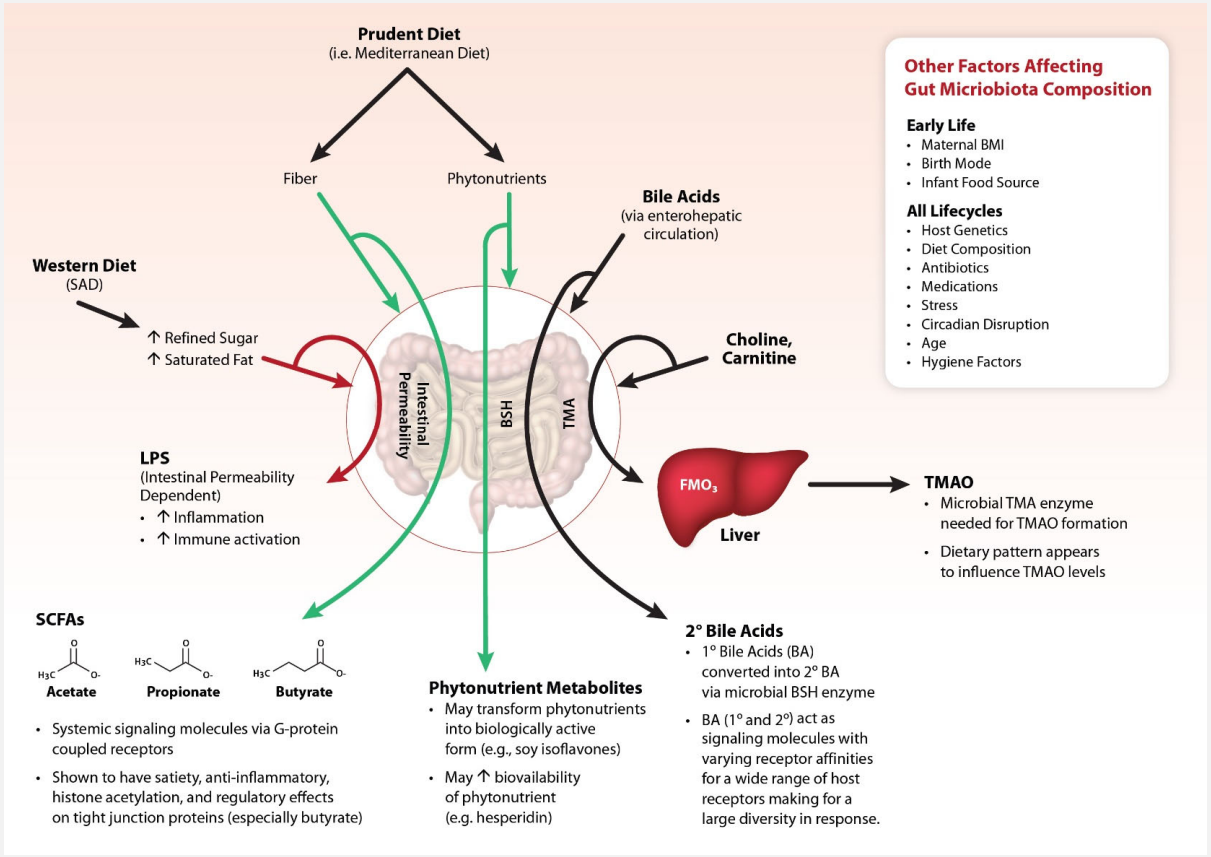
- The presence or absence of specific species of (or genes From) bacteria may be less important than the Gene expression, Proteome or Metabolome in the Gut.



THE BASIC ROLES OF GI MICROBIOTA

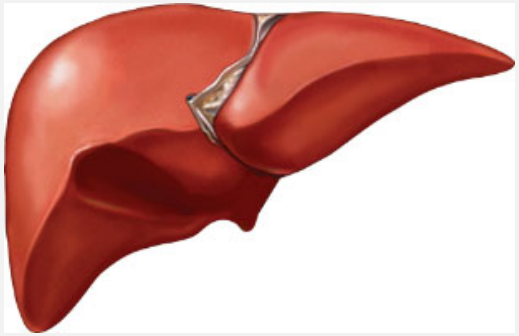


GUT MICROBIOME AND CVD

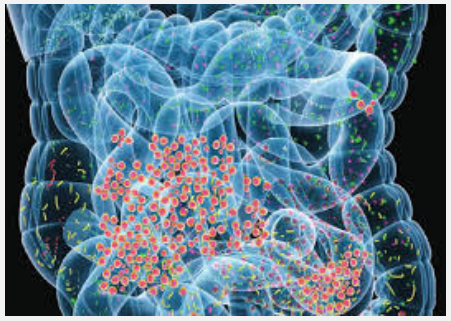


From: Guilliams TG: Cardiometabolic Risk Management- A Functional and Lifestyle Approach (Point Institute 2018)

THE POWER OF ADAPTABILITY

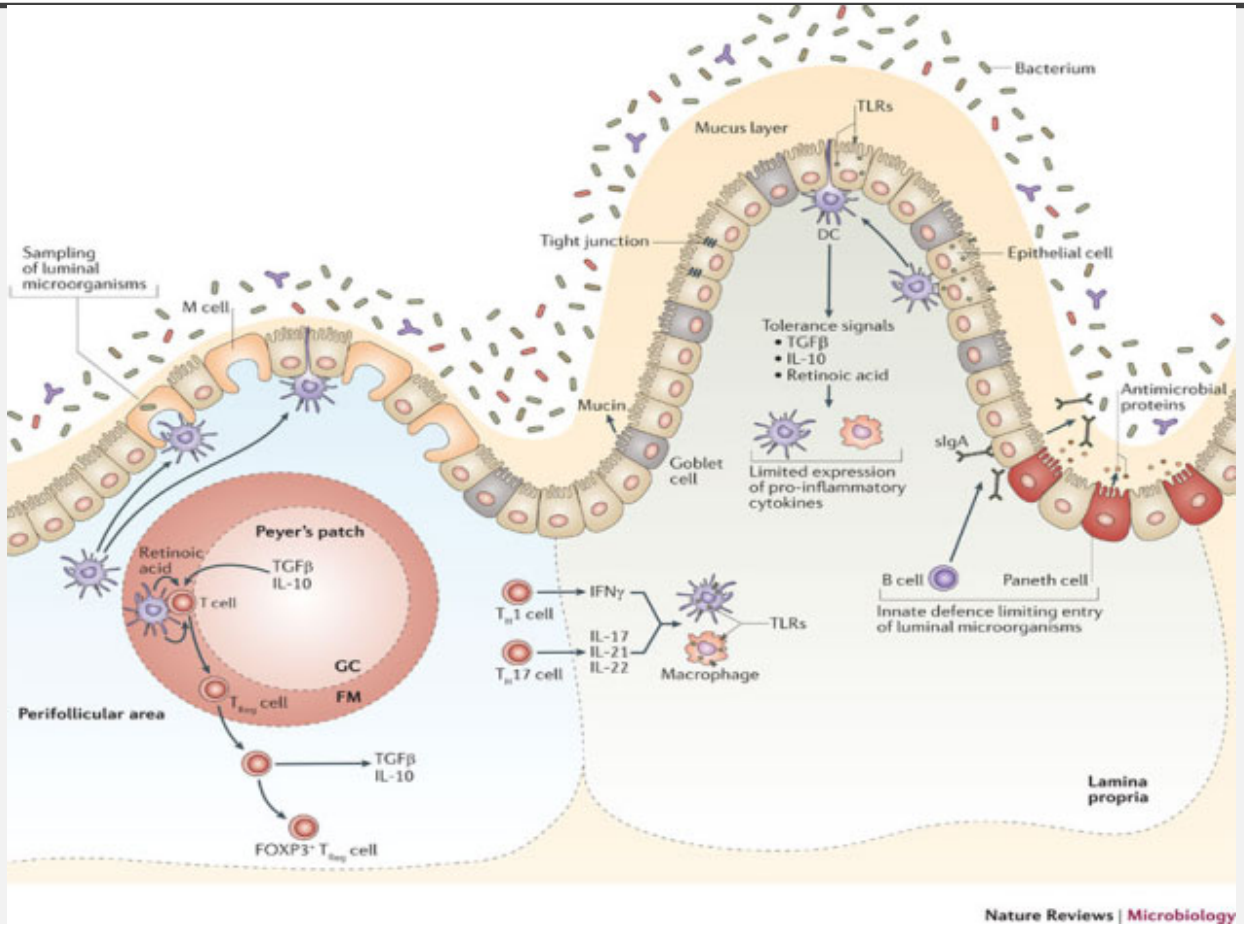


- 50-150 billion cells
- Fixed Genome, slowly change epigenome
- Influenced by genomic signals

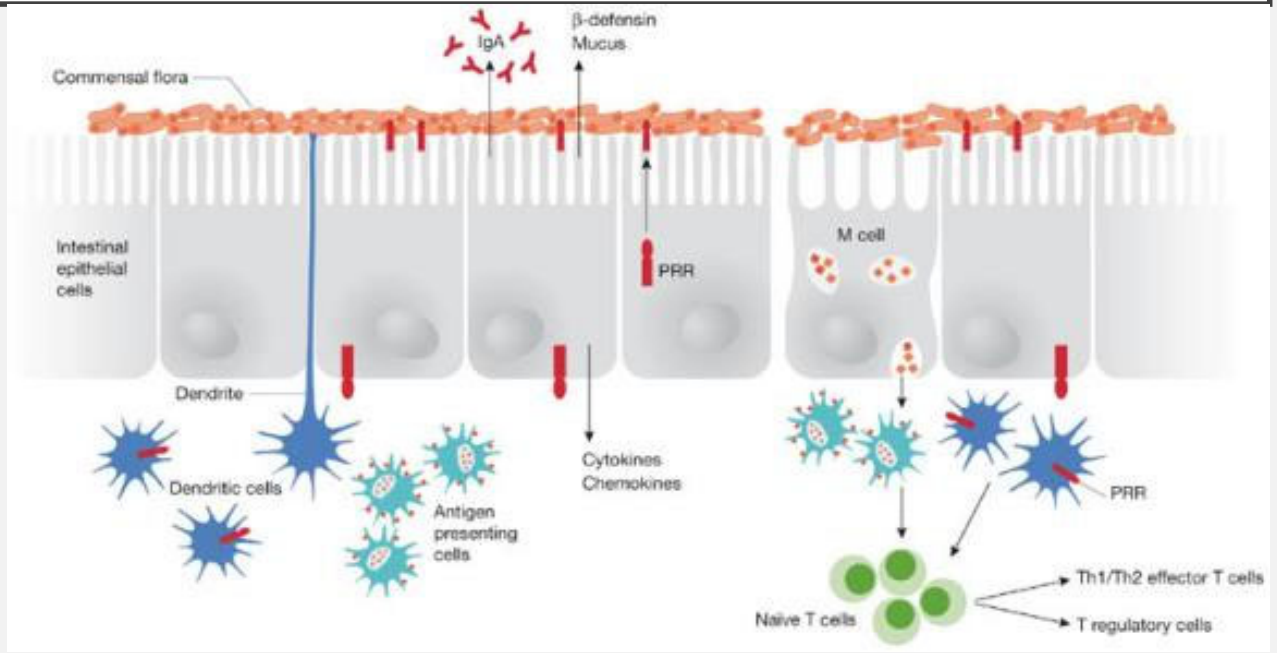


- ~1 Trillion Cells
- 30-40% altered genome in few days
- Influenced by genomic signals
- Epigenome?

MICROBIOME/IMMUNE INTERFACE

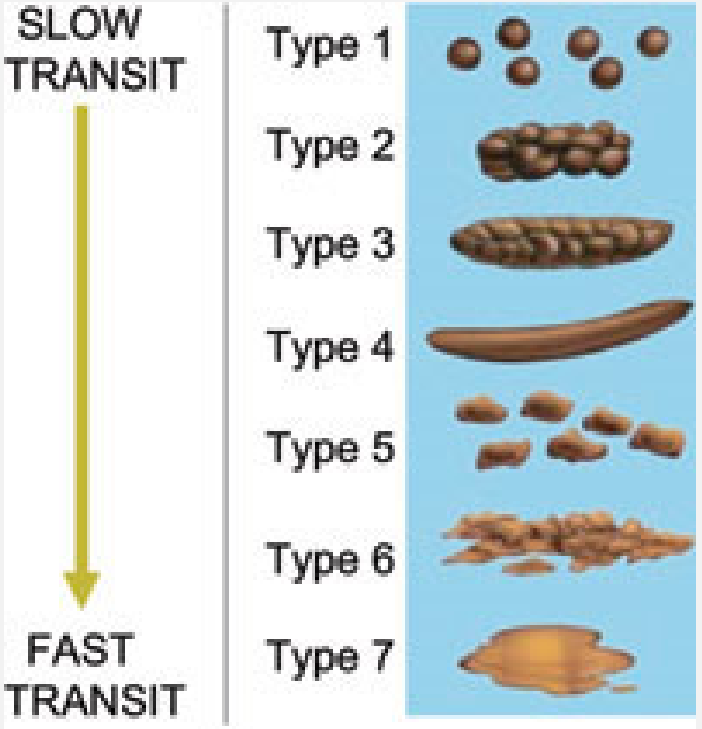


COMMENSAL FLORA AND IMMUNE CONTROL



IMMEDIATE FACTORS THAT INFLUENCE FECAL MICROBIOME SAMPLES.

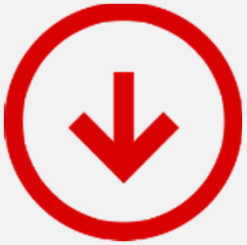
- Stool Morphology as measured by Bristol Stool Scale
  - Transit Time
  - Available time for fermentation/division
  - individuals with a short transit time may have greater amounts of fast-growing bacterial species, while those with slow transit times may instead select bacteria with greater adherence to host tissue.
- The Use of Medications
  - Antibiotics
  - Laxative
  - PPIs



Vandeputte D, Falony G, Vieira-Silva S, et al. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut*. 2016 Jan;65(1):57-62.

THE BIGGEST LEVERS TO CHANGE THE HUMAN GUT MICROBIOME

- Diverse Diet, variety of plants
  - Source of New Microbes
  - Food for Existing Microbes
- Fecal Microbial Transplants (short-term?)
- Probiotics (short-term)



- Processed Food Diet
- Broad spectrum Antibiotics

LETTER

doi:10.1038/nature12820

Diet rapidly and reproducibly alters the human gut microbiome

Lawrence A. David[1,2]†, Corinne F. Maurice[2], Rachel N. Carmody[1], David B. Gootenberg[1], Julie E. Button[1], Benjamin E. Wolfe[1], Alisha V. Ling[3], A. Sloan Devlin[4], Yug Varma[4], Michael A. Fischbach[4], Sudha B. Biddinger[3], Rachel J. Dutton[1] & Peter J. Turnbaugh[1]

Long-term dietary intake influences the structure and activity of the trillions of microorganisms residing in the human gut[1–4], but it remains unclear how rapidly and reproducibly the human gut microbiome responds to short-term macronutrient change. Here we show that the short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression. The animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila* and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*). Microbial activity mirrored differences between herbivorous and carnivorous mammals[5], reflecting trade-offs between carbohydrate and protein fermentation. Foodborne microbes from both diets transiently colonized the gut, including bacteria, fungi and even viruses. Finally, increases in the abundance and activity of *Bilophila wadsworthia* on the animal-based diet support a link between dietary fat, bile acids and the outgrowth of microorganisms capable of triggering inflammatory bowel disease[6]. In concert, these results demonstrate that the gut microbiome can rapidly respond to altered diet, potentially facilitating the diversity of human dietary lifestyles.

There is growing concern that recent lifestyle innovations, most notably the high-fat/high-sugar 'Western' diet, have altered the genetic composition and metabolic activity of our resident microorganisms (the human gut microbiome)[7]. Such diet-induced changes to gut-associated microbial communities are now suspected of contributing to growing epidemics of chronic illness in the developed world, including obesity[4,8] and inflammatory bowel disease[6]. Yet, it remains unclear how quickly and reproducibly gut bacteria respond to dietary change. Work in inbred mice shows that shifting dietary macronutrients can broadly and consistently alter the gut microbiome within a single day[7,9]. By contrast, dietary interventions in human cohorts have only measured community changes on timescales of weeks[10] to months[4], failed to find significant diet-specific effects[1], or else have demonstrated responses among a limited number of bacterial taxa[4,8].

We examined whether dietary interventions in humans can alter gut microbial communities in a rapid, diet-specific manner. We prepared two diets that varied according to their primary food source: a 'plant-based diet', which was rich in grains, legumes, fruits and vegetables; and an 'animal-based diet', which was composed of meats, eggs and cheeses (Supplementary Table 1). We picked these sources to span the global diversity of modern human diets, which includes exclusively plant-based and nearly exclusively animal-based regimes[11] (the latter being the case among some high-latitude and pastoralist cultures). Each diet was consumed *ad libitum* for five consecutive days by six male and four female American volunteers between the ages of 21 and 33, whose body mass indices ranged from 19 to 32 kg m^{-2} (Supplementary Table 2). Study volunteers were observed for 4 days before each diet arm to measure normal eating habits (the baseline period) and for 6 days after each diet arm to assess microbial recovery (the washout period; Extended Data Fig. 1). Subjects' baseline nutritional intake correlated well with their estimated long-term diet (Supplementary Table 3). Our study cohort included a lifetime vegetarian (see Extended Data Fig. 2, Sup-

days after the animal-based diet ended (Fig. 1e).

Analysis of the relative abundance of bacterial taxonomic groups supported our finding that the animal-based diet had a greater impact on the gut microbiota than the plant-based diet (Fig. 2). We hierarchically clustered species-level bacterial phylotypes by the similarity of their dynamics across diets and subjects (see Methods and Supplementary Tables 7, 8). Statistical testing identified 22 clusters whose abundance significantly changed while on the animal-based diet, whereas only 3 clusters showed significant abundance changes while on the plant-based diet ($q < 0.05$, Wilcoxon signed-rank test; Supplementary Table 9). Notably, the genus *Prevotella*, one of the leading sources of inter-individual gut microbiota variation[1,12] and hypothesized to be sensitive to long-term fibre intake[1,13], was reduced in our vegetarian subject during consumption of the animal-based diet (see Supplementary

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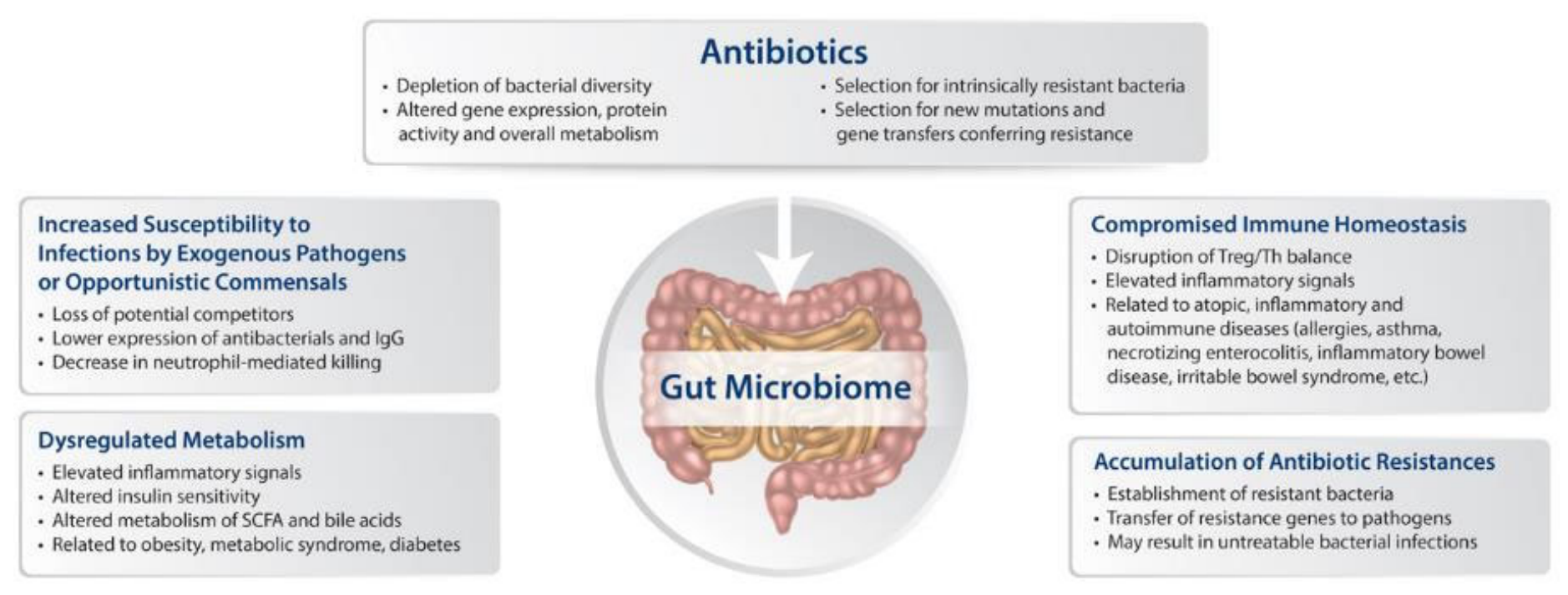
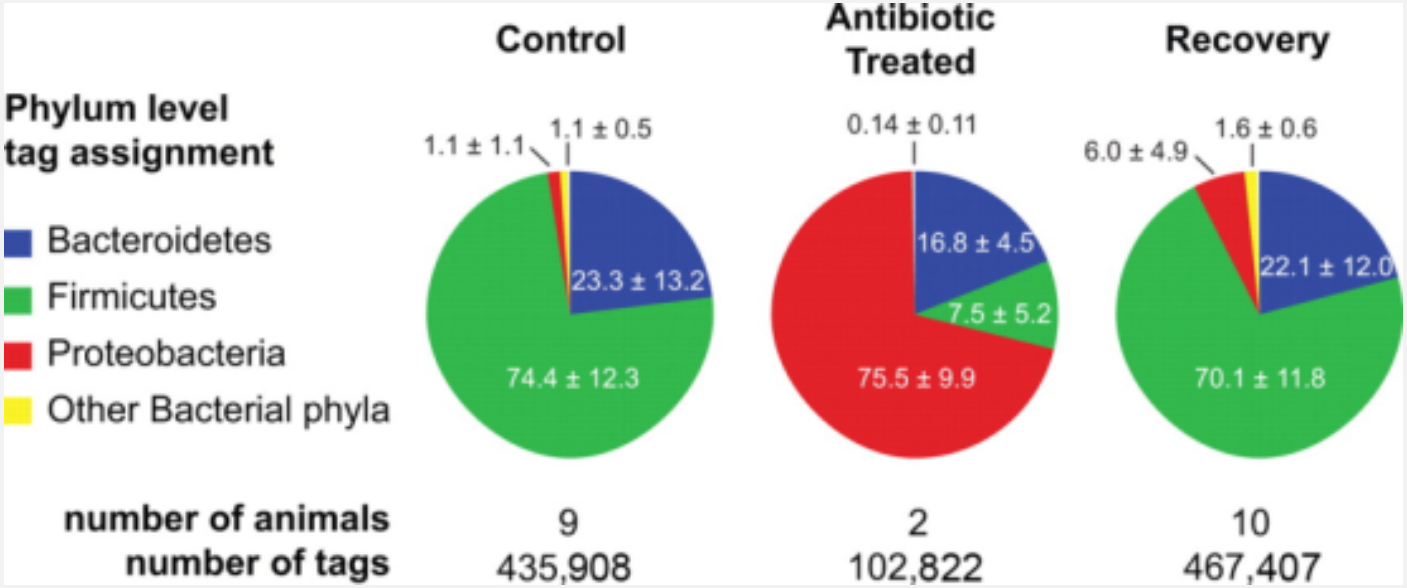
Here we show that the short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression. Microbial activity mirrored differences between herbivorous and carnivorous mammals, reflecting trade-offs between carbohydrate and protein fermentation. Foodborne microbes from both diets transiently colonized the gut, including bacteria, fungi and even viruses. In concert, these results demonstrate that the gut microbiome can rapidly respond to altered diet, potentially facilitating the diversity of human dietary lifestyles. *Nature*. 2014 Jan 23;505(7484):559-63.

THE HEAVY HAND OF ANTIBIOTIC THERAPY



THE RISK-REWARD OF RESCUE MEDICINE

- How much of the past 100 year shift in human metabolic function might have been caused by a massive shift in our microbiome due to the rampant use of antibiotics?



Phylum-level changes in animals given antibiotics

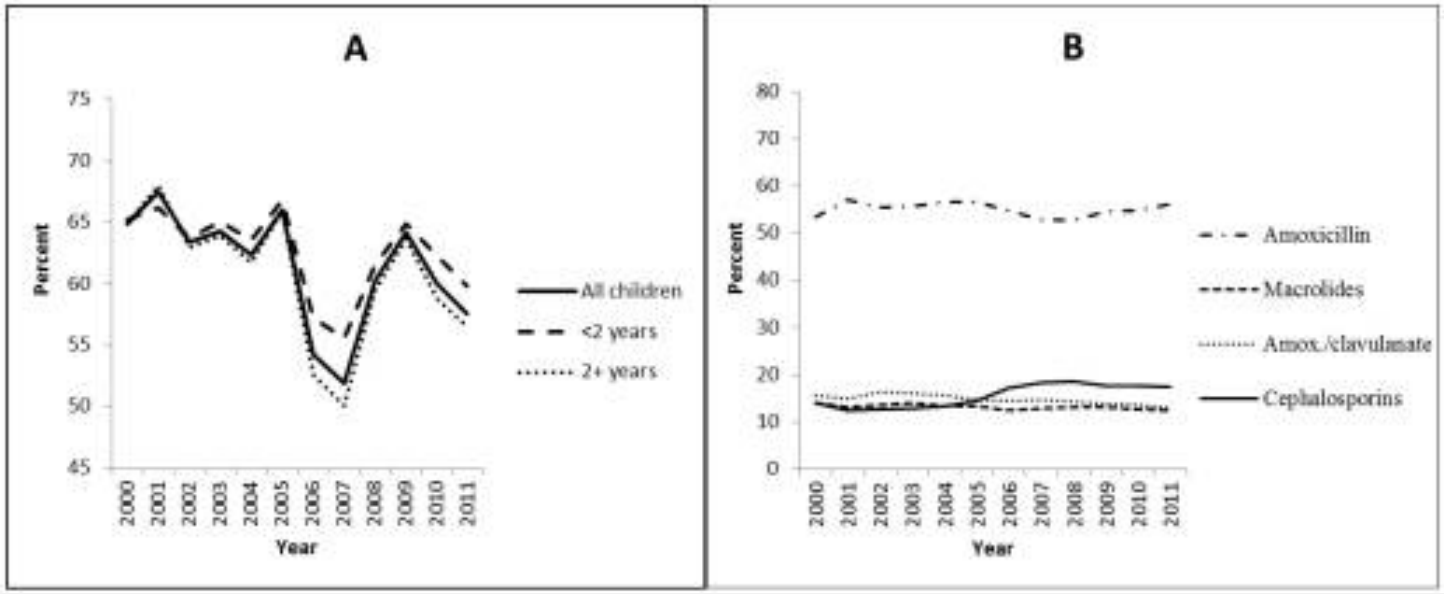
OPEN ACCESS Freely available online

PLOS ONE

Trends in Antibiotic Treatment of Acute Otitis Media and Treatment Failure in Children, 2000–2011

Leah J. McGrath[1]*, Sylvia Becker-Dreps[2], Virginia Pate[1], M. Alan Brookhart[1]

1 Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, United States of America, **2** Department of Family Medicine, University of North Carolina, Chapel Hill, North Carolina, United States of America



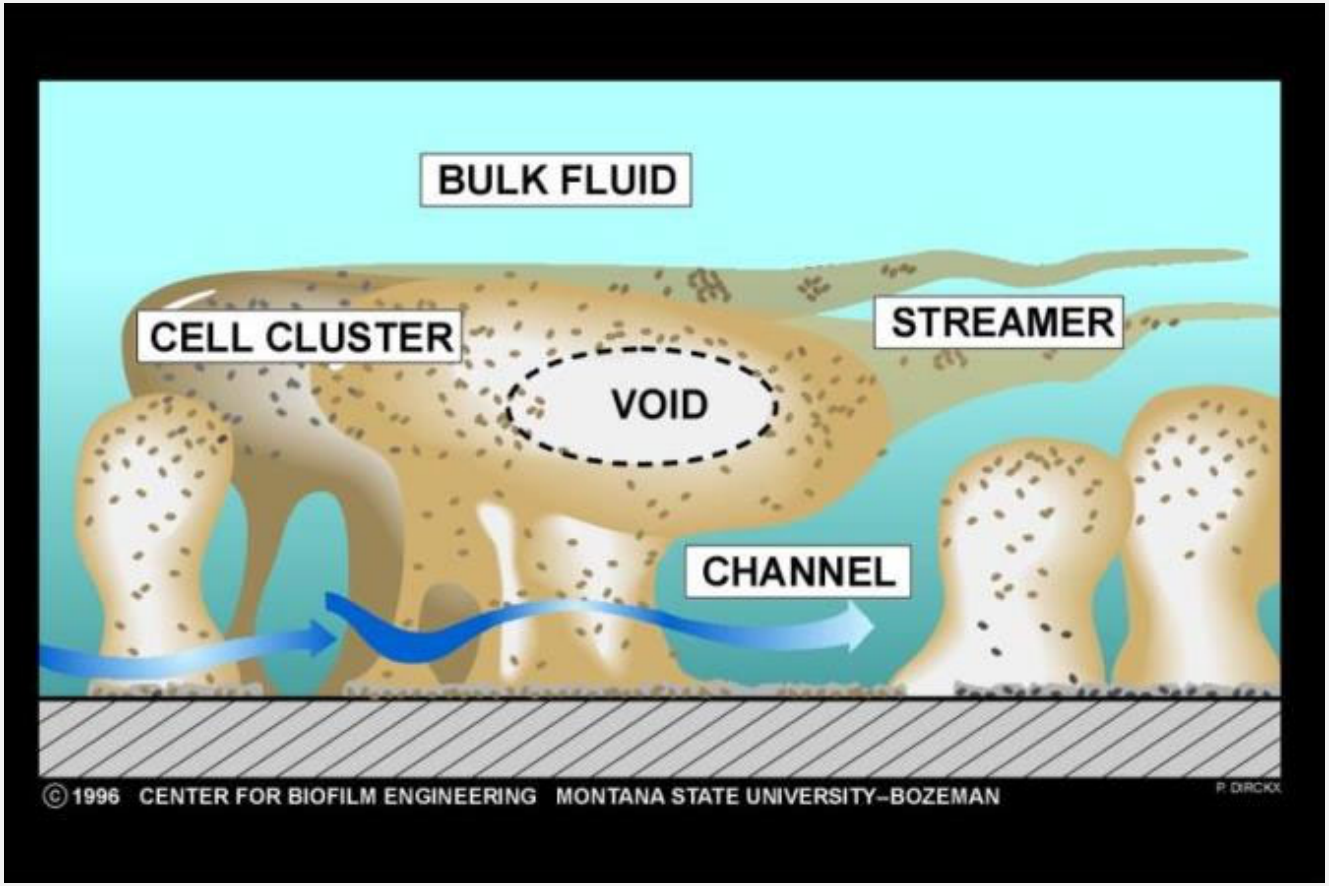
Failure Rate (need for new antibiotic within 2-18 days of first prescription) was ~10%

C. DIFF AND ANTIBIOTICS

- Taking antibiotics is the top risk factor for developing *C. difficile* infections for both children and adults.
- Researchers found that 71 percent of cases of *Clostridium difficile* infection among American children aged 1 to 17 occurred shortly after they took antibiotics that were prescribed in doctors' offices to treat other conditions.
- Most of the children received antibiotics for problems such as ear, sinus or upper respiratory infections. Previous research has shown that at least 50 percent of antibiotics prescribed to children in doctors' offices are for respiratory infections, most of which do not require antibiotics.

U.S. Centers for Disease Control and Prevention, news release, March 7, 2014

BIOFILM: A CLASSIC APPROACH, BUT NOT LIKELY A MODEL FOR GI ORGANISMS



Pathogens and Disease ISSN 2049-632X

MINIREVIEW

Microbial biofilms and gastrointestinal diseases

Erik C. von Rosenvinge[1,2], Graeme A. O'May[3], Sandra Macfarlane[4], George T. Macfarlane[4] & Mark E. Shirtliff[3]

1 Department of Gastroenterology and Hepatology, University of Maryland School of Medicine, Baltimore, MD, USA
2 Department of Veterans Affairs, VA Maryland Health Care System, Baltimore, MD, USA
3 Department of Microbial Pathogenesis, University of Maryland School of Dentistry, Baltimore, MD, USA
4 Microbiology and Gut Biology Group, University of Dundee, Ninewells Hospital Medical School, Dundee, UK

This timely review on the significance of microbial biofilms and gastrointestinal disease will stimulate research in this field.

Keywords
biofilm; microbiota; gastrointestinal disease; gastrointestinal tract.

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Received: 9 September 2012; revised 12 December 2012; accepted 12 December 2012. Final version published online 29 January 2013.

doi:10.1111/2049-632X.12020

Editor: Ake Forsberg

Abstract

The majority of bacteria live not planktonically, but as residents of sessile biofilm communities. Such populations have been defined as 'matrix-enclosed microbial accretions, which adhere to both biological and nonbiological surfaces'. Bacterial formation of biofilm is implicated in many chronic disease states. Growth in this mode promotes survival by increasing community recalcitrance to clearance by host immune effectors and therapeutic antimicrobials. The human gastrointestinal (GI) tract encompasses a plethora of nutritional and physicochemical environments, many of which are ideal for biofilm formation and survival. However, little is known of the nature, function, and clinical relevance of these communities. This review summarizes current knowledge of the composition and association with health and disease of biofilm communities in the GI tract.

Introduction

The human gastrointestinal (GI) tract extends from the esophagus through the stomach, small intestine, and large intestine (colon) and terminates in the rectum (Fig. 1). The small intestine is divided proximally-to-distally into the duodenum, jejunum, and ileum. This collection of interconnected organs harbors a diversity of microhabitats that are colonized by microorganisms to varying degrees, depending on local environmental conditions. For the purposes of this article, the oral and nasal cavities will not be regarded as being part of the GI tract, although these anatomical spaces also contain great microbiological complexity (Ledder *et al.*, 2007).

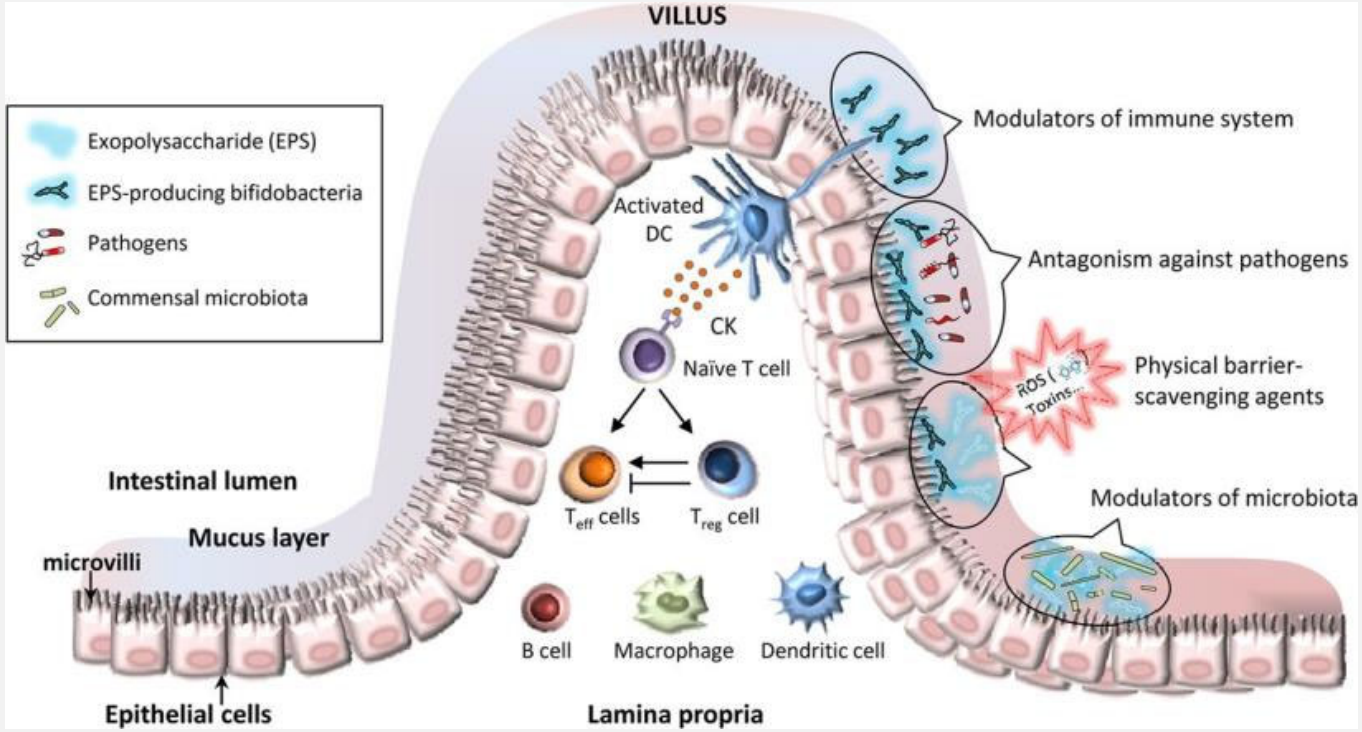
There exists in the GI tract a gradient of colonization, from the relatively sparsely populated esophagus and stomach to the much more heavily colonized colon, which can contain up to 10^{10} culturable bacteria per gram luminal contents (Hopkins *et al.*, 2002). Evolution has dictated that the GI tract possess a large surface area to facilitate efficient nutrient uptake, its primary physiological role in the body. This coupled to high nutrient availability and a constant influx of microorganisms, together with stable autochthonous populations, makes the GI tract an ideal site for the development of sessile microbial biofilm communities. The microbiome of the gut has recently been determined in 124 subjects, and the microbial diversity indicates that the entire cohort harbors only between 1000 and 1150 prevalent bacterial species and each individual at least 160 such species (Qin *et al.*, 2010). In addition, there were common microbial flora in subjects tested with 75 species common to > 50% of individuals and 57 species common to > 90%.

Those microorganisms in closest proximity to host tissues have the most opportunity for interaction with host physiology, immunity, and metabolism; thus, mucosal populations are arguably the most important component of any host–microbiota interaction, whether beneficial or detrimental. The GI tract microbiota has been implicated in disease states such as inflammatory bowel disease (IBD; Macpherson *et al.*, 1996), colon cancer (Horie *et al.*, 1999a, b), gastric cancer (Björkholm *et al.*, 2003), and irritable bowel syndrome (IBS; Swidsinski *et al.*, 2005). In

- Excellent Review of biofilm
- Reveals we still know very little about biofilm's beneficial and detrimental functions- and what we can do about it.

- Pathog Dis. 2013 Feb;67(1):25-38

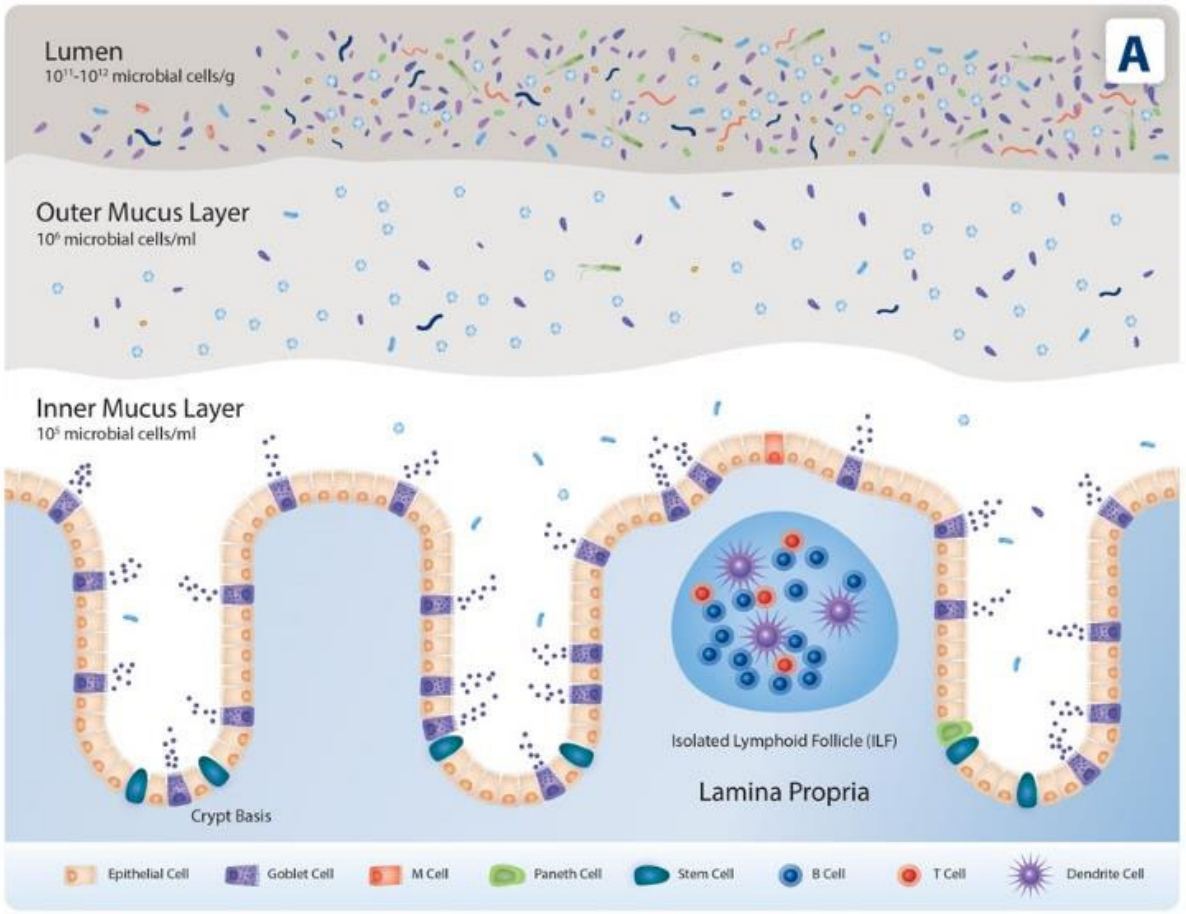
Beneficial activities potentially attributed to some exopolysaccharides synthesized by Bifidobacterium.



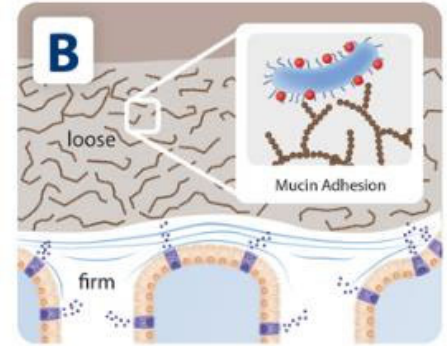
Hidalgo-Cantabrana C et al. Appl. Environ. Microbiol. 2014;80:9-18

Applied and Environmental Microbiology

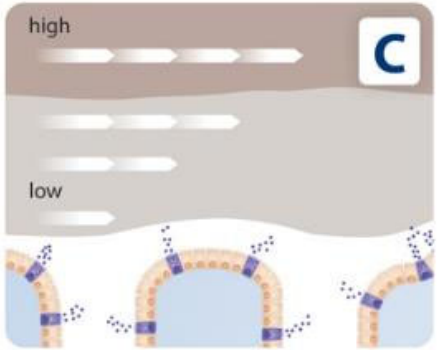
THE MUCOSAL MICRO-ENVIRONMENTS



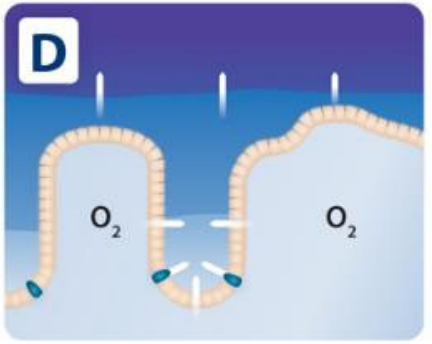
MUCUS FACTORS THAT INFLUENCE MICROENVIRONMENTS



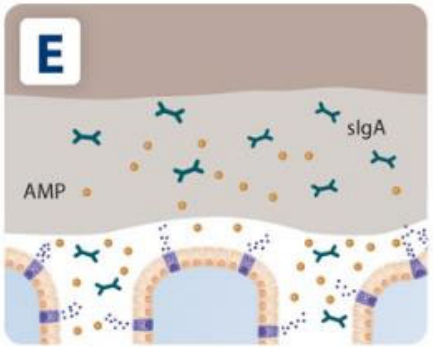
Mucus Rigidity



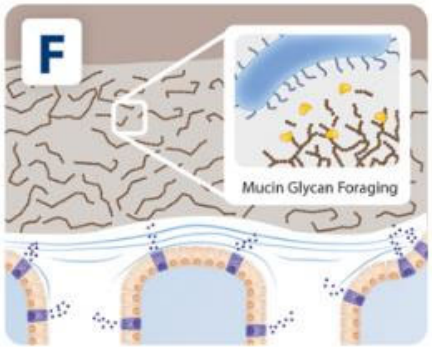
Fluid Shear Gradients



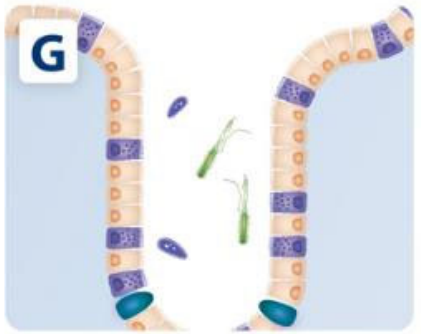
Oxygen Gradients



Host Defense Molecules



Mucosal Nutrient Platform



Crypt Niche

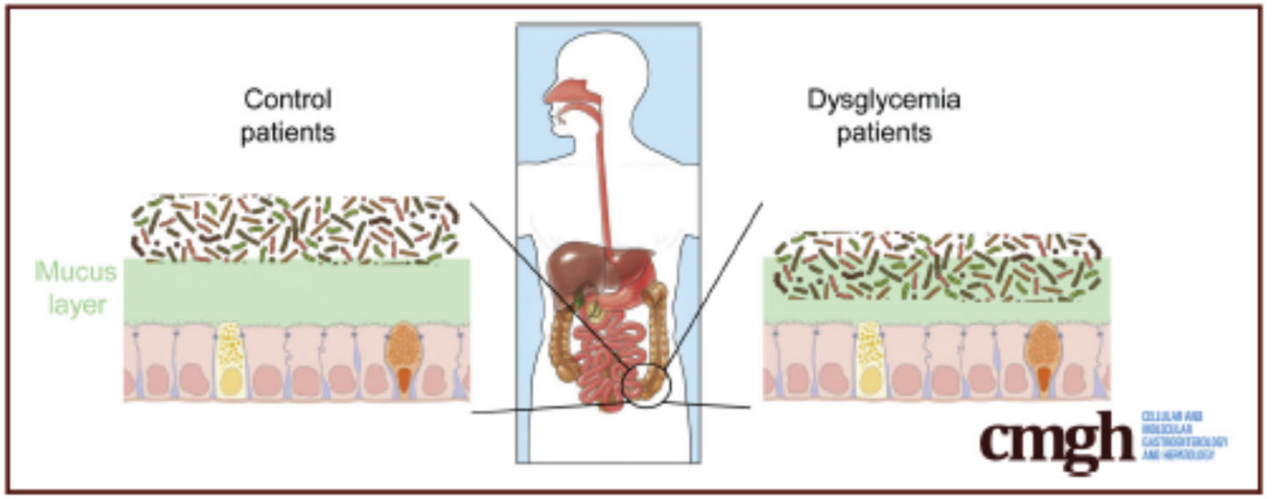
cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Colonic Microbiota Encroachment Correlates With Dysglycemia in Humans

Benoit Chassaing,[1] Shreya M. Raja,[2,3] James D. Lewis,[4] Shanthi Srinivasan,[2,3] and Andrew T. Gewirtz[1,2]

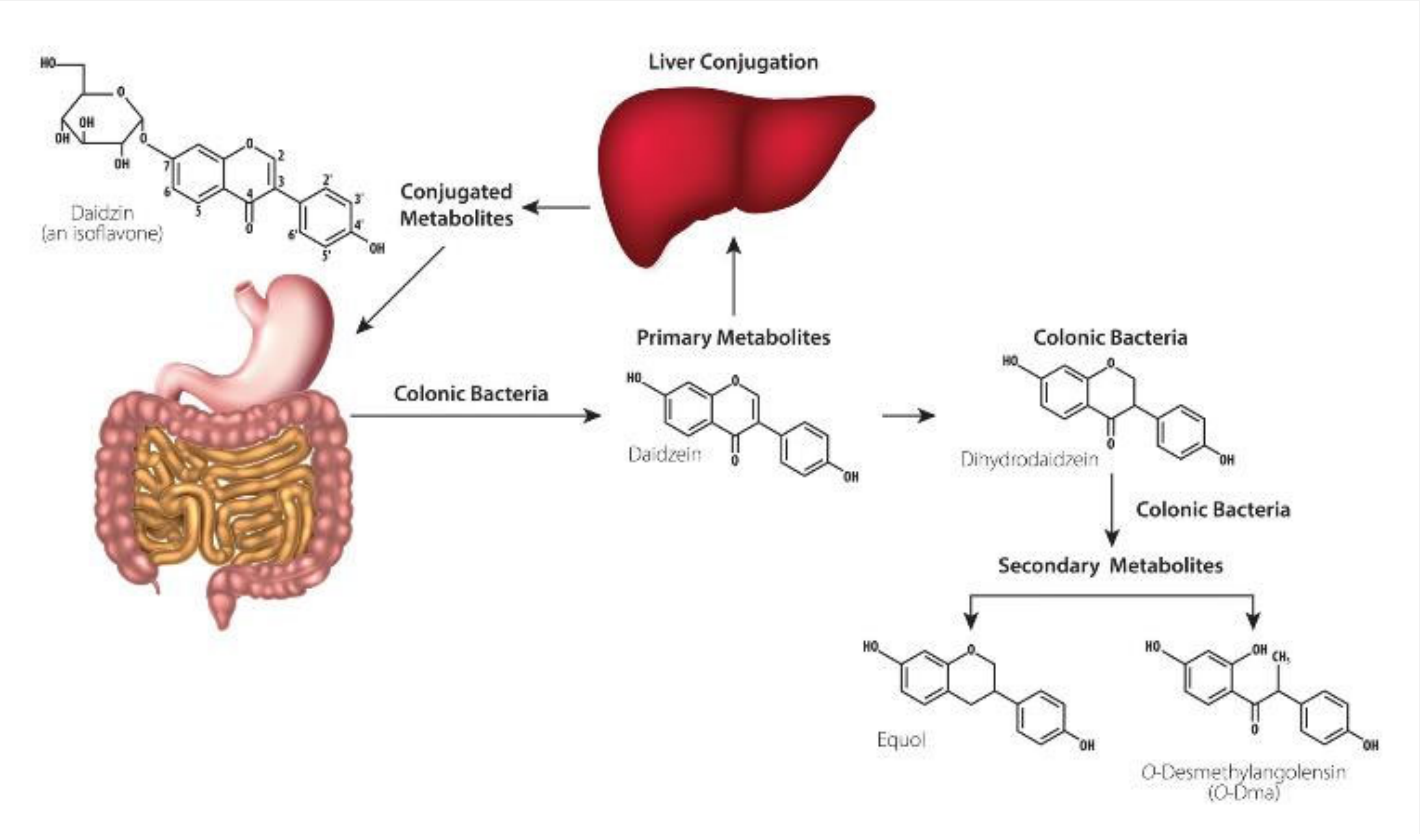
[1]Center for Inflammation, Immunity and Infection, Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia; [2]Digestive Diseases Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia; [3]Atlanta VA Medical Center, Decatur, Georgia; [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania



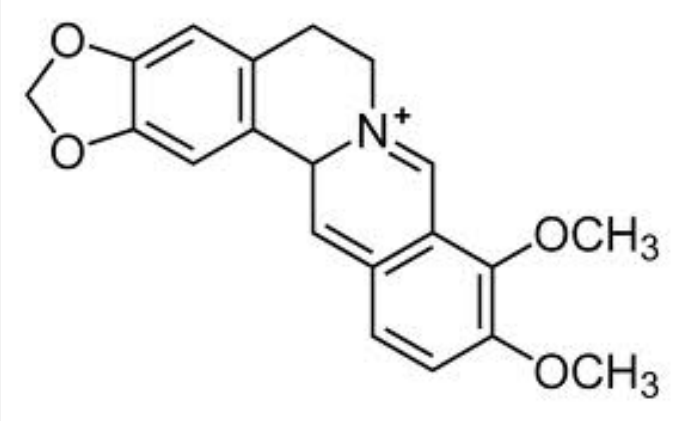
INHIBITING BIOFILMS IN GI?

- Several natural agents (and synthetic analogs) are known to disturb biofilm formation or inhibit quorum sensing in laboratory tests, but we are not aware of any research specific to mucosal biofilms generally, or gastrointestinal outcomes specifically.
- We are very cautious on recommending GI "biofilm-disrupting" therapies because biofilm communities within the GI mucosa contain heterogeneous mixtures of mostly beneficial organisms along with those that are potentially harmful
- Biofilm disruption is unlikely to discriminate between the good and the bad.
- Furthermore, virtually no evidence exists to guide the clinician in the selection of agents, doses and length of treatment for GI biofilm disrupting therapies related to GI-related clinical outcomes.
- The use of ingredients that perform well in *in vitro* tests of biofilm disruption of monocultures, have yet to be shown effective or beneficial for GI-related outcomes (though some are marketed as if they have).

THE MICROBIOME AND PHYTOTHERAPY



BERBERINE- A COMPREHENSIVE METABOLIC SIGNALING MOLECULE.



An isoquinoline alkaloid found in plants like *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Berberis aristata* (tree turmeric)), *Hydrastis canadensis* (goldenseal), *Xanthorhiza simplicissima* (yellowroot) and Coptis chinensis (Chinese goldthread)- the common source for commercial berberine HCL/Sulfate

nature medicine

Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins

Weijia Kong[1,5], Jing Wei[2,5], Parveen Abidi[3,5], Meihong Lin[3], Satoru Inaba[3], Cong Li[3], Yanling Wang[4], Zizheng Wang[2], Shuyi Si[1], Huaining Pan[2], Shukui Wang[2], Jingdan Wu[2], Yue Wang[4], Zhuorong Li[1], Jingwen Liu[3] & Jian-Dong Jiang[1,4]

We identify berberine (BBR), a compound isolated from a Chinese herb, as a new cholesterol-lowering drug. Oral administration of BBR in 32 hypercholesterolemic patients for 3 months reduced serum cholesterol by 29%, triglycerides by 35% and LDL-cholesterol by 25%. Treatment of hyperlipidemic hamsters with BBR reduced serum cholesterol by 40% and LDL-cholesterol by 42%, with a 3.5-fold increase in hepatic *LDLR* mRNA and a 2.6-fold increase in hepatic LDLR protein. Using human hepatoma cells, we show that BBR upregulates LDLR expression independent of sterol regulatory element binding proteins, but dependent on ERK activation. BBR elevates LDLR expression through a post-transcriptional mechanism that stabilizes the mRNA. Using a heterologous system with luciferase as a reporter, we further identify the 5′ proximal section of the *LDLR* mRNA 3′ untranslated region responsible for the regulatory effect of BBR. These findings show BBR as a new hypolipidemic drug with a mechanism of action different from that of statin drugs.

The expression of liver low-density lipoprotein receptor (LDLR) regulates human plasma LDL cholesterol (LDL-c) homeostasis[1,2]. Increased hepatic LDLR expression results in improved clearance of plasma LDL-c through receptor-mediated endocytosis, which has been strongly associated with a decreased risk of developing cardiovascular disease in humans[3,4]. LDLR expression is predominantly regulated at the transcriptional level through a negative feedback mechanism by the intracellular cholesterol pool. This regulation is controlled through specific interactions of sterol-regulatory element (SRE-1) of the LDLR promoter[5,6] and SRE binding proteins (SREBPs)[7–9]. In the inactive state, SREBP resides in the endoplasmic reticulum (ER) and associates with another transmembrane protein, SREBP-cleavage activating protein (SCAP) which provides conditional chaperone activity to the SREBP[10–12]. SCAP contains a cholesterol-sensing domain, which responds to the depletion of sterol with activation of the SCAP-SREBP transporting activity[13–15]. Under cholesterol-depleted conditions, SCAP transports SREBP to the Golgi apparatus, where the N-terminal transcription activation domain of the SREBP is released from the precursor protein through specific cleavages[11]. The active form of the SREBP translocates to the nucleus, binds to its cognate SRE-1 site and activates transcription of the LDLR gene. In contrast, under cholesterol-replete conditions, the SCAP-SREBP complex remains in an inactive form in the ER through active repression by sterols and *LDLR* gene transcription is maintained at a minimal constitutive level.

Clinically, statins have been the most widely prescribed drugs for hypercholesterolemia[3,4]. Statins inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. Inhibition of cholesterol biosynthesis leads to a depletion of intracellular cholesterol and an activation of the SCAP-SREBP transporting activity, thereby resulting in upregulation of the LDLR and subsequent lowering of the LDL-c in blood. Statins effectively lower the plasma concentration of LDL-c and reduce mortality and morbidity from coronary artery disease[16,17]. Recent studies showed additional benefits of statin beyond its cholesterol-lowering effects[18]. But despite the success of treatment with statins, there is a need for new therapies to reduce LDL-c. Some patients do not tolerate statins well, and more importantly, many patients under statin treatment alone do not achieve the LDL-c goal

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and Laboratory of Molecular Medicine, First Hospital of Nanjing City, Nanjing Medical U
Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, 94304, USA. 4Departmer
[5]These authors contributed equally to this work. Correspondence should be addressed t

Published online 7 November 2004; doi:10.1038/nm1135

500 mg BID

Table 1 Effects of BBR on serum lipids in the subgroup of hypercholesterolemic patients who were not taking other medication before or during BBR treatment.

Treatment (3 months)		BBR[a] Hypercholesterolemia (>5.2 mmol/L, $n = 32$)	Placebo Hypercholesterolemia (>5.2 mmol/L, $n = 11$)
Serum level of cholesterol			
Cholesterol	Before	5.9 ± 0.7	6.1 ± 0.6
	After	4.2 ± 0.9*	6.0 ± 0.8
Triglyceride	Before	2.3 ± 1.8	2.2 ± 0.8
	After	1.5 ± 0.9*	2.1 ± 0.9
HDL-c	Before	1.1 ± 0.3	1.2 ± 0.5
	After	1.1 ± 0.3	1.2 ± 0.4
LDL-c	Before	3.2 ± 0.7	3.7 ± 0.7
	After	2.4 ± 0.6***	3.7 ± 0.8

[a]Statistical analysis of the baselines of cholesterol, triglyceride, HDL-c and LDL-c showed that there were no significant differences between the BBR and placebo groups before therapy ($P > 0.05$). ***$P < 0.0001$, as compared to the baselines of 'before treatment' group.

Table 2 Effect of BBR on liver and kidney functions of the subgroup of hypercholesterolemic patients who were not taking other medication before or during BBR treatment.

	n	ALT(U/L)	AST(U/L)	GGT (U/L)	Bil-T(μM/L)	Cr (μM/L)	BUN (mM/L)
BBR group:							
Before treatment	32	44.9 ± 21.8	39.3 ± 22.2	53.7 ± 24.4	17.4 ± 8.8	75.5 ± 14.6	5.76 ± 1.2
After treatment	32	23.6 ± 11.1**	26.6 ± 8.2*	31.7 ± 15.2**	13.8 ± 6.3**	72.6 ± 18.7	5.79 ± 1.2
Placebo group:							
Before treatment	11	45.7 ± 17	39.6 ± 19.2	52.2 ± 21.4	17.0 ± 6.0	72.6 ± 17.1	5.60 ± 1.4
After treatment	11	44.8 ± 10.2	38.8 ± 8.3	52.0 ± 14.8	17.3 ± 5.3	73.1 ± 19	5.66 ± 1.3
Normal range[a]		0–40.0	0–40.0	10.0–50.0	3.4–26.6	39.8–134.4	2.1–7.9

[a]"National Clinical Laboratory Manual" issued by The Ministry of Health of the People's Republic of China, with minor modifications. *$P < 0.01$; **$P < 0.001$, as compared to those 'before treatment.' ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, gamma glutamyl transpeptidase; Bil-T, total bilirubin; Cr, creatinine; BUN, blood urea nitrogen.

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Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression

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[b]Department of Medicine, Nanjing First Hospital, Nanjing 210006, China
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Abstract

Our previous work demonstrated that berberine (BBR) increases insulin receptor (InsR) expression and improves glucose utility both in vitro and in animal models. Here, we study the InsR–up-regulating and glucose-lowering activities of BBR in humans. Our results showed that BBR increased InsR messenger RNA and protein expression in a variety of human cell lines, including CEM, HCT-116, SW1990, HT1080, 293T, and hepatitis B virus–transfected human liver cells. Accordingly, insulin-stimulated phosphorylations of InsR β-subu and Akt were increased after BBR treatment in cultured cells. In the clinical study, BBR significantly lowered fasting blood gluc (FBG), hemoglobin A_{1c}, triglyceride, and insulin levels in patients with type 2 diabetes mellitus (T2DM). The FBG- and hemoglobin A lowering efficacies of BBR were similar to those of metformin and rosiglitazone. In the BBR-treated patients, the percentages of periph blood lymphocytes that express InsR were significantly elevated after therapy. Berberine also lowered FBG effectively in chronic hepat B and hepatitis C patients with T2DM or impaired fasting glucose. Liver function was improved greatly in these patients by show reduction of liver enzymes. Our results confirmed the activity of BBR on InsR in humans and its relationship with the glucose-lower effect. Together with our previous report, we strongly suggest BBR as an ideal medicine for T2DM with a mechanism different fr metformin and rosiglitazone.

1. Introduction

The insulin receptor (InsR) is a membrane-spanning glycoprotein that is essential for the action of insulin. Binding of insulin to InsR in the liver, muscles, or adipose tissues triggers multiple intracellular pathways that cause glycogen synthesis and glucose uptake increase, as well hepatic/muscle glucose output reduction. The blood gluc level is thus lowered [1,2]. This is one of the ma mechanisms for the human body to keep glucose homeos sis. Disruption of the expression of InsR generates hyperglycemic phenotype in mice [3]. Type 2 diabe mellitus (T2DM) is a human hyperglycemic state charact ized by insulin resistance in peripheral tissues, particula the liver, muscles, adipocytes, and pancreatic β-cells [1,4, About 92% of the patients with T2DM show insu resistance [6]. Individuals with insulin resistance have eit... decreased levels or absence of InsR expression [7-9]. Thus, InsR is considered as a potential target to treat T2DM and insulin resistance, in which the intrinsic tyrosine kinase could be activated for insulin signaling. At the present time, small-molecular weight compounds that mimic insulin

There is no conflict of interest in this work.

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Table 1
Effects of BBR, metformin, and rosiglitazone in T2DM patients

Measurement (reference range)	Treatment (2 mo)	BBR (1 g/d, n = 50)	Metformin (1.5 g/d, n = 26)	Rosiglitazone (4 mg/d, n = 21)
FBG	Before	10.4 ± 0.4	10.9 ± 0.5	9.1 ± 0.8
(3.9-5.6 mmol/L)	After	7.7 ± 0.3†	7.6 ± 0.3†	7.5 ± 0.6*
HbA_{1c}	Before	8.3 ± 0.3	9.4 ± 0.5	8.3 ± 0.4
(4.0%-6.0%)	After	6.8 ± 0.2†	7.2 ± 0.3†	6.8 ± 0.3*
TG	Before	1.7 ± 0.1	1.7 ± 0.2	1.9 ± 0.3
(<1.7 mmol/L)	After	1.4 ± 0.1*	1.6 ± 0.1	1.6 ± 0.1

Values are mean ± SEM.
* $P < .01$ compared with that before treatment by paired t test.
† $P < .001$ compared with that before treatment by paired t test.

I gram BBR per day

EXCELLENT REVIEW OF BERBERINE'S MECHANISMS AND CLINICAL RESULTS

Atherosclerosis 243 (2015) 440–461

Contents lists available at ScienceDirect

Atherosclerosis

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Review article

Berberine, a plant alkaloid with lipid- and glucose-lowering properties: From *in vitro* evidence to clinical studies

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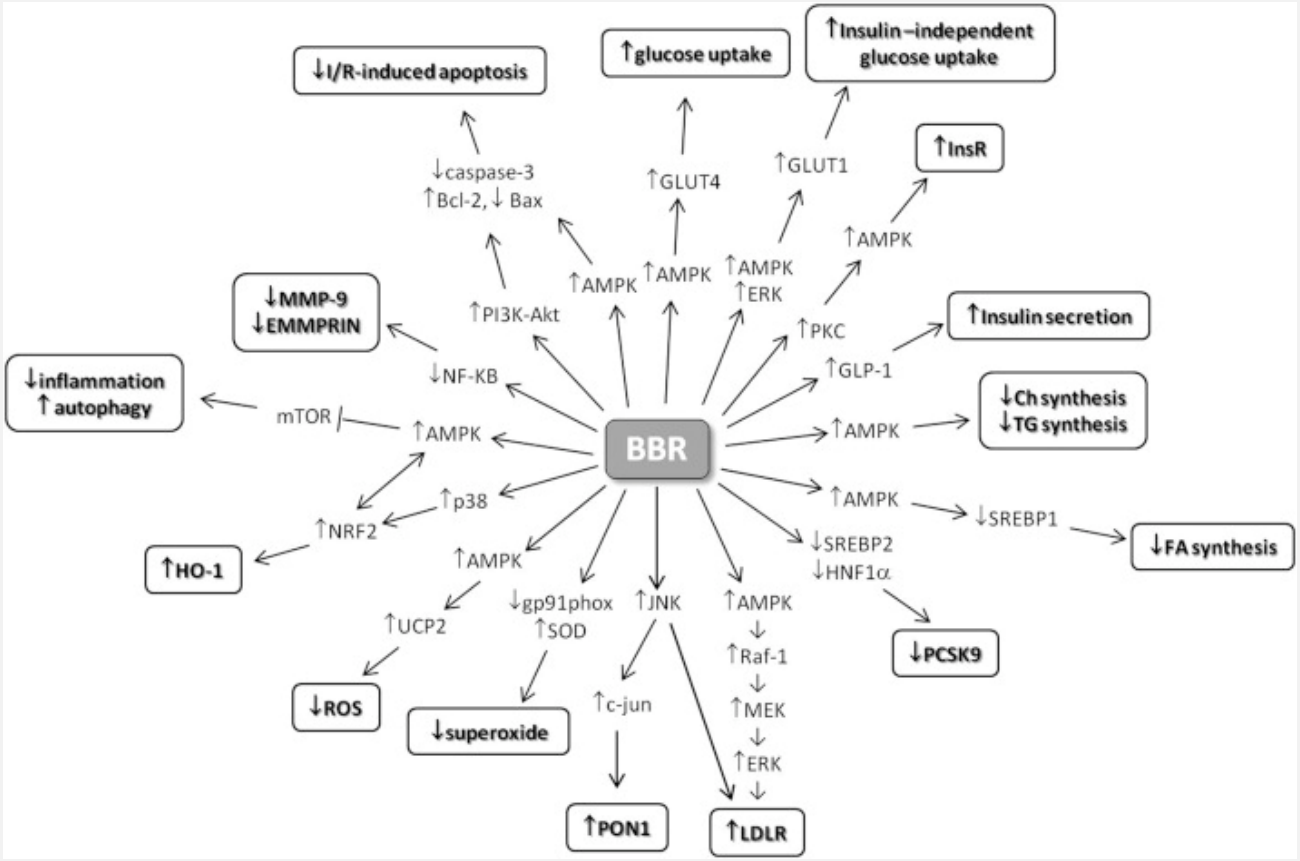
ABSTRACT

Berberine (BBR) is an isoquinoline plant alkaloid endowed with several pharmacological activities, including anti-microbial, glucose- and cholesterol-lowering, anti-tumoral and immunomodulatory properties. The main mechanism by which BBR exerts a protective role in atherosclerosis relates to its cholesterol-lowering activity. BBR significantly increases hepatic low density lipoprotein receptor (LDLR) expression and reduces the expression and secretion of the LDLR modulator proprotein convertase subtilisin/kexin type 9 (PCSK9). In addition to this, several other atheroprotective effects have been ascribed to BBR, including anti-inflammatory and anti-oxidant properties, inhibition of vascular smooth muscle cell proliferation and improvement of endothelial dysfunction. BBR also increases glucose utilization in adipocytes and myocytes, while decreases glucose absorption in intestinal cells, resulting in a net hypoglycemic effect. In hypercholesterolemic animals, BBR significantly decreases LDL-C and total cholesterol (TC) levels and reduces aortic lesions, an effect similar to that of statins. In diabetic animals, BBR significantly reduces glucose levels, improves glucose tolerance, reduces body weight gain and adipose tissue mass. Several clinical studies have also tested the efficacy of BBR in humans. In hypercholesterolemic subjects, BBR induces a significant reduction of TC, triglycerides and LDL-C levels and a significant increase of HDL-C levels, without major adverse effects. BBR also reduces glycemia and plasma cholesterol in diabetic patients, improves lipid and glucose profile and decreases body mass index and waist circumference in subjects with metabolic syndrome. These findings, together with the good tolerability, suggest that BBR administration might be considered a potential therapeutic approach for the treatment of hypercholesterolemia or diabetes. Given the level of evidence available to date well-designed randomized controlled trials to test safety and efficacy of BBR are warranted.

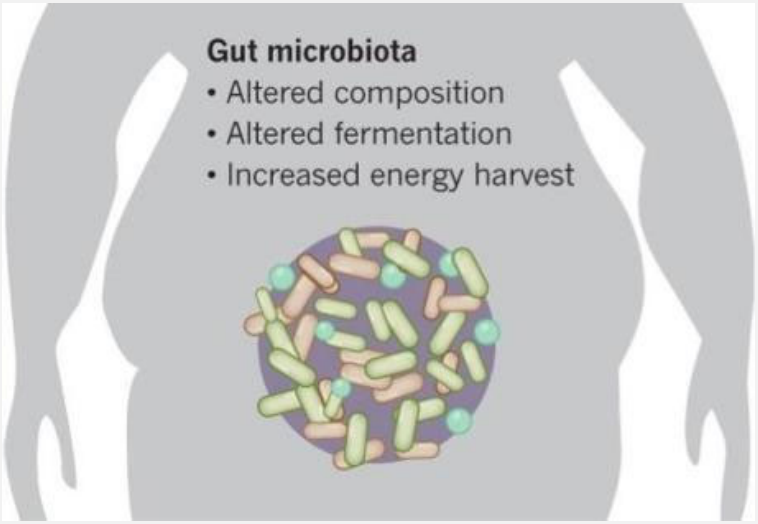
Atherosclerosis 243 (2015): 440-461

SIGNALING PATHWAYS TRIGGERED BY BERBERINE



BERBERINE AND THE GUT MICROBIOME HOW THEY AFFECT EACH OTHER.

- One the one hand: Berberine appears to affect physiology, partly by modulating gut microbiome.



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PLoS one

Structural Changes of Gut Microbiota during Berberine-Mediated Prevention of Obesity and Insulin Resistance in High-Fat Diet-Fed Rats

Xu Zhang[1], Yufeng Zhao[2], Menghui Zhang[1], Xiaoyan Pang[1], Jia Xu[1], Chaoying Kang[2], Meng Li[2], Chenhong Zhang[1], Zhiguo Zhang[3], Yifei Zhang[3], Xiaoying Li[3], Guang Ning[3], Liping Zhao[1,2]*

1 State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, PR China, **2** Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, PR China, **3** Shanghai Clinical Center for Endocrine and Metabolic Diseases and Division of Endocrine and Metabolic Diseases, Rui Jin Hospital, Shanghai Jiao Tong University, Shanghai, PR China

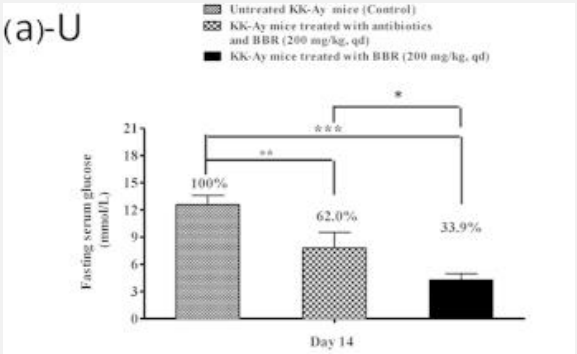
Abstract

Berberine, a major pharmacological component o
bacterial diarrhea, has recently been demonstrated
revealed that berberine effectively prevented the c
rats, which showed decreased food intake. Increas
chemoattractant protein-1, and leptin and decrease
were also significantly retarded by the co-ad
pyrosequencing of the V3 region of 16S rRNA ge
berberine-treated rats. UniFrac principal coordinat
berberine-treated rats away from that of the contro
taxonomic units (OTUs), most of which were esser
producing bacteria, including *Blautia* and *Allobac*
concentrations. Partial least square regression moc
the adiposity index, body weight, leptin and adipo
might have a close association with the host m
prevention of obesity and insulin resistance by
modulation of the gut microbiota, which may help
host and elevating SCFA levels in the intestine.

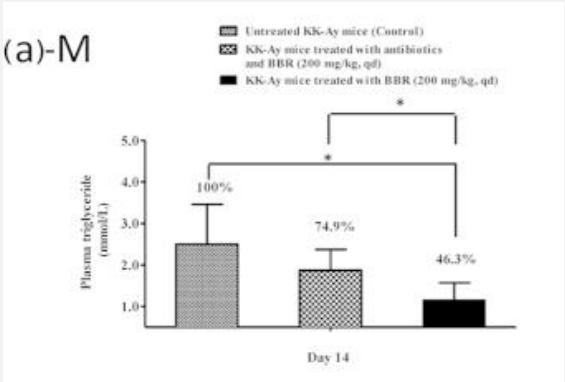
Taken together, our findings suggest that the prevention of obesity and insulin resistance by berberine in HFD-fed rats is at least partially mediated by structural modulation of the gut microbiota, which may help to alleviate inflammation by reducing the exogenous antigen load in the host and elevating SCFA levels in the intestine.

Citation: Zhang X, Zhao Y, Zhang M, Pang X, Xu J, et al. (2012) Structural Changes of Gut Microbiota during Berberine-Mediated Prevention of Obesity and Insulin Resistance in High-Fat Diet-Fed Rats. PLoS ONE 7(8): e42529. doi:10.1371/journal.pone.0042529

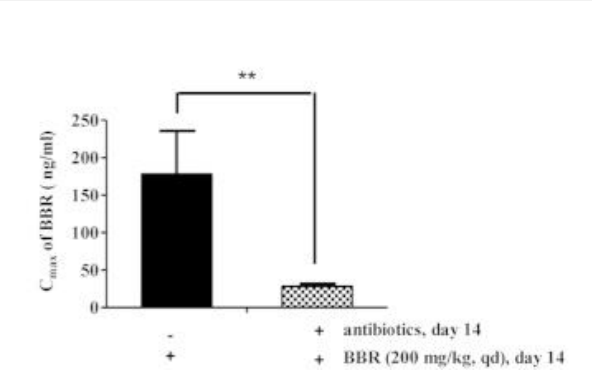
ANTIBIOTICS INHIBIT THE BENEFIT AND BIOAVAILABILITY OF BERBERINE IN ANIMAL MODELS



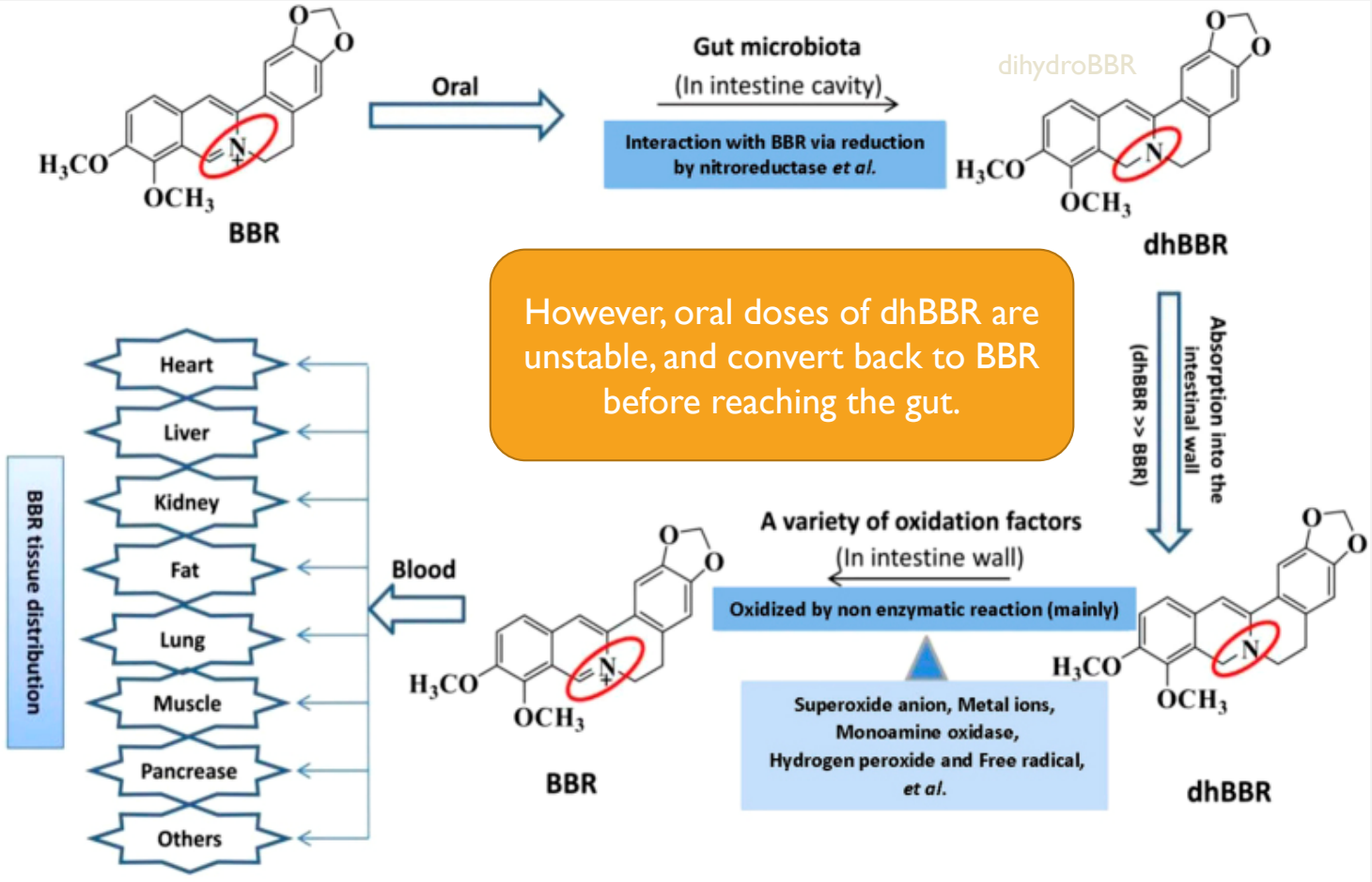
Change in FBG



Change in TG



Sci Rep. 2015 Jul 15;5:12155.



Transforming berberine into its intestine-absorbable form by the gut microbiota.
Sci Rep. 2015 Jul 15;5:12155.

SCIENTIFIC REPORTS

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Significant pharmacokinetic differences of berberine are attributable to variations in gut microbiota between Africans and Chinese

Raphael N. Alolga[1], Yong Fan[1], Zhuo Chen[1], Li-Wei Liu[1], Yi-Jing Zhao[1], Jin Li[1], Yan Chen[2], Mao-De Lai[1], Ping Li[1] & Lian-Wen Qi[1]

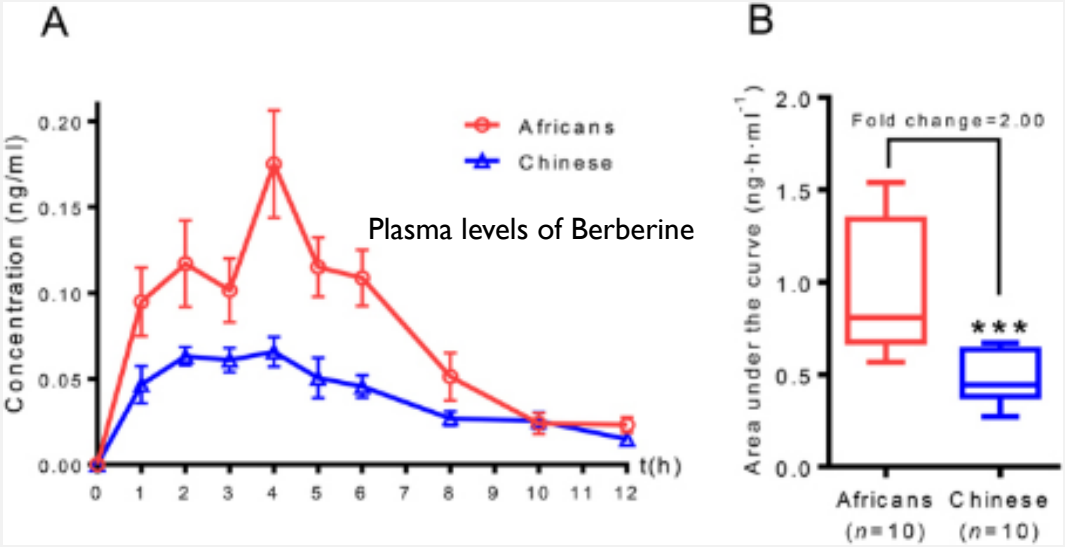
We investigated the influence of gut microbiotal metabolism on the pharmacokinetics of berberine in healthy male Africans and Chinese. The C_{max} and *AUC* in the Africans were 2.67-fold and 2.0-fold higher than the Chinese, respectively. Microbiotal compositions by 16S rRNA pyrosequencing showed higher abundance of the genera *Prevotella*, *Bacteroides*, and *Megamonas* (34.22, 13.88, and 10.68%, respectively) in the Chinese than the Africans (30.08, 9.43, and 0.48%, respectively). Scatter plot showed a strong negative correlation between the microbiotal abundance and the berberine *AUC*, especially for the genus *Prevotella* ($r = -0.813$) and its species. A more extensive metabolism was observed in Chinese with 1.83-fold higher metabolites, possibly contributing to the lower *AUC* than the Africans. In conclusion, significant PK differences of berberine were observed between Africans and Chinese, which is partly attributable to variations in gut microbiota and its corresponding metabolic capacity.

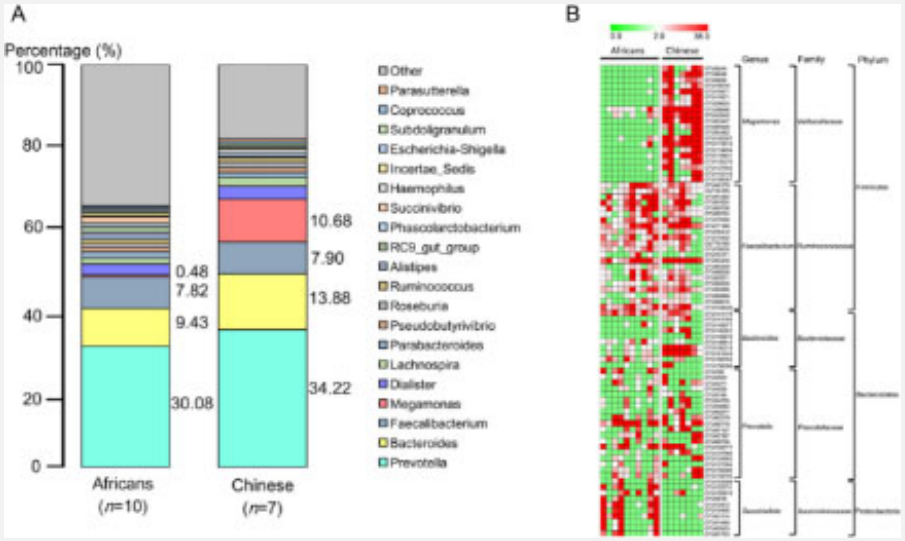
Berberine is an active constituent present in many medicinal plants. It has long been used in traditional medicine to treat many health concerns, and recently has been widely tested due to its diverse clinical and pharmacological activities[1–5]. It has now become one of the world's most widely used natural products. It is estimated that, approximately 20 billion pills of berberine are consumed every year in Asian countries. Because of its low cost, berberine has also been recognized and has gained entry in many African countries. In USA, it is widely marketed as a powerful dietary supplement.

Pharmacokinetic (PK) profile of berberine is necessary data to design a rational dosage regimen. Berberine has low rates of absorption when taken orally. High oral doses may cause intestinal side-effects, including constipation, stomach upset, and cramping[4]. For this reason, choosing the right dose of berberine for a specific population is of significance to avoid potential side-effects. Though berberine is largely used in many African countries, PK studies of the drug in such races are lacking. Most African countries are bedeviled with myriad of problems and with little to no technological know-how to conduct their independent PK studies. They are usually left with no option than follow the dosage regimen gotten from other races.

PK differences in response to drugs have been of concern in different racial populations and ethnic groups. Pharmaceutical companies do include specific genetic or racial information to some marketed drugs to guide their usage by various populations. Isosorbide dinitrate-hydralazine is known to be effective for use in times of heart failure in black patients[6]. Warfarin and rosuvastatin are required in a lower dose by Asians[7,8], while tacrolimus is needed at a higher dose by blacks[9]. Some of the observed racial differences may be explained by the genetic differences[10]. Other possible mechanisms for the differences remain unknown. Understanding these differences is of clinical significance for individualized treatments.

[1]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, Jiangsu, China. [2]Department of Emergency Center, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China. Correspondence and requests for materials should be addressed to P.L. (email: liping2004@126.com) or L.-W.Q. (email: Qilw@cpu.edu.cn).





HOW MANY PHYTOCHEMICALS NEED A HEALTHY MICROBIOTA FOR ACTIVATION?

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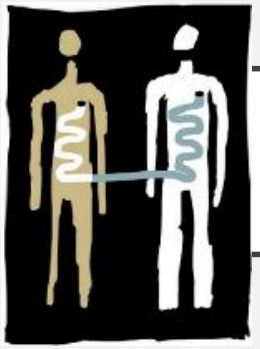
Review

Could the gut microbiota reconcile the oral bioavailability conundrum of traditional herbs?

Feng Chen *, Qi Wen, Jun Jiang, Hai-Long Li, Yin-Feng Tan, Yong-Hui Li, Nian-Kai Zeng

Hainan Provincial Key Laboratory of R&D of Tropical Herbs, School of Pharmacy, Hainan Medical College, Haikou 571199, China

- In many cases "microbiota availability" may actually be the target of phytochemicals that are known to have poor human bioavailability
- Our desire to increase bioavailability of important phytochemicals may actually miss the very target of their therapy, or at least alter that relationship substantially.



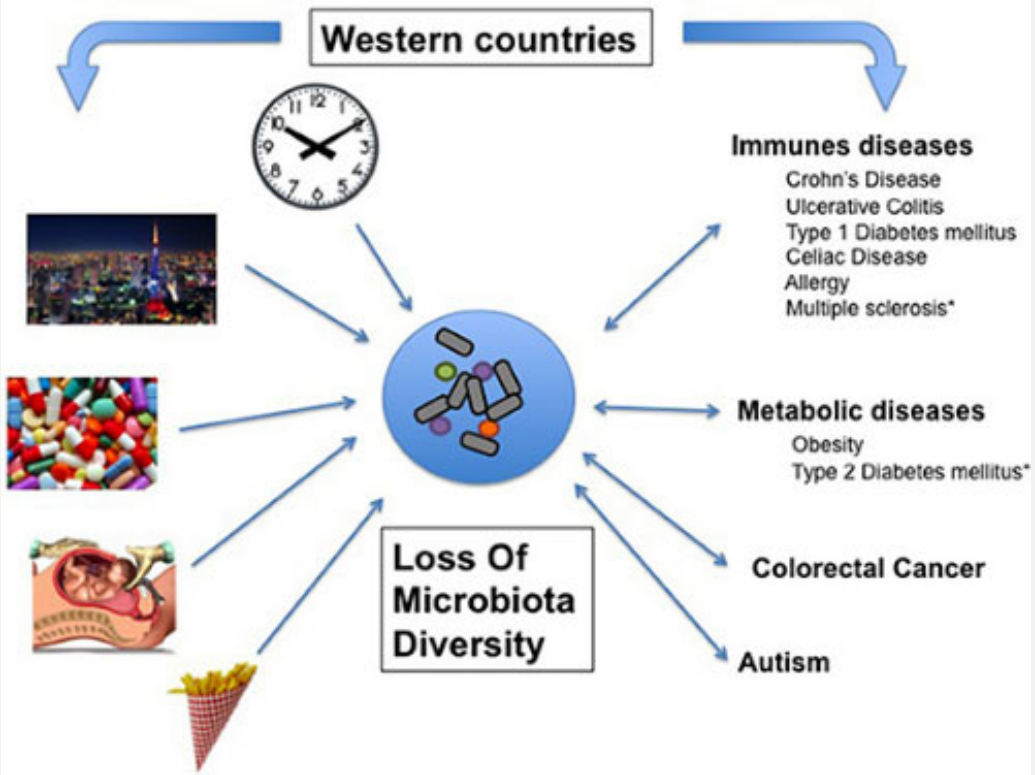
FECAL MICROBIOTA TRANSPLANTS

- FMT are now recognized globally as highly successful for recurrent *C. difficile* infections (CDI/CDAD)
- In appropriate subjects (with appropriate donors), successful remission is >90% (Fresh or Frozen!)
- Success in children for CDI is similar, though fewer studies have been performed. *Pediatr Res*. 2016 Jul;80(1):2-6.
- FMT studies on other GI conditions (IBD, IBS, etc.) and non-GI conditions: obesity, immune-related outcomes, autism etc. are ongoing with some success in small trials. *Dig Dis Sci*. 2017 May;62(5):1131-1145.

WHAT ABOUT PROBIOTICS?

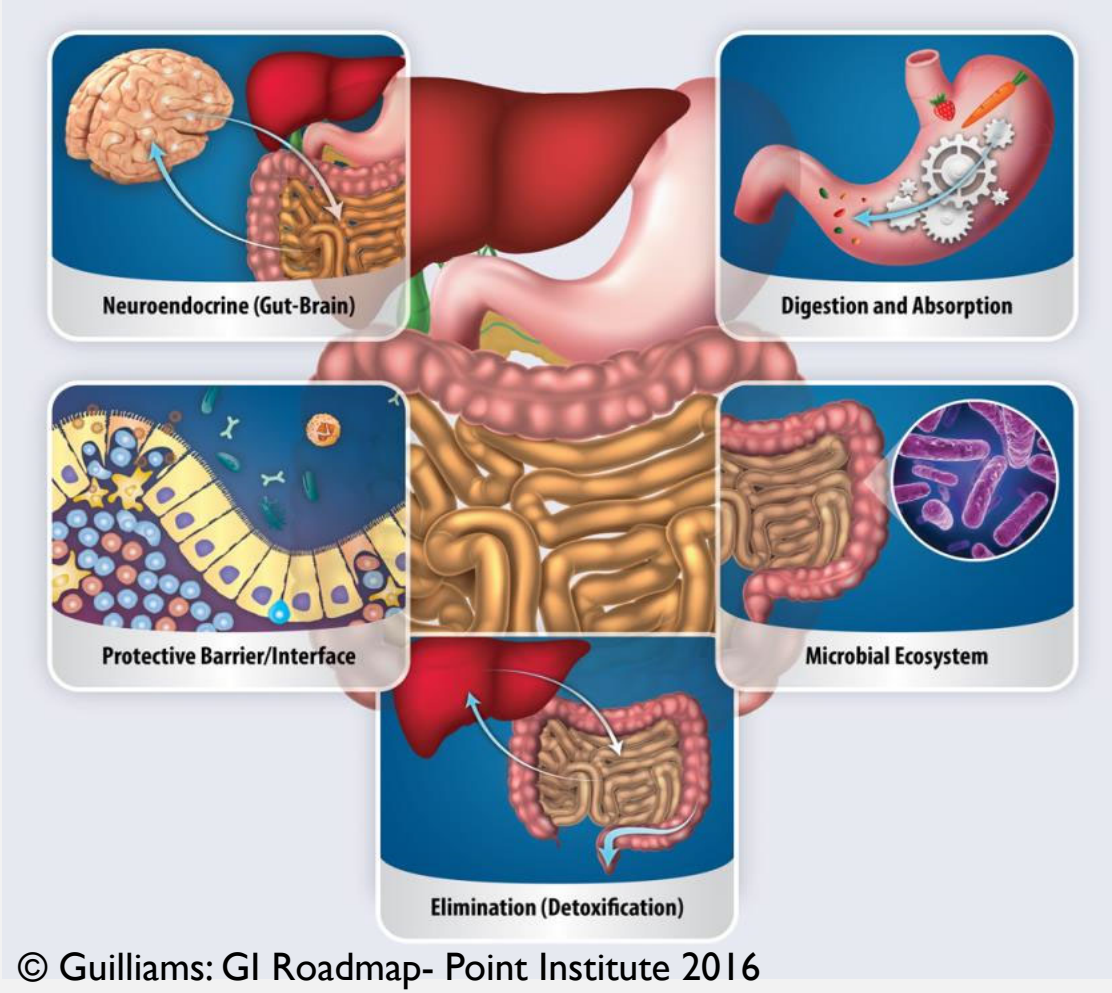
Stay Tuned…..

MOST IMPORTANT TAKEAWAY



- Most of what we know is good (or Bad) for our microbiome(s) has already been shown to be good (or Bad) for Us-With few exceptions.
- the Microbiome revolution helps explain how and why certain lifestyle interventions may work, but rarely contradicts what we know about healthy diets, physical activity, stress, Hygiene etc.

CHRONIC DISEASE MANAGEMENT REQUIRES SUPPORTING THE CORE FUNCTIONS OF GI



CORE FUNCTIONS VS 4R (OR 5R)