A Novel Overview of GI Functions in Health & Disease

Mapping GI Symptoms to Create Better Management

Michael Chapman, ND

Director of Product Innovation

Objectives

- Understand the major underlying functions of the GI tract
- Be able to look at GI dysfunction from a systems-medicine approach
- Be able to accurately assess what root cause is driving your patients' symptoms
- Integrate novel therapeutics to address the cause of symptoms

“All disease begins in the gut.”

Hippocrates

c.460 - c.370 BC

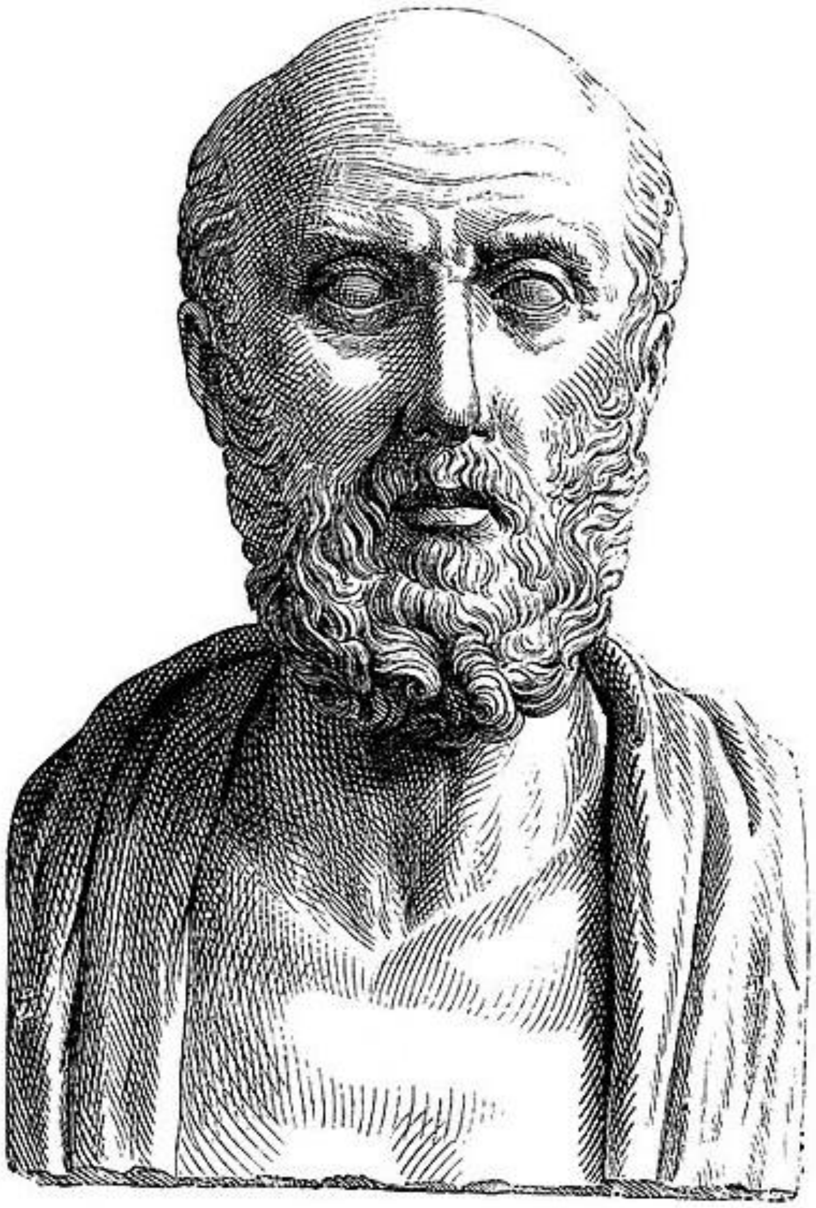

Image: https://commons.wikimedia.org/wiki/File:Hippocrates.jpg

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Conventional Silo Medicine

Functional Gastroenterology

- What exactly is the role of the GI tract?
  - Digestion
  - Absorption
  - Inflammation modulation
  - Immune response
  - Microbiome balance
  - Intestinal permeability
  - Neuroendocrine
  - Detoxification
- These are functions, but they are also methods to achieve a larger goal

The GI tract's role in the system is to maximize assimilation of essential nutrition at the highest efficiency…

While…

Limiting risks of exposure to external dangers.

“Nutrients are, like, important and stuff.”

~Michael Chapman, ND

c. 2019-2022

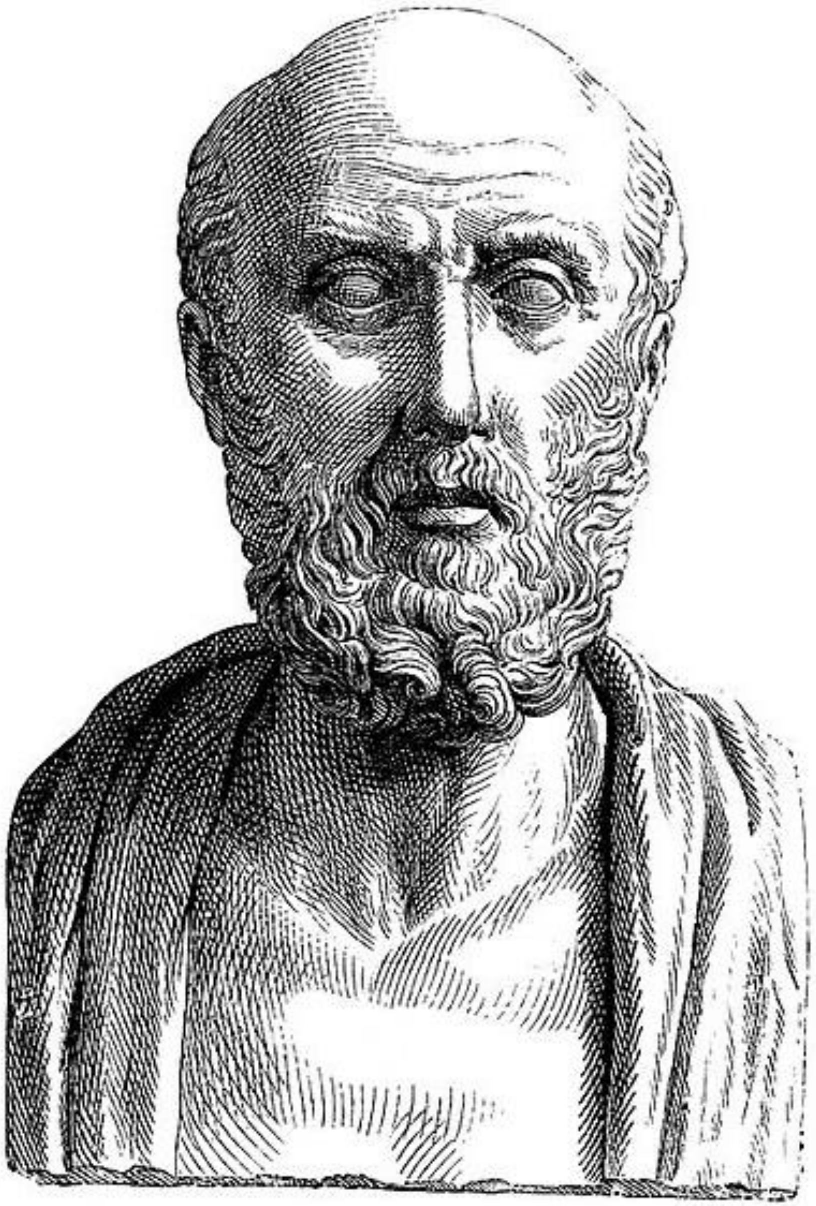

Image: https://commons.wikimedia.org/wiki/File:Hippocrates.jpg

Breaking Down These Roles

Assimilation of Nutrients

- Digestion
  - Enzymatic
  - Physical/Mastication
  - Fermentation
- Absorption
  - Passive/Active/Facilitated
  - Surface area maximization
  - Motility

Minimize Risk to Organism

- Assessment
  - Surveillance
- Tolerance
  - Immune inactivation
- Activation
  - Immune recruitment
  - Inflammation
  - Motility

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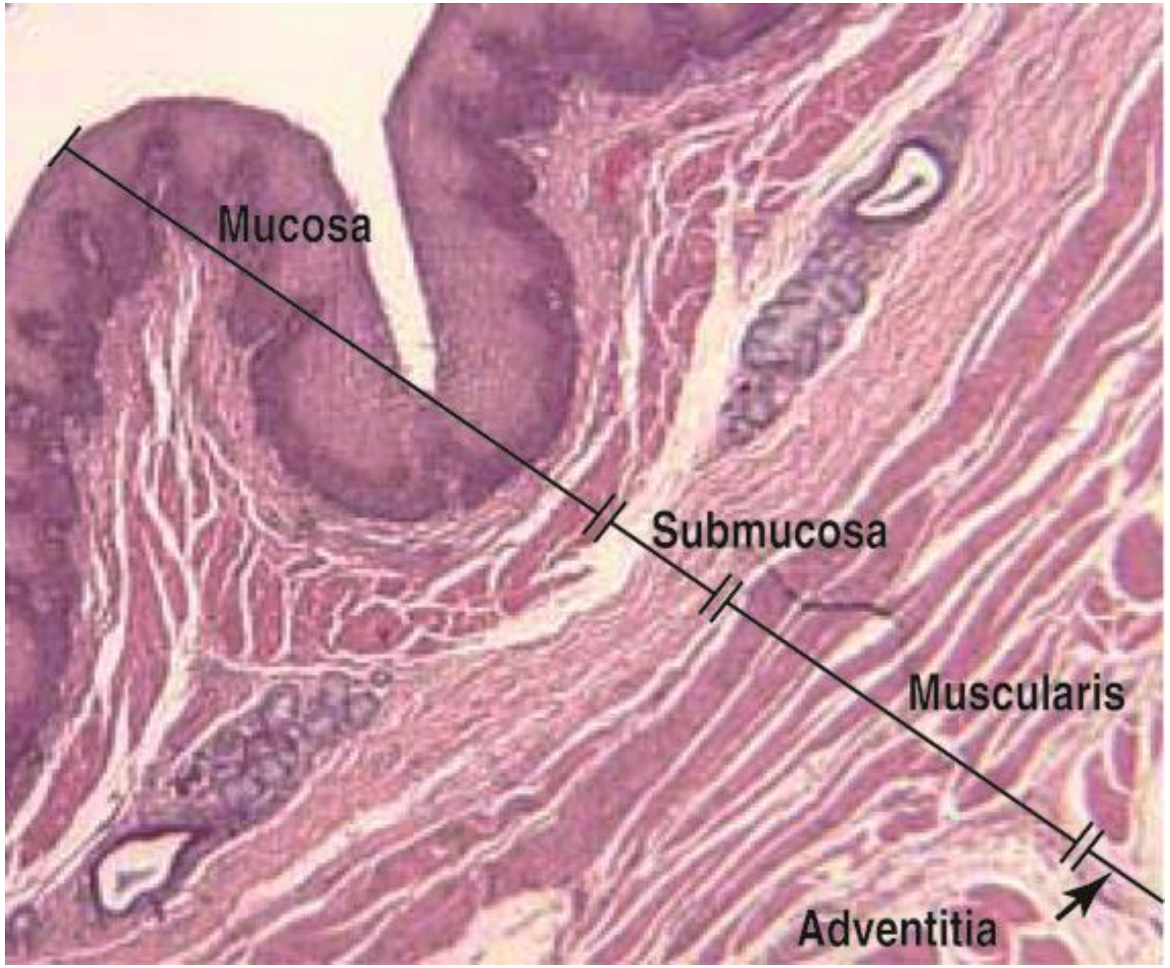
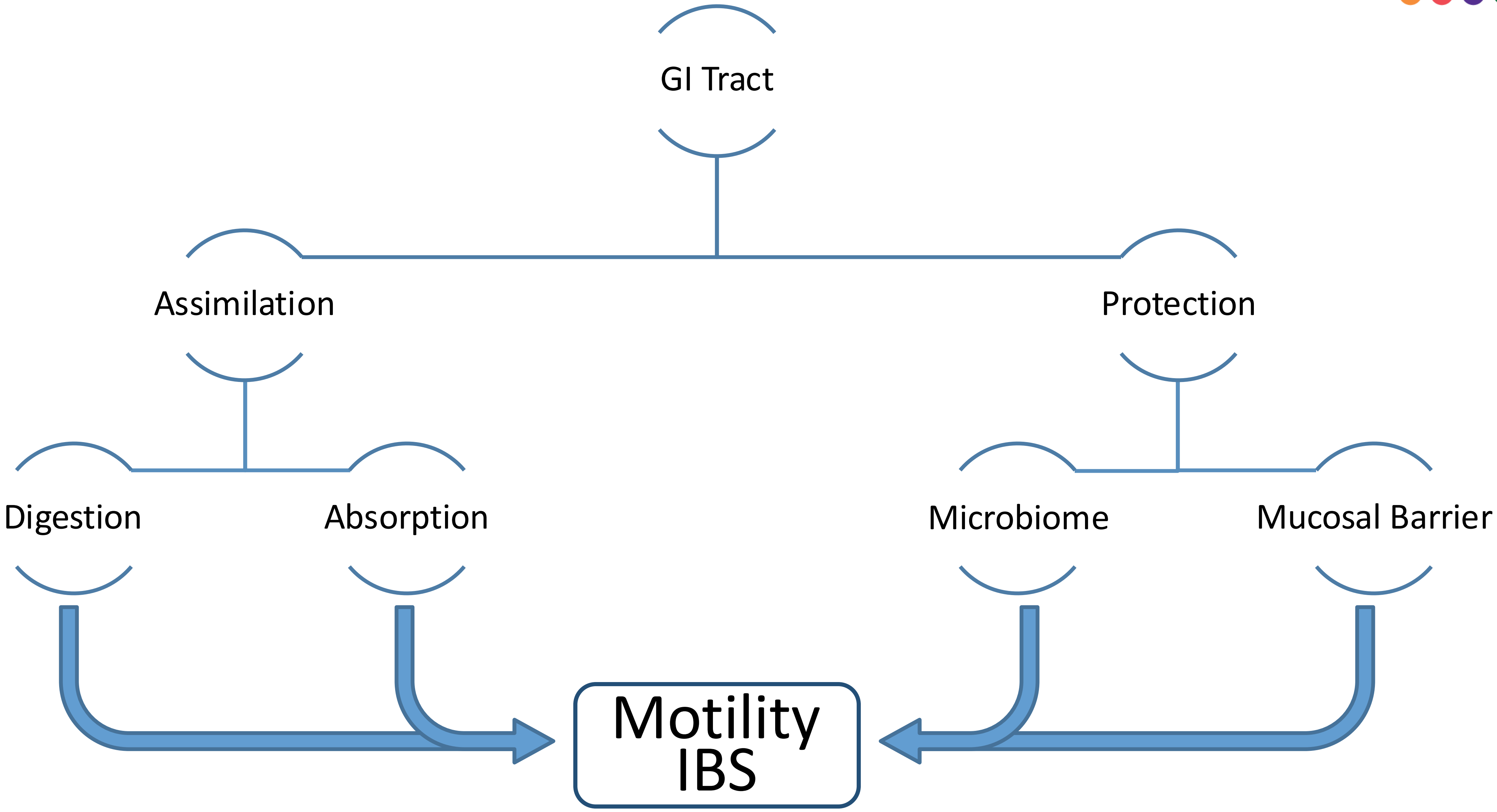
“The truth of the story lies in the details” ~ Paul Auster

The Gut Lumen

- Remember, the gut lumen is not a part of us!
- Think of the GI Tract as being *outside world,* much like the skin

The Interface With The Outside World




GI Tract
Assimilation
Protection
Digestion
Absorption
Microbiome
Mucosal Barrier
Motility
IBS

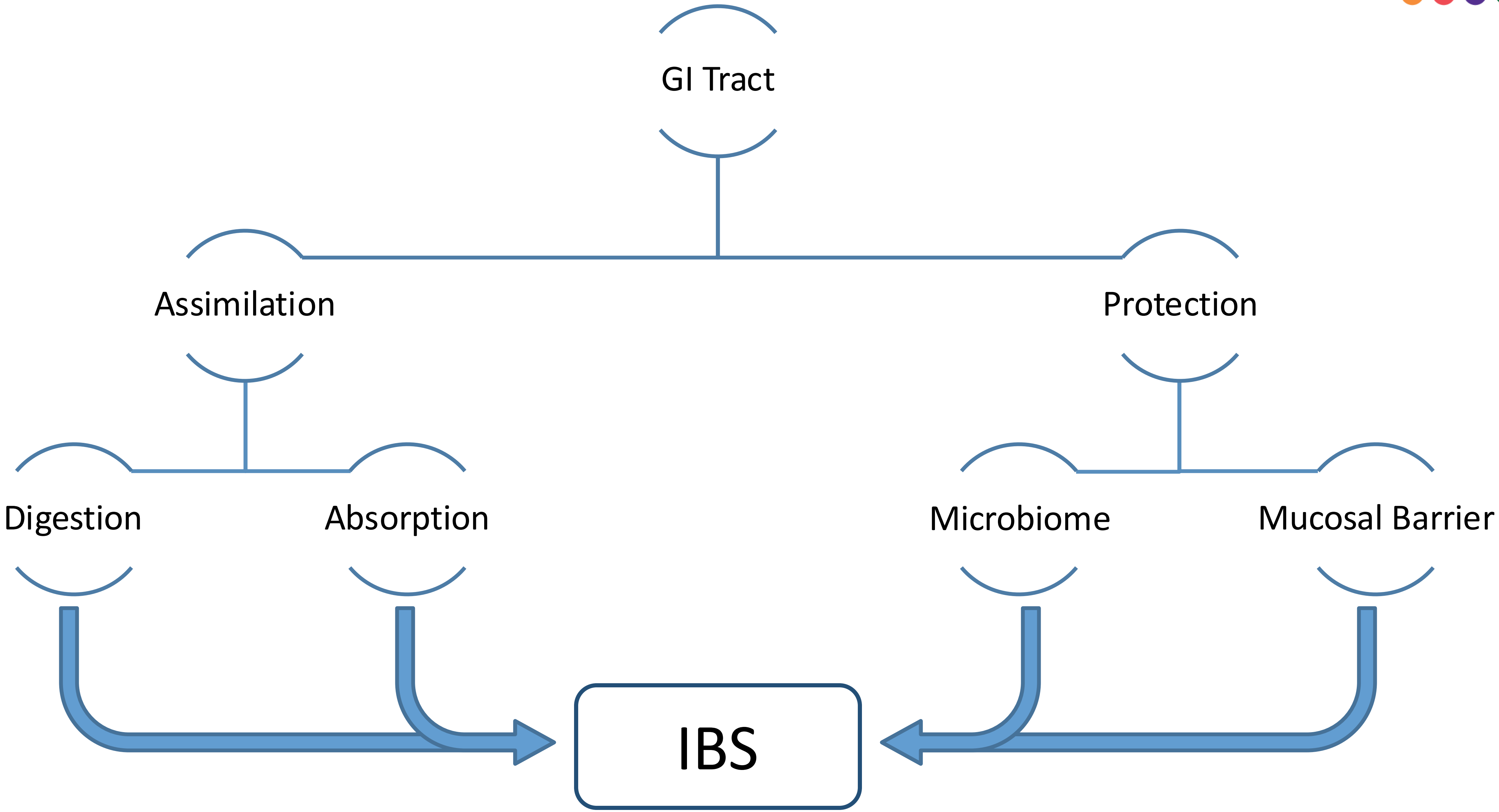
Recalculating GI Pathology

- If we can clinically focus on the categories of assimilation and protection, we can easily begin to uncover the root-cause(s) of most GI chronic conditions
  - IBS – which is a motility issue
  - IBD – which is multifactorial, but is a protection issue conceptually

- One additional factor hovers in the background
  - Direct insult to GI function by dietary influence
    - Alcohol directly impairing lower esophageal function
    - Other foods/medications that directly damage the mucosa

The Diet Factor

- We need to think about diet differently as well, because it can have multiple impacts:
  - Direct insult on mucosal function
  - Alteration of microbiome composition
  - Activation of immune response due to poor tolerance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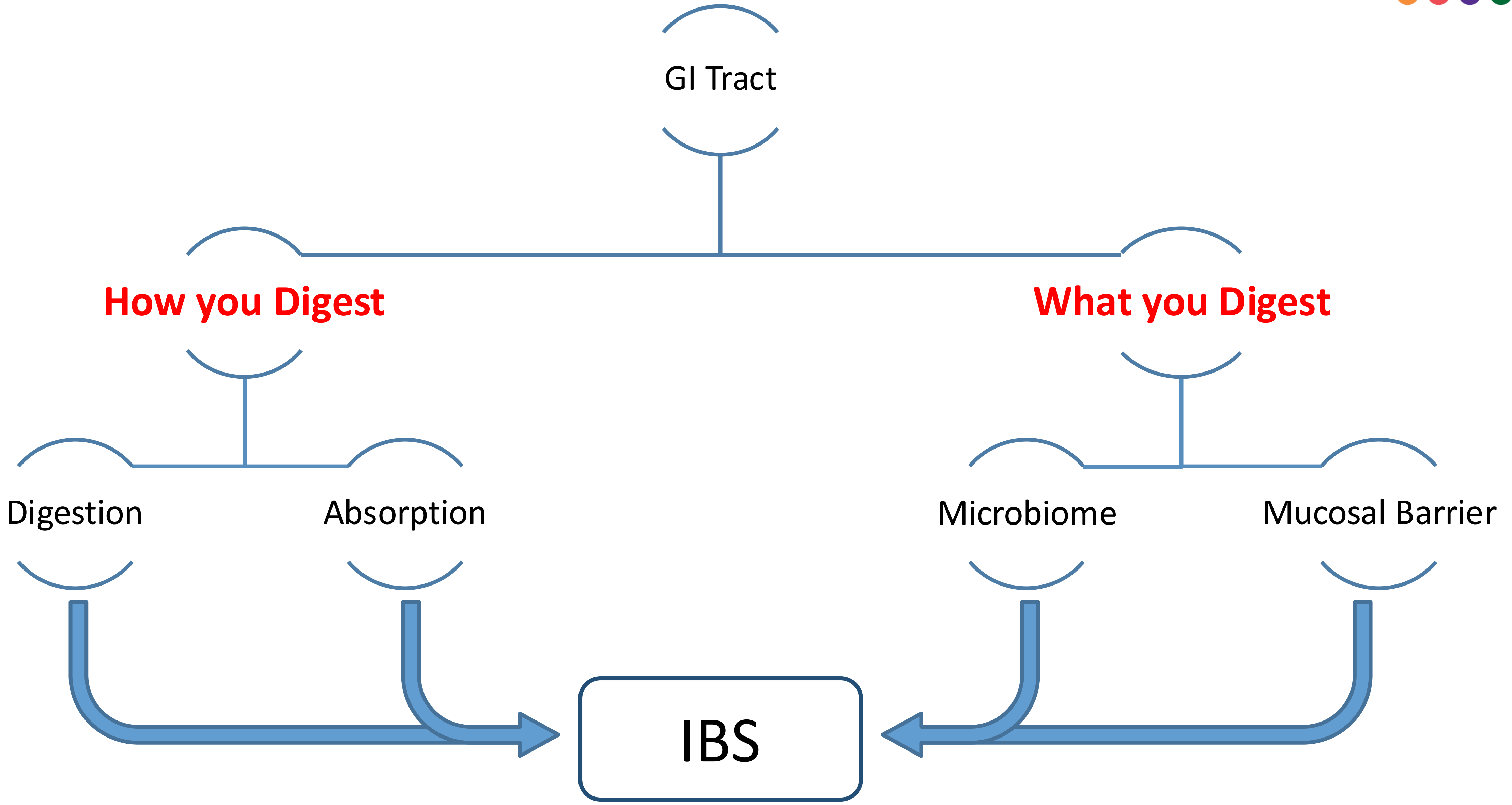

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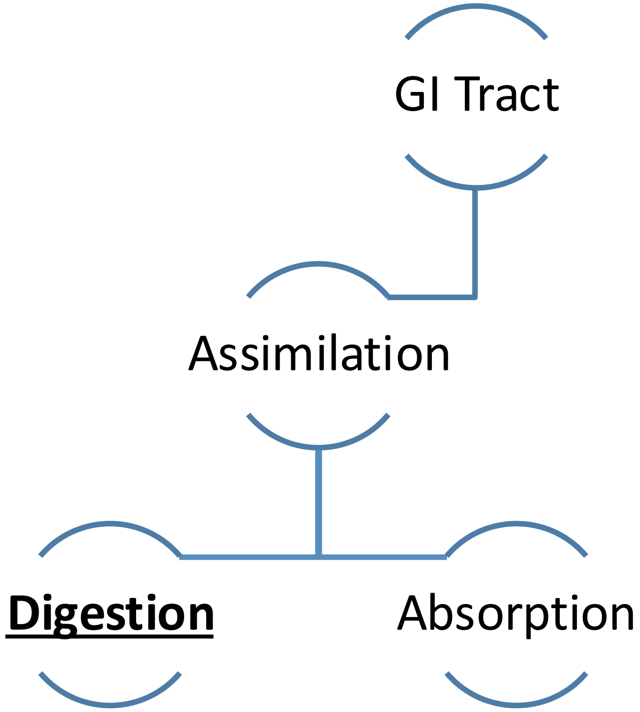
Digestion



- Breaking down foods into macro and micro-nutrients
  - Enzymatic
  - Mechanical

- How often do we talk to our patients about chewing their food?

Evidence-Based Mastication

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INVITED MEDICAL REVIEW

WILEY ORAL DISEASES

Salivary functions in mastication, taste and textural perception, swallowing and initial digestion

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Saliva exerts multiple functions in relation to the initial digestive processes taking place in the upper parts of the gastrointestinal tract. Ingestion of food and beverages, in turn, is a strong stimulus for secretion of saliva with a differential composition depending on the neuronal stimulation pattern. This review paper provides insight into the mechanisms by which saliva acts in relation to taste, mastication, bolus formation, enzymatic digestion and swallowing. Also, the protective functions of saliva including maintenance of dental and mucosal integrity will be discussed as they indirectly influ-

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Research is Always Mixed

Swallowing food without chewing; a simple way to reduce postprandial glycaemia

By N. W. READ, I. McL. WELCH, C. J. AUSTEN, C. BARNISH, C. E. BARTLETT, A. J. BAXTER, G. BROWN, M. E. COMPTON, K. E. HUME, I. STORIE and J. WORLDING

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(*Received 25 March 1985 – Accepted 2 August 1985*)

1. The degree to which disruption by mastication affects the glycaemic response to four different carbohydrate foods was investigated in healthy human volunteers; each food was eaten by six subjects.

2. Subjects ate meals of sweetcorn, white rice, diced apple or potato on two occasions; on one occasion they chewed the food thoroughly, on the other occasion they swallowed each mouthful without chewing it.

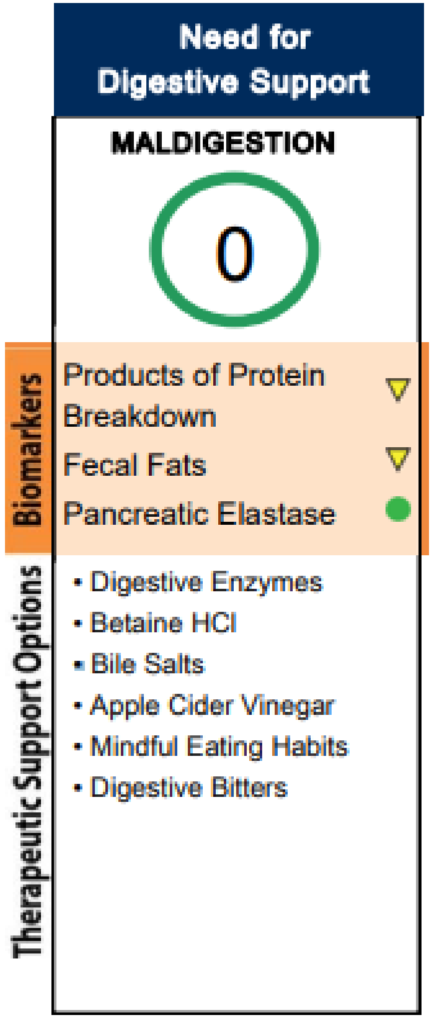
3. When the foods were chewed the postprandial blood glucose levels rose to levels which varied according to the food ingested.

4. Swallowing without chewing reduced the glycaemic response to each food, achieving a similar effect as administration of viscous polysaccharides or ‘slow-release’ carbohydrates.

Ingestion of foods which contain equivalent amounts of carbohydrate yield widely different effects on postprandial blood glucose levels and insulin release (Crapo *et al*. 1977, 1980; Jenkins *et al*. 1981), and it seems likely that the form in which food is ingested influences the rate of digestion and absorption of carbohydrates, the metabolic response to a meal and subsequent food intake. Carbohydrates in the form of sugars or potato starch are rapidly absorbed, yield high postprandial glycaemic and plasma insulin responses and may

Improving Digestion Without Having to Chew

- Three components:
  - pH
  - Enzymes
  - Bile
- Oh, and we can't do it ourselves, we have friends!
  - Fermentation from the microbiome



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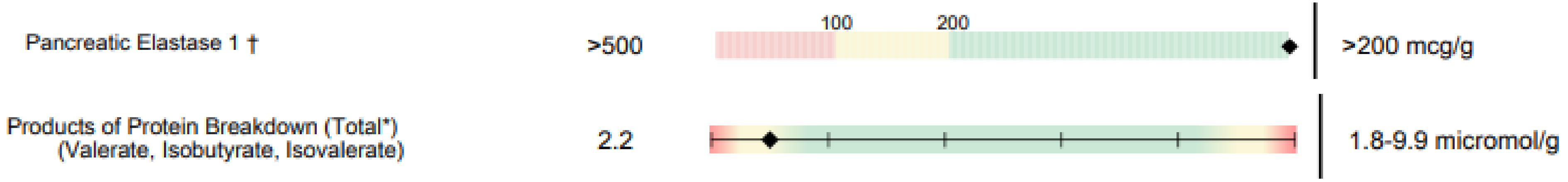
Gastric pH

- Critical in breaking down protein into polypeptides
- Don’t forget that any digestive enzyme has a specific pH window that it needs to operate at
- If the patient doesn’t have appropriate pH, all enzymes will not function as well, promoting poor digestive capacity
  - Protease
  - Lipase



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Eating Hygiene

Causes of Hypochlorhydria

- Zinc Deficiency
- B-Vitamin Deficiency
- H.pylori
- Stress
- Decreased Production with Age
  - Between 25%-40% achlorhydria after 50yo

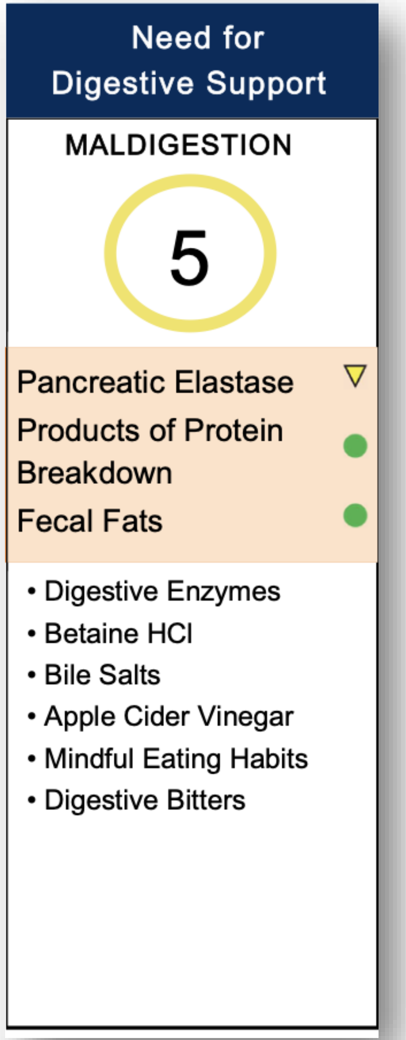
Grossman et al. *Gastroenterology* 1963;45:15-26.
Sharp GS et al. *J Amer Ger Soc* 1967;15:786-791.

Supporting pH

- Improve Zinc and B-vitamin status
- Eating behaviors that stimulate HCl release
- Betaine HCl
- Gentian drops
- Apple cider vinegar?
  - Not a lot of literature support and has potential drawbacks

Enzymes & Pancreatic Insufficiency (EPI)

- Evaluation & Management
  - Low Pancreatic Elastase
  - High Protein Products (could also be pH/bacterial overgrowth)
  - High Stool Fats
  - Radioactively labelled ^{13}C-MTG breath test
- Pancreatic Enzyme Replacement
  - This is a bit of a bandage until underlying root causes can be worked on



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Domínguez-Muñoz, et al. *J Gastroenterol. Hepatol.* 26 (2011): 12-16.

Root Cause & EPI

Direct Causes of EPI

- Cystic Fibrosis
- Chronic pancreatitis (CP)
- Pancreatic resection
- Autoimmune pancreatitis
- Gallstones
- Pancreatic tumor/cancer
- GI surgery (i.e., gastric bypass, pancreatic resection)

Other Factors Associated with EPI

- Celiac disease
- Inflammatory Bowel Disease (IBD)
- Excessive alcohol consumption
- Small Intestinal Bacterial Overgrowth
- Smoking
- Obesity
- Vegan/vegetarian diets
- Diabetes
- Infectious enteritis

Shades of Pancreatitis

- The pancreas is strongly susceptible to oxidative stress
  - Alcohol, smoking
  - Insulin resistance, diabetes
  - Inflammation

- SIBO has been shown to create inflammation and potential damage which reduces pancreatic exocrine output through feedback mechanisms
  - Damaged mucosa produces less cholecystokinin (CCK) reducing pancreatic stimulation

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Bile

- Has some sneaky roles
  - Activates pancreatic lipase to break down fats
  - Is also antimicrobial to a degree
  - Critical in excreting bilirubin and toxins

- Not going into all the details, but….a few things to note:
  - Bile is secreted in direct response to fat in the meal (and in the diet overall)
  - Bile is secreted in response to cholecystokinin (CCK)
  - >95% of bile salts are reabsorbed

Bile Acid Malabsorption

- Thought to be significantly under-diagnosed
- Could represent at least 30-50% of IBS-D cases

- Mechanisms of bile acid malabsorption:
  - Increased secretion of sodium/water into the gut lumen
  - Direct stimulation of peristalsis and defecation
  - Increased mucus secretion
  - Damage to intestinal mucosa → thereby further altering CCK

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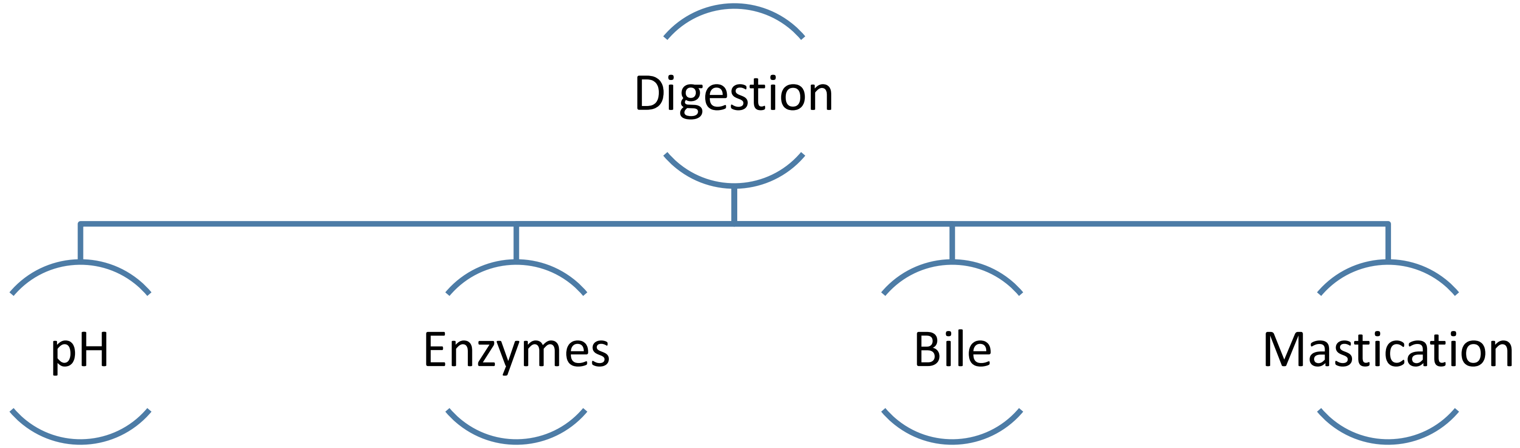
Pattni, et al. *Br Med Bull* 92.1 (2009): 79-93.

Causes of BAM

- Type 1
  - Crohn's Disease
  - Ileal Resection
- Type 2
  - "Idiopathic"
- Type 3
  - Cholecystectomy
  - Celiac Disease
  - SIBO
  - Pancreatic Insufficiency

Fecal Fat (Total*)	6.6	3.2-38.6 mg/g
Triglycerides	0.7	0.3-2.8 mg/g
Long-Chain Fatty Acids	4.6	1.2-29.1 mg/g
Cholesterol	0.8	0.4-4.8 mg/g
Phospholipids	0.5	0.2-6.9 mg/g



Digestion and Absorption

Test	Result		Reference Range
Pancreatic Elastase 1 †	158	L	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	6.0		1.8-9.9 micromol/g
Fecal Fat (Total*)	19.5		3.2-38.6 mg/g
Triglycerides	1.1		0.3-2.8 mg/g
Long-Chain Fatty Acids	12.9		1.2-29.1 mg/g
Cholesterol	0.5		0.4-4.8 mg/g
Phospholipids	5.0		0.2-6.9 mg/g

Need for Digestive Support

MALDIGESTION

5

- Pancreatic Elastase ▽
- Products of Protein Breakdown
- Fecal Fats

- Digestive Enzymes
- Betaine HCl
- Bile Salts
- Apple Cider Vinegar
- Mindful Eating Habits
- Digestive Bitters

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Absorption

Passive/Active

Surface Area

Motility

Digestion and Absorption

Pancreatic Elastase 1 †	158 L	100 / 200	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	6.0		1.8-9.9 micromol/g
Fecal Fat (Total*)	19.5		3.2-38.6 mg/g
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MALDIGESTION

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Lots of Room To Learn

- Passive and Active Absorption
  - Incredible dynamics at play with various genomic differences person to person

- Surface area
  - The amount of inflammation and damage plays a huge role on microvillous blunting

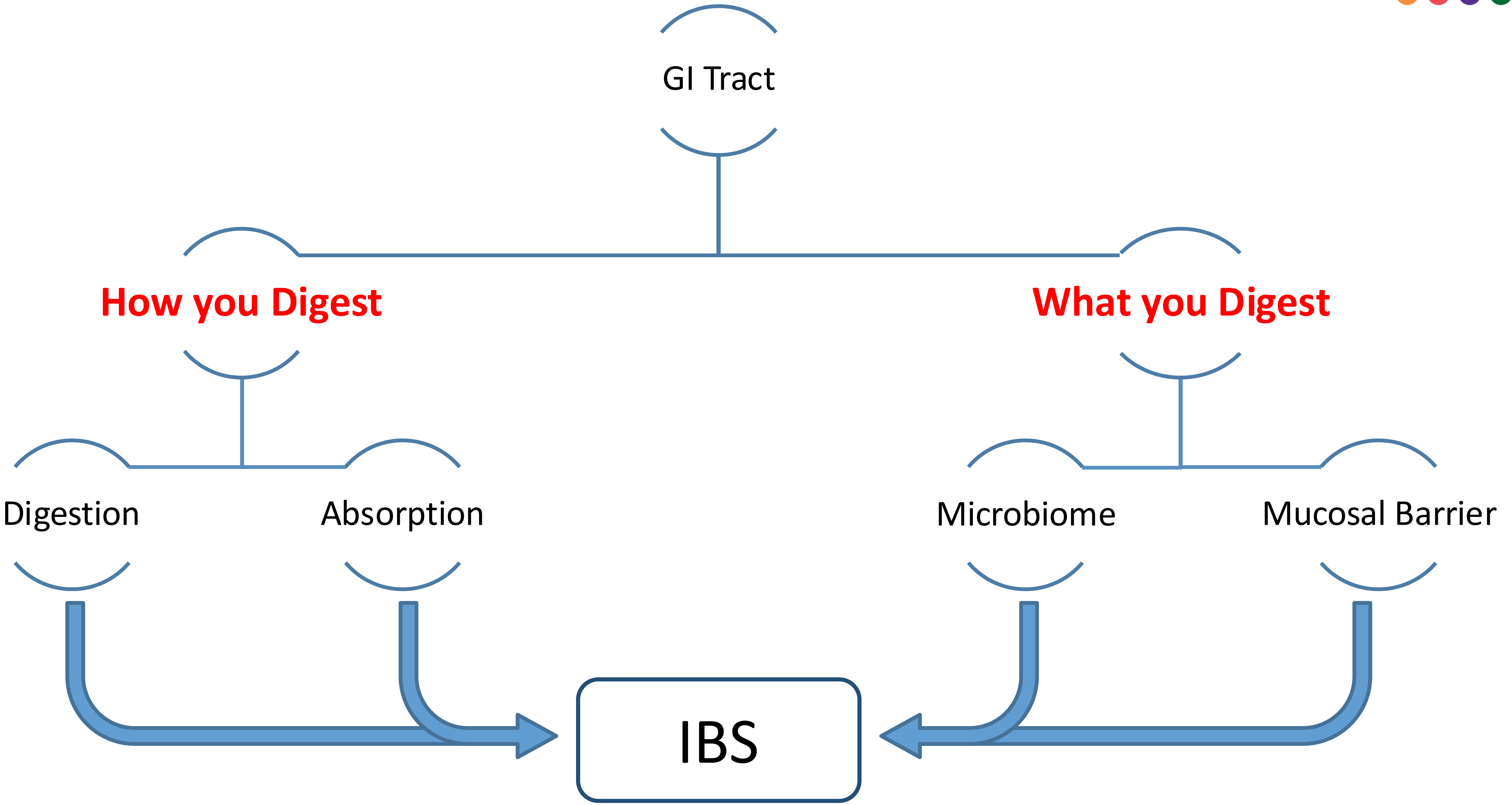
- Motility
  - Vicious cycle…poor absorption causes motility issues, and it creates motility issues (back to Bile Acid Malabsorption)

How can we Address Motility Clinically

- Fluid dynamics in the intestines
  - Fiber plays a crucial role
    - Soluble vs insoluble

- Migrating motor complex
  - Major insights to be gleaned
  - Serotonin as a local regulator of the MMC
  - Look toward enterochromaffin cells and support serotonin in IBS-C and SIBO


It often looks complicated, until you learn the system.



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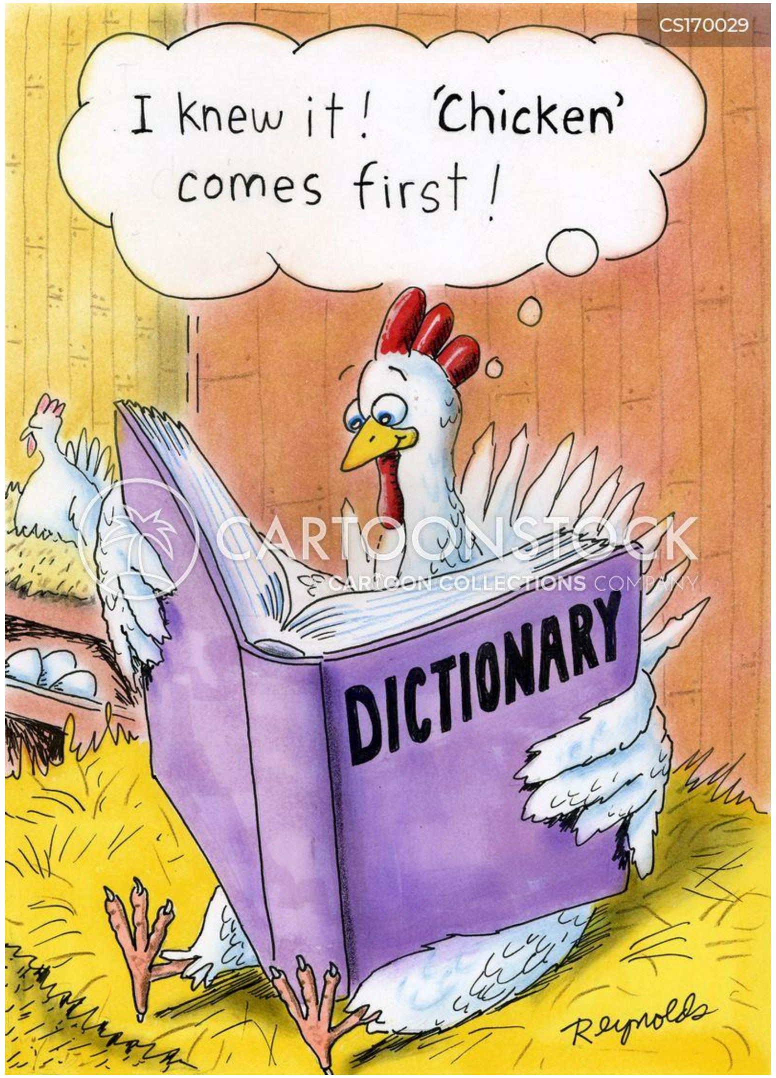
The Microbiome

<u>What comes first?</u>

The Microbiome

Or

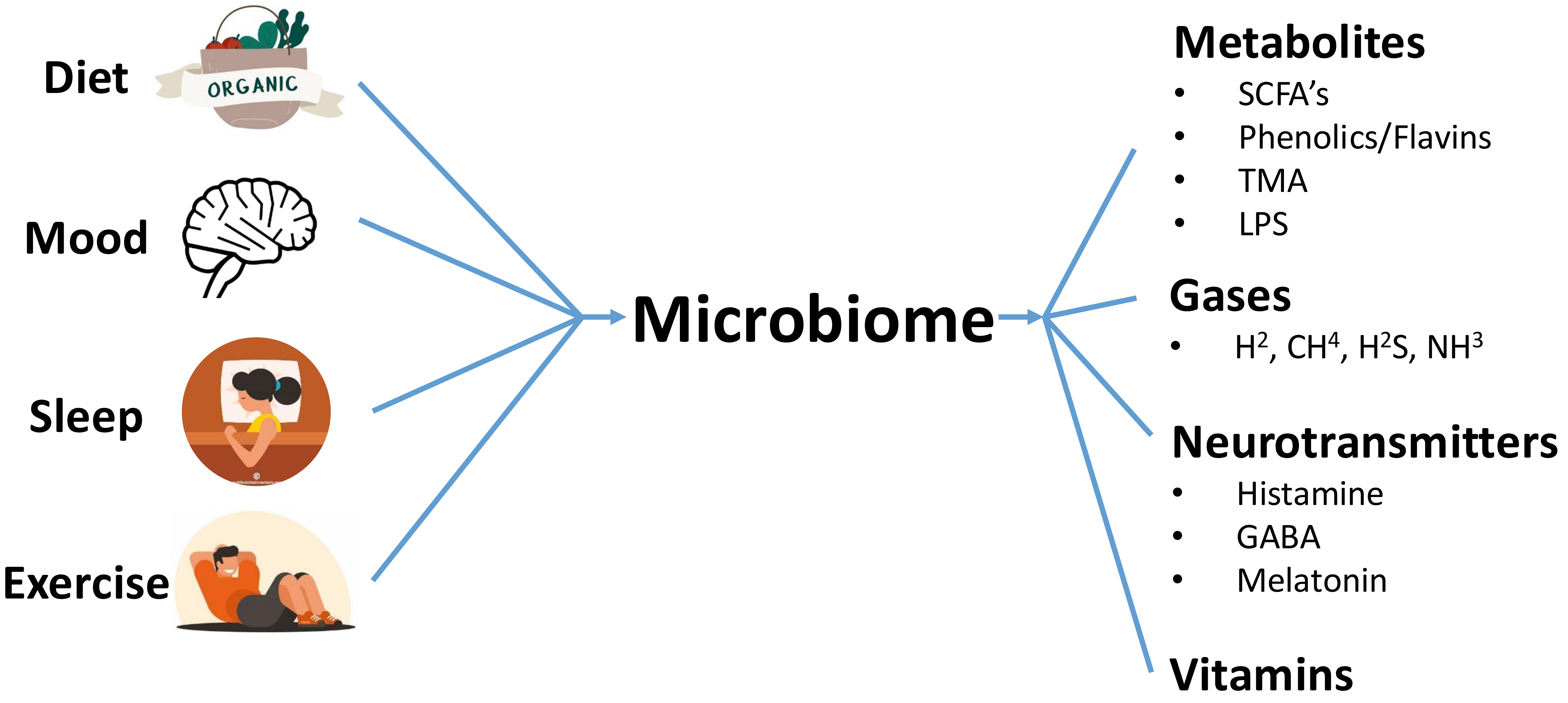
The Disease



The Microbiome

- Throughout the literature there is often mixed clinical associations with various commensal bacteria
- Correlation vs Causation
- What is the real root cause?

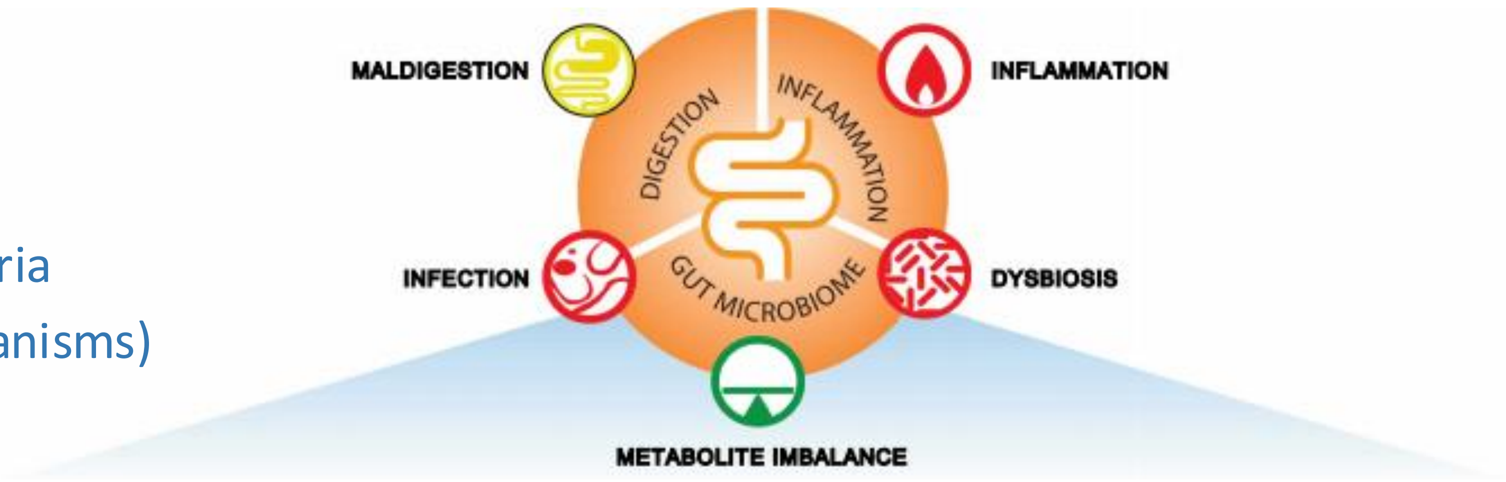
Commensal Bacteria	Out of Reference Range	IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Bacteroidetes Phylum									
Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus		↑			↑	↑		↑	↑
Barnesiella spp.									
Odoribacter spp.									
Prevotella spp.	L	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis		↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus									
Clostridium spp.									
Coprococcus eutactus		↑			↑	↑		↑	↑
Faecalibacterium prausnitzii		↑				↑			↑
Lactobacillus spp.									
Pseudoflavonifractor spp.		↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.			↓						
Ruminococcus spp.	L	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.		↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.	H								
Bifidobacterium longum									
Collinsella aerofaciens	L	↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger									↑
Escherichia coli		↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes		↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii		↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.		↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila	L	↓	↓	↓	↓	↓	↓	↓	↓



Diet
ORGANIC
Mood
Sleep
Exercise
Microbiome
Metabolites
SCFA’s
Phenolics/Flavins
TMA
LPS
Gases
H^2, CH^4, H^2S, NH^3
Neurotransmitters
Histamine
GABA
Melatonin
Vitamins

The Microbiome

- Infection
  - Bacterial
  - Fungal
  - Parasitic
- Dysbiosis
  - Imbalance in commensal bacteria
  - Pathobionts (opportunistic organisms)
  - Overgrowth/Deficiency
- Microbial Metabolites
  - What is the microbiome producing?
    - SCFA’s, gases, endotoxins, etc



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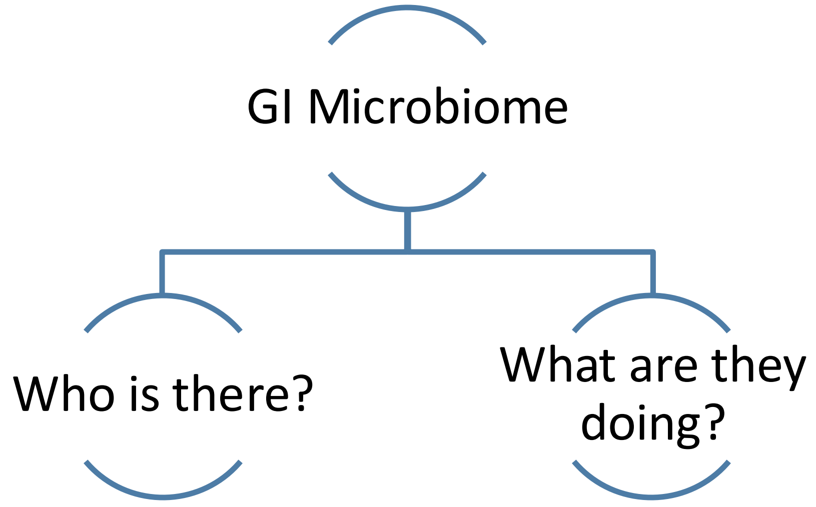
Two Root Approach To Microbiome

- Who is there?
  - Pathogens
  - Pathobionts
  - Dysbiosis

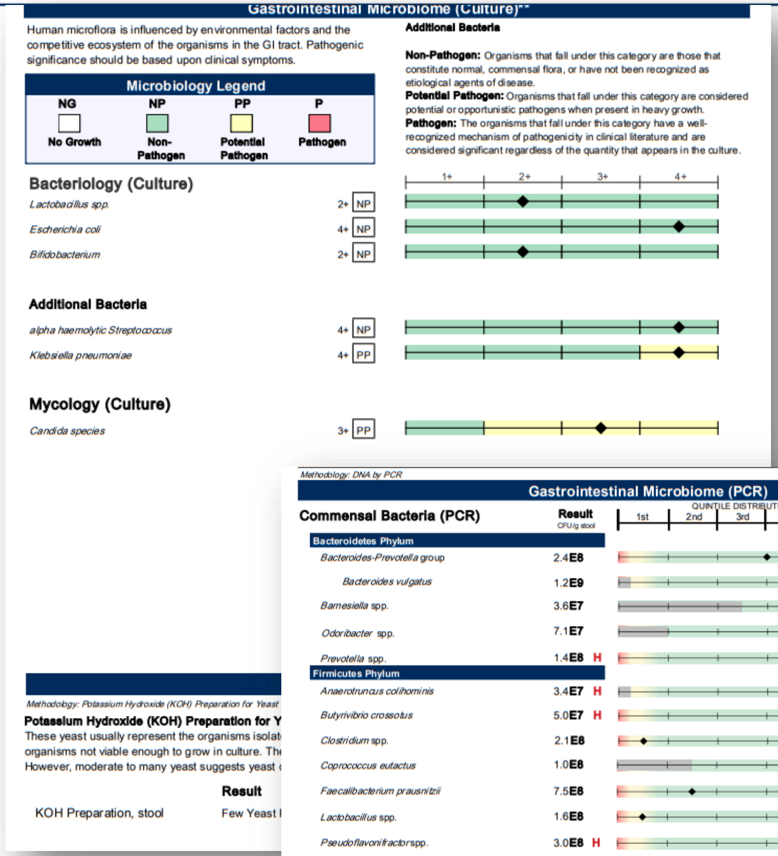
- What are they doing?
  - Microbial Metabolites



Microbiome Methods

- Bacteriological Culture
  - MALDI-TOF identification
  - Pros: Detects living viable organisms; useful in antimicrobial sensitivity testing
  - Cons: Mostly relevant to aerobic microbes

- 16s PCR
  - Can be qPCR or standard PCR
  - Pros: Quantitative Data for analysis; not limited to aerobic organisms; provides data to fuel dysbiosis patterns
  - Cons: requires 1 probe for each organism



Gastrointestinal Microbiome (Culture)**

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

Microbiology Legend

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

Additional Bacteria

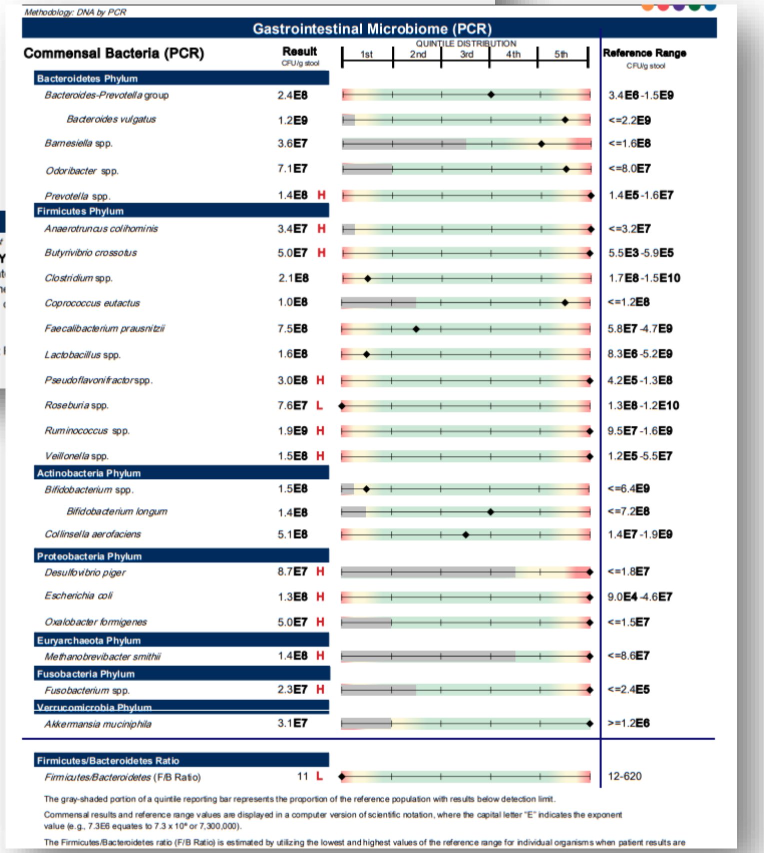
Non-Pathogen: Organisms that fall under this category are those that constitute normal, commensal flora, or have not been recognized as etiological agents of disease.
Potential Pathogen: Organisms that fall under this category are considered potential or opportunistic pathogens when present in heavy growth.
Pathogen: The organisms that fall under this category have a well-recognized mechanism of pathogenicity in clinical literature and are considered significant regardless of the quantity that appears in the culture.

Bacteriology (Culture)			1+ 2+ 3+ 4+
Lactobacillus spp.	2+	NP	
Escherichia coli	4+	NP	
Bifidobacterium	2+	NP	
Additional Bacteria			
alpha haemolytic Streptococcus	4+	NP	
Klebsiella pneumoniae	4+	PP	
Mycology (Culture)			
Candida species	3+	PP	

Methodology: Potassium Hydroxide (KOH) Preparation for Yeast

Potassium Hydroxide (KOH) Preparation for Y
These yeast usually represent the organisms isolat
organisms not viable enough to grow in culture. The
However, moderate to many yeast suggests yeast

	Result
KOH Preparation, stool	Few Yeast



Methodology: DNA by PCR

Gastrointestinal Microbiome (PCR)

Commensal Bacteria (PCR)	Result CFU/g stool		QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range CFU/g stool
Bacteroidetes Phylum				
Bacteroides-Prevotella group	2.4E8			3.4E6-1.5E9
Bacteroides vulgatus	1.2E9			<=2.2E9
Barnesiella spp.	3.6E7			<=1.6E8
Odoribacter spp.	7.1E7			<=8.0E7
Prevotella spp.	1.4E8	H		1.4E5-1.6E7
Firmicutes Phylum				
Anaerotruncus colihominis	3.4E7	H		<=3.2E7
Butyrivibrio crossotus	5.0E7	H		5.5E3-5.9E5
Clostridium spp.	2.1E8			1.7E8-1.5E10
Coprococcus eutactus	1.0E8			<=1.2E8
Faecalibacterium prausnitzii	7.5E8			5.8E7-4.7E9
Lactobacillus spp.	1.6E8			8.3E6-5.2E9
Pseudoflavonifractor spp.	3.0E8	H		4.2E5-1.3E8
Roseburia spp.	7.6E7	L		1.3E8-1.2E10
Ruminococcus spp.	1.9E9	H		9.5E7-1.6E9
Veillonella spp.	1.5E8	H		1.2E5-5.5E7
Actinobacteria Phylum				
Bifidobacterium spp.	1.5E8			<=6.4E9
Bifidobacterium longum	1.4E8			<=7.2E8
Collinsella aerofaciens	5.1E8			1.4E7-1.9E9
Proteobacteria Phylum				
Desulfovibrio piger	8.7E7	H		<=1.8E7
Escherichia coli	1.3E8	H		9.0E4-4.6E7
Oxalobacter formigenes	5.0E7	H		<=1.5E7
Euryarchaeota Phylum				
Methanobrevibacter smithii	1.4E8	H		<=8.6E7
Fusobacteria Phylum				
Fusobacterium spp.	2.3E7	H		<=2.4E5
Verrucomicrobia Phylum				
Akkermansia muciniphila	3.1E7			>=1.2E6
Firmicutes/Bacteroidetes Ratio				
Firmicutes/Bacteroidetes (F/B Ratio)	11	L		12-620

The gray-shaded portion of a quintile reporting bar represents the proportion of the reference population with results below detection limit.

Commensal results and reference range values are displayed in a computer version of scientific notation, where the capital letter "E" indicates the exponent value (e.g., 7.3E6 equates to 7.3×10^6 or 7,300,000).

The Firmicutes/Bacteroidetes ratio (F/B Ratio) is estimated by utilizing the lowest and highest values of the reference range for individual organisms when patient results are

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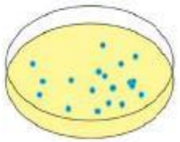
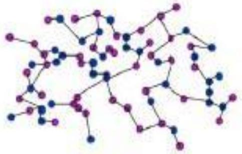
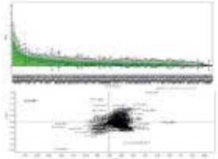
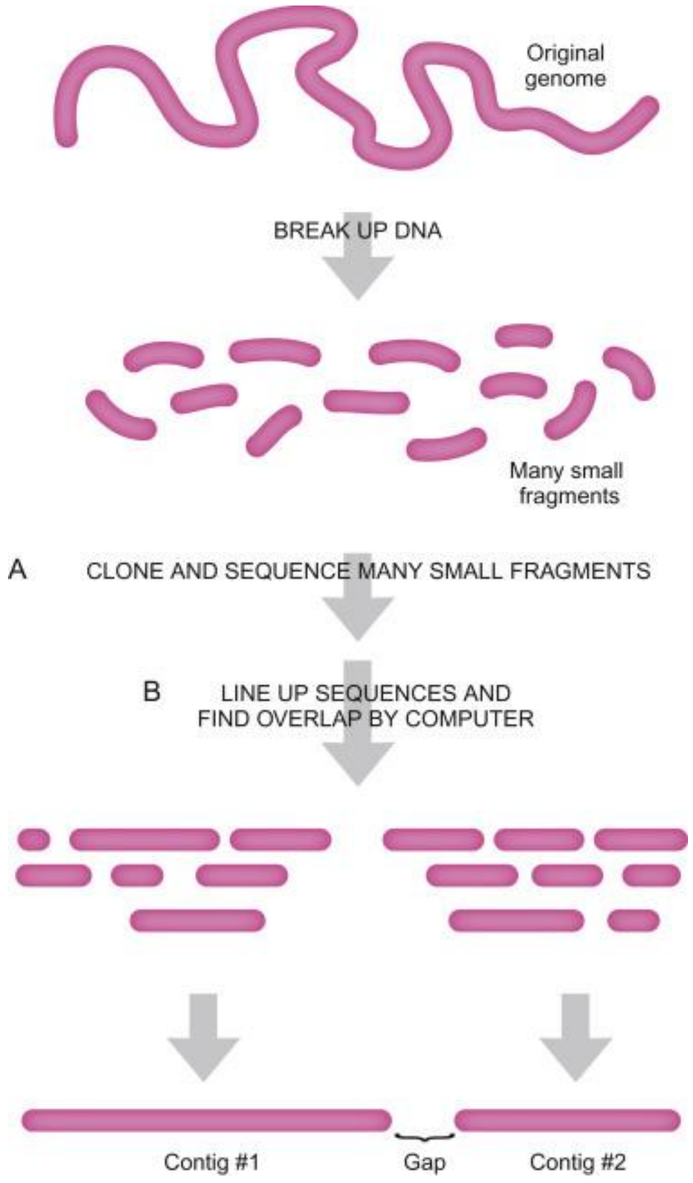
			Characteristics	Limitations	Applications
Microbiome	Cultivation		Visible bacterial colonies Low costs	Can not detect uncultured microbiota	Clinical diagnosis Obtaining target bacterial colonies
DNA	PCR-DGGE		Using 16S rDNA Revealing bacterial diversity Detecting microbial dysbiosis	Only baxonomic information Chimera production and PCR bias Except archaea and virus	Microbial composition dysbiosis Identifying healthy or disease specific species
	16S rDNA sequencing		Using 16S rDNA Revealing bacterial diversity Detecting microbial dysbiosis	Only baxonomic information Except archaea and virus	Microbial composition dysbiosis Identifying healthy or disease specific species
	Metagenomics		Sequencing the total genes Uncovering microbial diversity Finding the novel genes	No microbial expressed functions Complex bioinformatic analysis Consuming costs and time	Revealing functional dysbiosis Finding disease specific microbial genes Identifying functional based studies
mRNA	Metatranscriptomics		Obtaining gene expression profiling Revealing different microbial gene expression among different physiological conditions	Poor stability of bacterial mRNA Requiring multiple purification steps Insufficient reference databases No unique protocol	Revealing functional dysbiosis Finding microbial activity kinetics Specific monitoring active bacteria
Proteins	Metaproteomics		Obtaining protein profiles Comparing microbial proteins among different physiological conditions	Insufficient reference databases Hard to extract total protein No unique protocol	Confirming microbial function Identifying eucaryotes-procaryotes analogs Clinical protein biomarkers
Metabolites	Metabolomics		Obtaining metabolic profiles Identifying metabolites among different physiological conditions	Insufficient reference databases Difficult to identify host or microbial metabolites No unique protocol	Revealing and confirming new pathways Identifying novel metabolic biomarkers

Whole Genome/Shotgun Sequencing

- Identification of (nearly) entire microbiome
- Assesses genes that encode for metabolite production or degradation

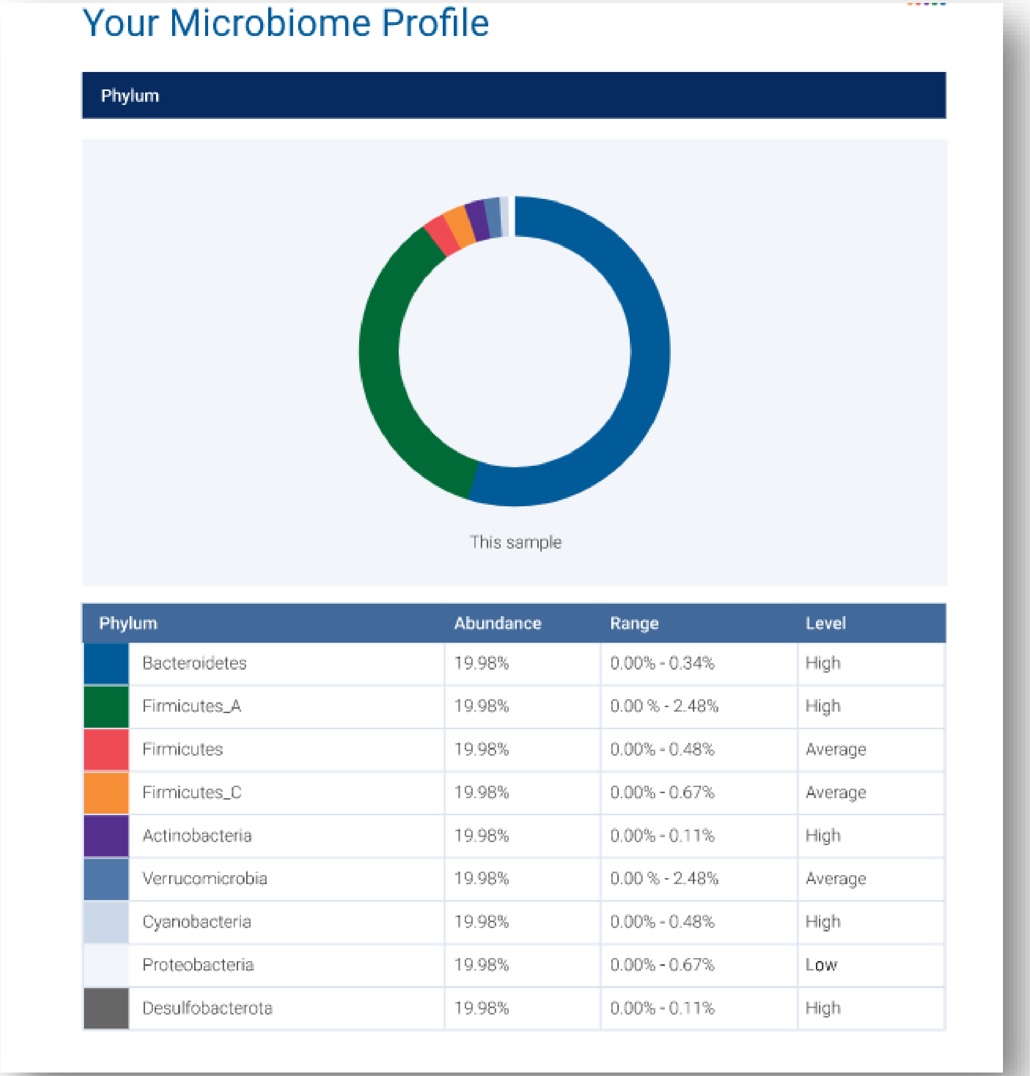


<u>**Factors that affect accuracy:**</u>

- Depth of sequencing
- Library comparison for ID
- Reference population used for ranges

What can we glean?

- Shannon Diversity
- Phylum Balance
- Pathogenic or Opportunistic Organisms
  - Bacteria
  - Archaea
  - Fungi
  - Parasites



Your Microbiome Profile

Phylum

Phylum	Abundance	Range	Level
Bacteroidetes	19.98%	0.00% - 0.34%	High
Firmicutes_A	19.98%	0.00 % - 2.48%	High
Firmicutes	19.98%	0.00% - 0.48%	Average
Firmicutes_C	19.98%	0.00% - 0.67%	Average
Actinobacteria	19.98%	0.00% - 0.11%	High
Verrucomicrobia	19.98%	0.00 % - 2.48%	Average
Cyanobacteria	19.98%	0.00% - 0.48%	High
Proteobacteria	19.98%	0.00% - 0.67%	Low
Desulfobacterota	19.98%	0.00% - 0.11%	High

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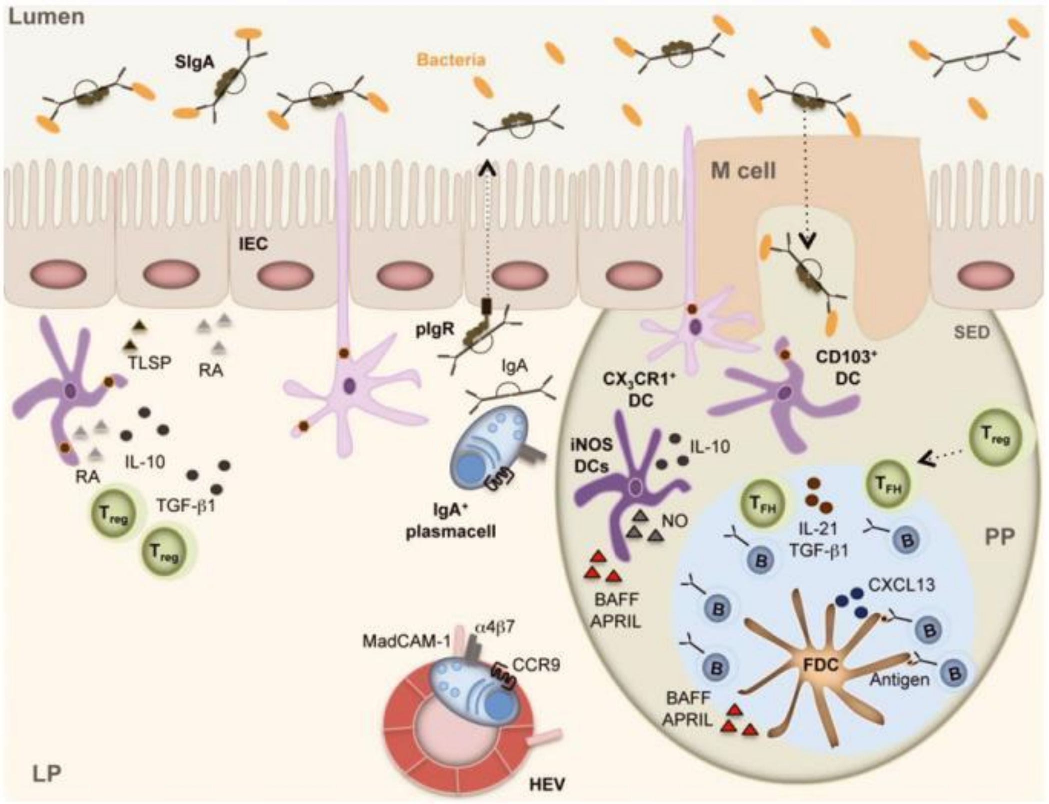
Clinical Relevance of Microbiome Profile

- Pathogenic organisms cause direct harm to GI function
  - Endotoxin secretion leading to mucosal damage
- This allows us to determine if we need to eradicate
  - Secretory IgA acts as primary line of defense

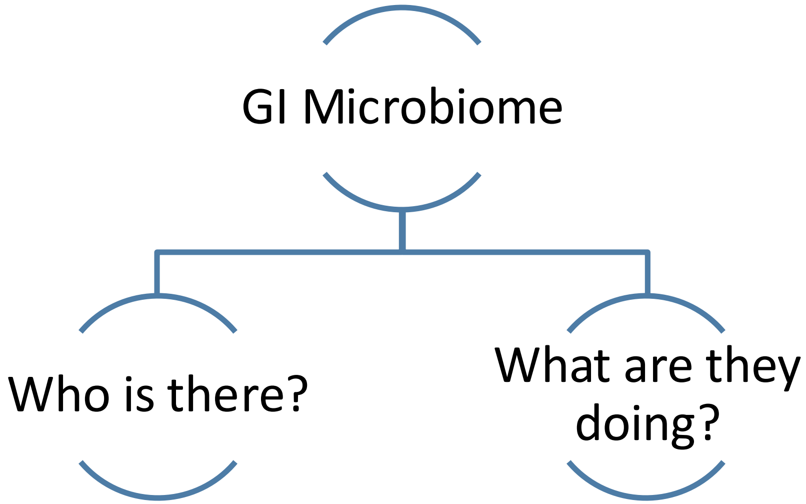


Gutzeit C, et al. ***Immunol Rev***. 2014 Jul;260(1):76-85.

GENOVA DIAGNOSTICS

Dysbiosis

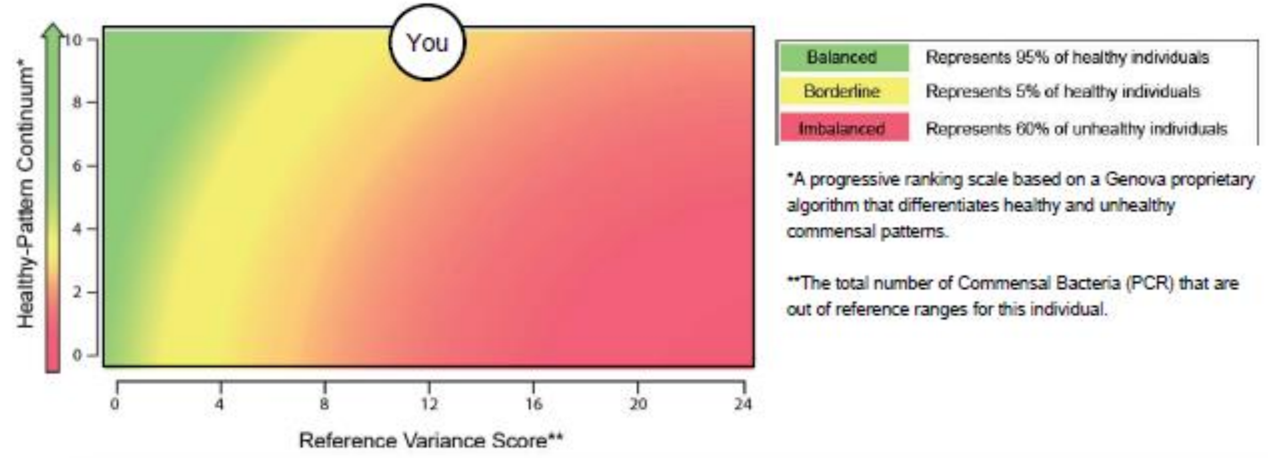
- Who is there?
  - Pathogens
  - Pathobionts
  - **Dysbiosis**

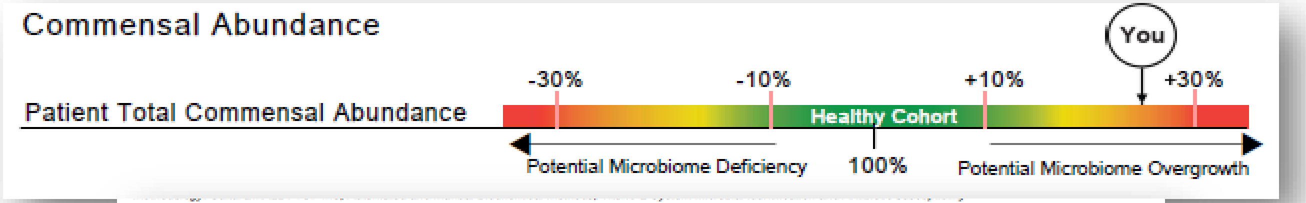


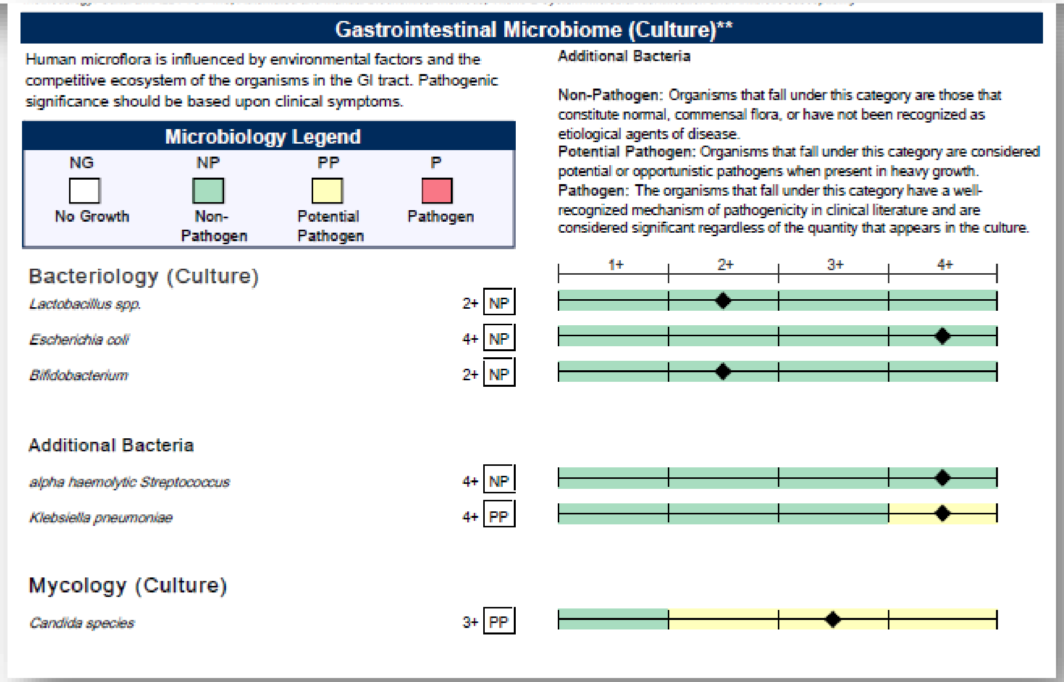
Defining Dysbiosis

- An alteration of the microbiome from a person's baseline?
- An alteration of the microbiome from healthy cohort?

- Overgrowth/Deficiency
- Pathogens and Pathobionts







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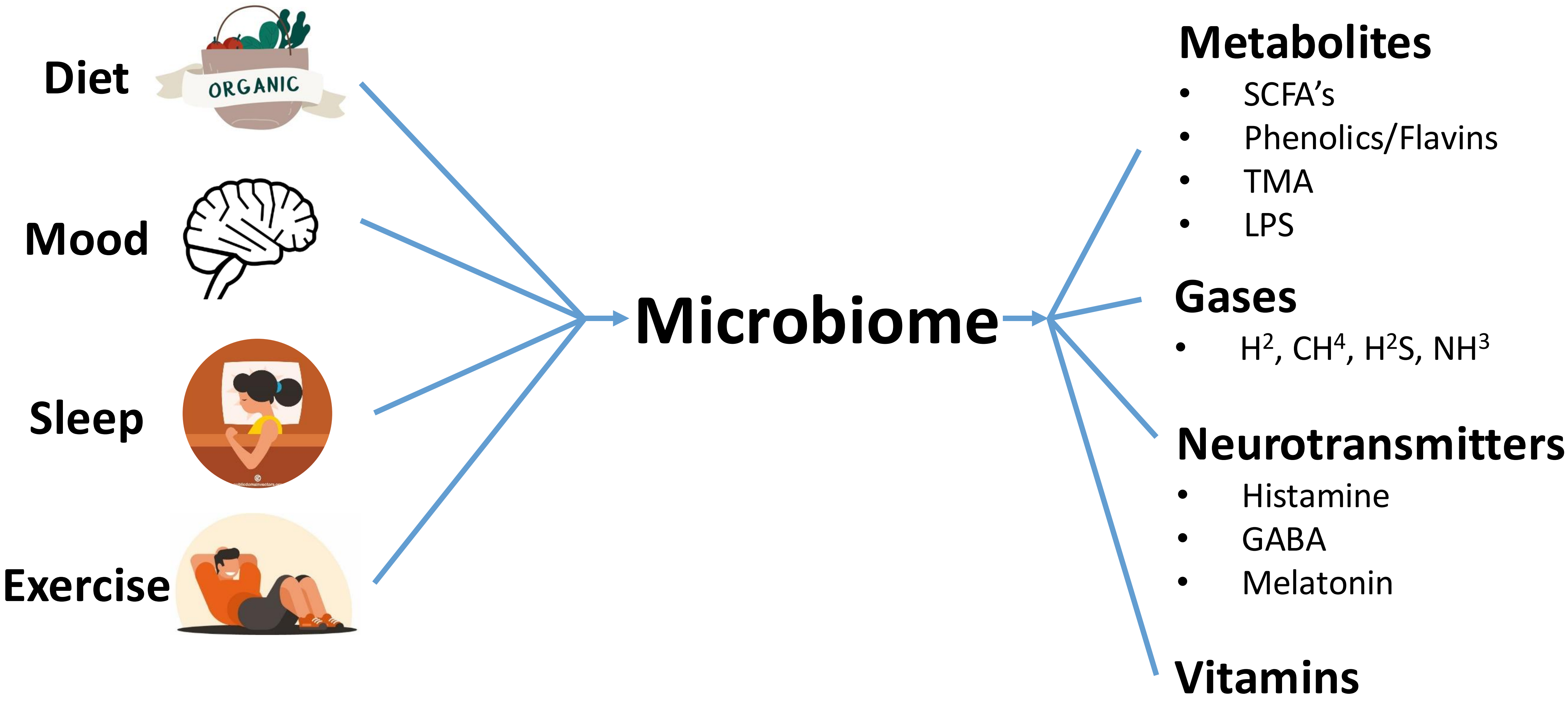

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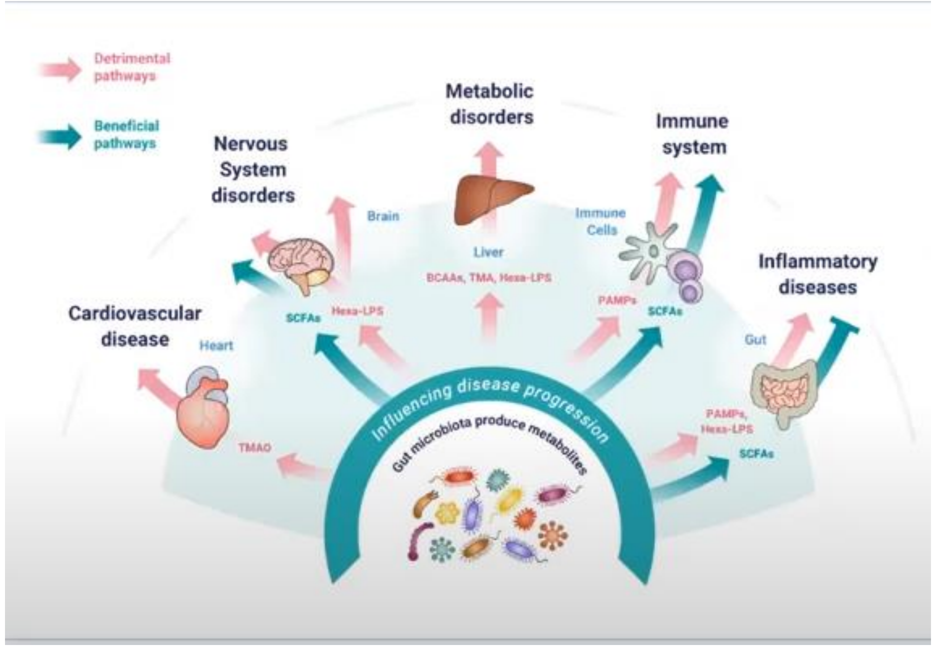
Refining Dysbiosis

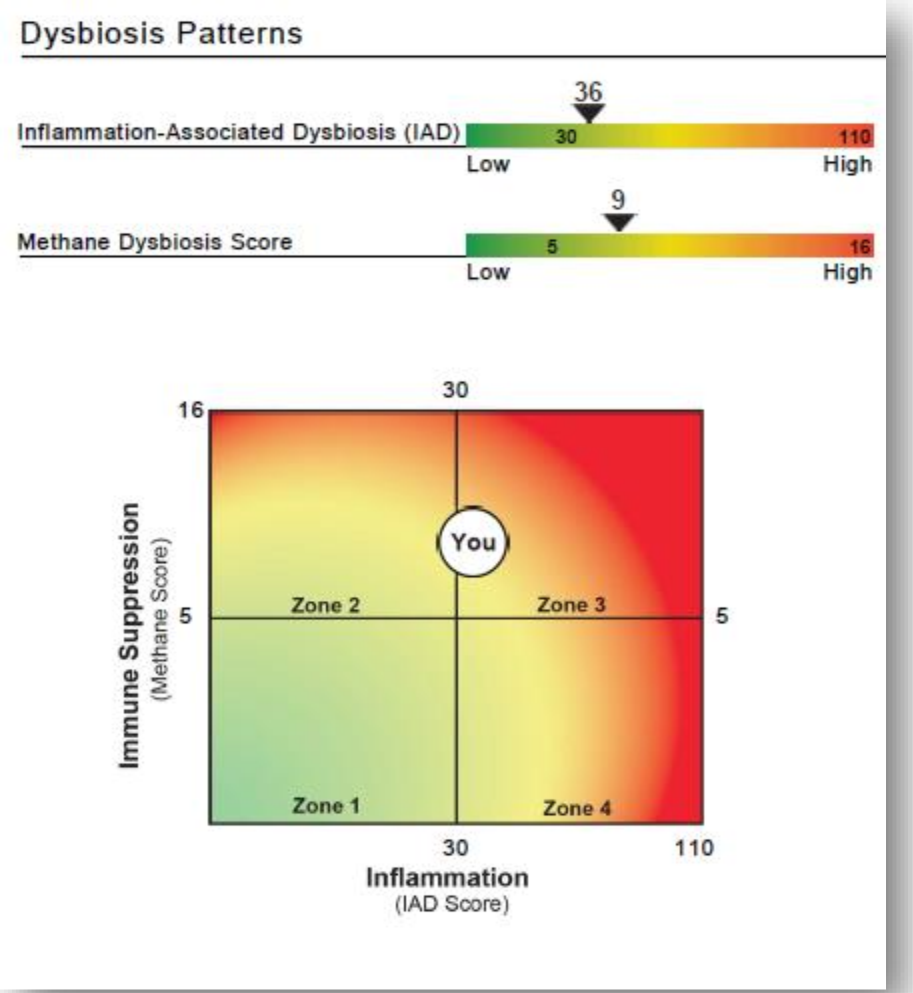
- The real importance of “dysbiosis” is not in who is present or absent…
- It’s about what metabolites are being created!
- It begs the question whether dysbiosis is a real thing?



"I'm pretty sure he just said there's no such thing as dysbiosis."

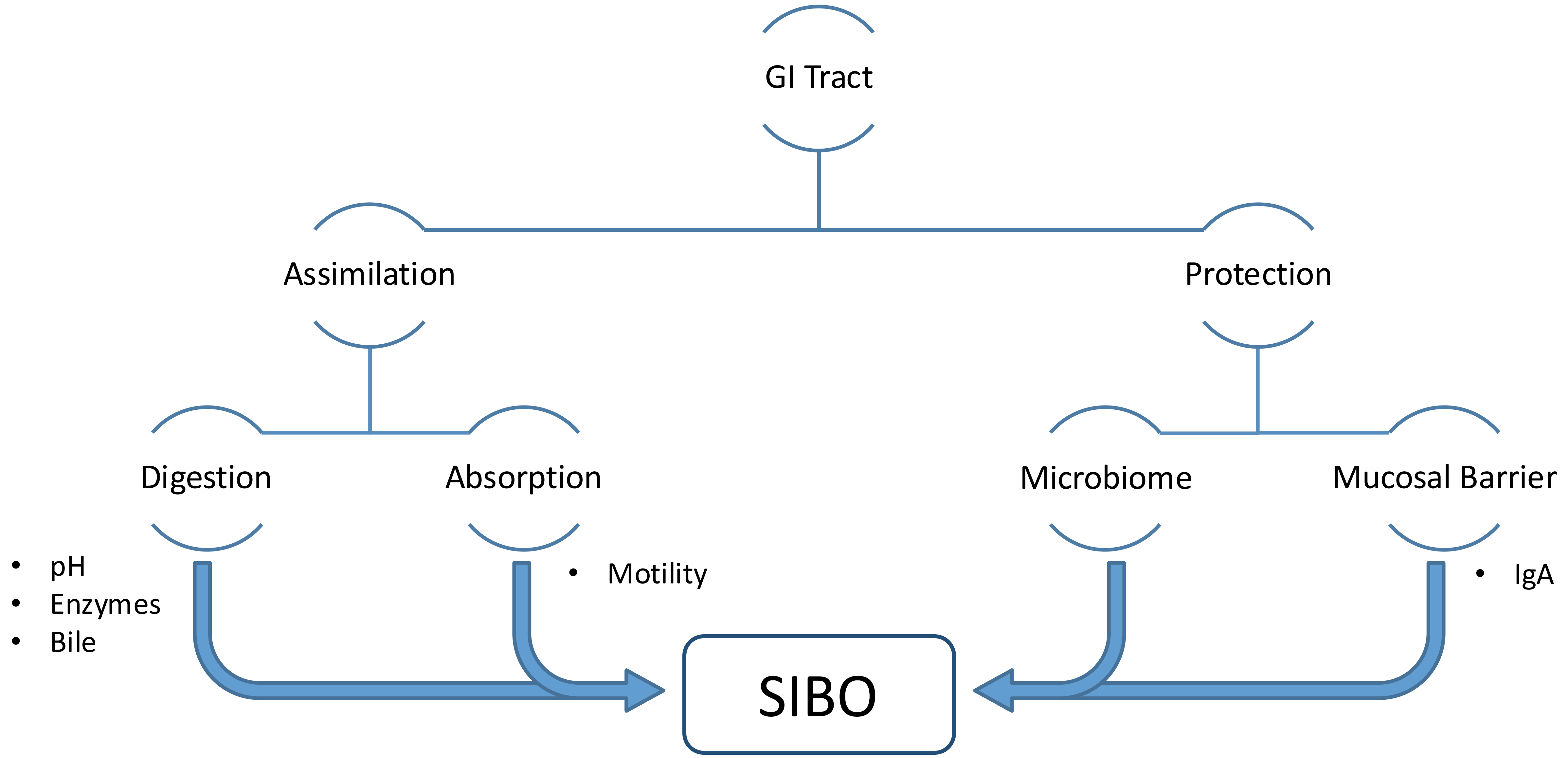
Refining Dysbiosis

- Dysbiosis is not specific to any single bacteria, or even a genus or a phylum
- Dysbiosis does not create any consistent pattern of symptoms
- We need to reclassify dysbiosis into functional categories
  - Inflammatory dysbiosis
  - Immunosuppression dysbiosis (methane dysbiosis)
  - Metabolic dysbiosis
  - Etc…



What about SIBO?

- Causes of SIBO:
  - Low pH
  - Low Enzyme/Bile Secretion
  - Migrating Motor Complex
  - IgA Deficiency
- Root Causes of SIBO:
  - Causes of Hypochlorhydria
  - Causes of EPI
  - Causes of Motility
  - Causes of IgA Deficiency



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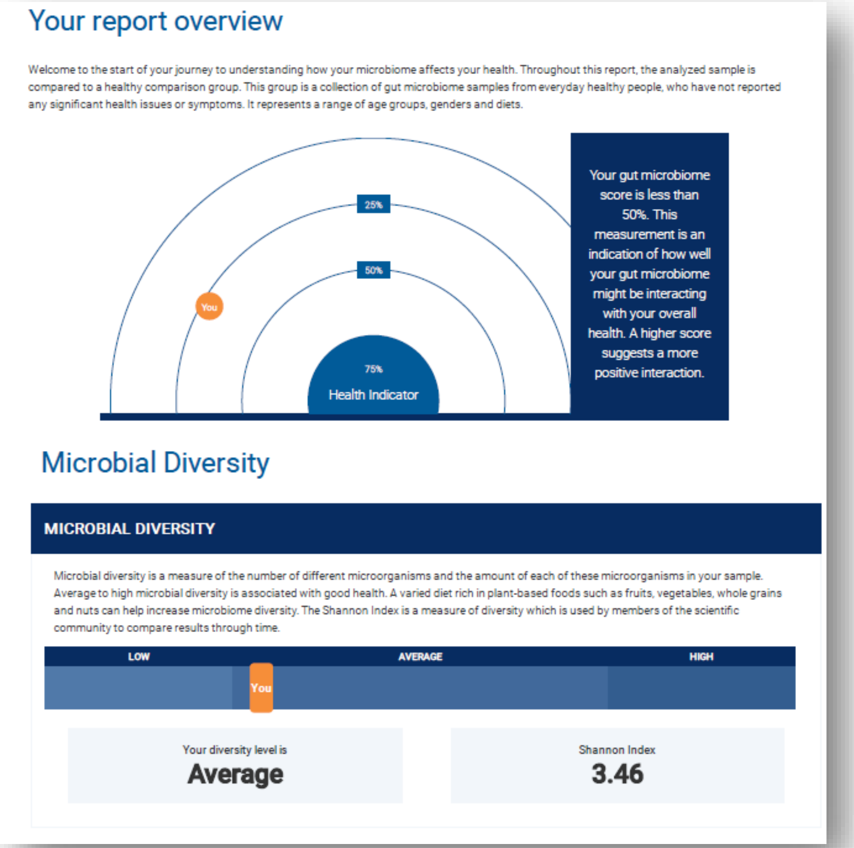
Microbiome Diversity

- Measuring Shannon Diversity Index
  - alpha-diversity
- Microbial richness
  - The number of different species are present
- Microbial evenness/dominance
  - Whether the abundance is more spread out or dominated by few organisms



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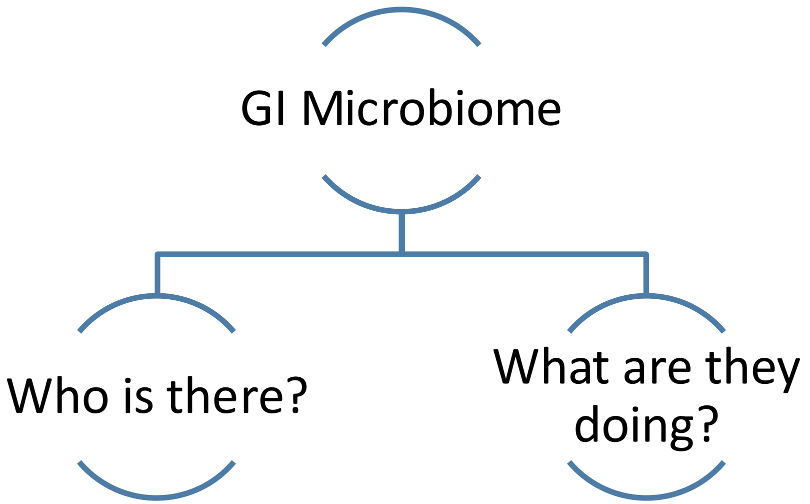
The Real Power of Shotgun Sequencing

- Instead of obsessing about “Who is there,”…
- We can identify with much more clarity, “What they do.”



Diet

Mood

Sleep

Exercise

Microbiome

Metabolites

- SCFA’s
- Phenolics/Flavins
- TMA
- Hexa-LPS

Gases

- H^2, CH^4, H^2S, NH^3

Neurotransmitters

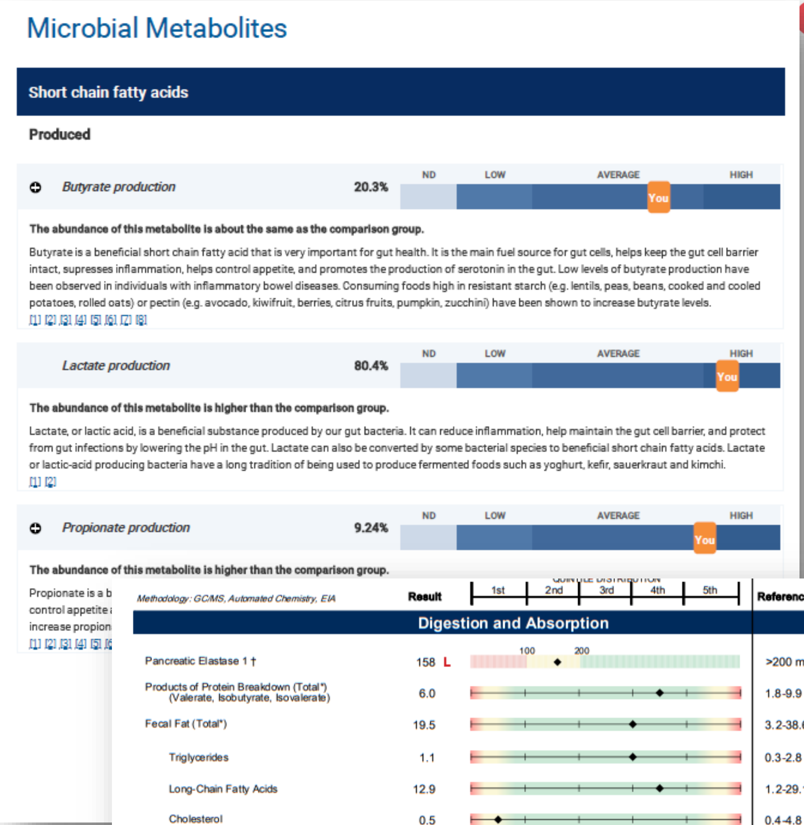
- Histamine
- GABA
- Melatonin

Vitamins

Microbial Metabolite	Brief Clinical Application
Hexa-LPS	Inflammation marker
Trimethylamine	Cardiovascular risk factor (TMAO)
Methane, Hydrogen Sulfide	Gas production, inflammation, motility
Ammonia (Urease)	Intestinal Permeability
B. fragilis toxin	Infectious diarrhea
Beta-glucuronidase	Detoxification
Oxalate consumption	Insight into kidney stone formation
Neurotransmitters (GABA, IPA, Histamine)	Gut-brain axis
SCFA's	Potential to produce beneficial short-chain fatty acids
Vitamin production	B2, B7, B9, B12, Vitamin K
Branched chain amino acids	Metabolic dysfunction

One Thing to Note

- Whole-Genome Sequencing is measuring the DNA within the microbiome that encodes for metabolite production
- This means that it is looking at DNA potential rather than a direct measurement
- Phenotypic biomarkers measure direct concentrations
  - n-Butyrate, Calprotectin, PE1, etc



Microbial Metabolites

Short chain fatty acids

Produced

		ND	LOW	AVERAGE	HIGH
Butyrate production	20.3%			You	

The abundance of this metabolite is about the same as the comparison group.

Butyrate is a beneficial short chain fatty acid that is very important for gut health. It is the main fuel source for gut cells, helps keep the gut cell barrier intact, supresses inflammation, helps control appetite, and promotes the production of serotonin in the gut. Low levels of butyrate production have been observed in individuals with inflammatory bowel diseases. Consuming foods high in resistant starch (e.g. lentils, peas, beans, cooked and cooled potatoes, rolled oats) or pectin (e.g. avocado, kiwifruit, berries, citrus fruits, pumpkin, zucchini) have been shown to increase butyrate levels.
[1] [2] [3] [4] [5] [6] [7] [8]

		ND	LOW	AVERAGE	HIGH
Lactate production	80.4%				You

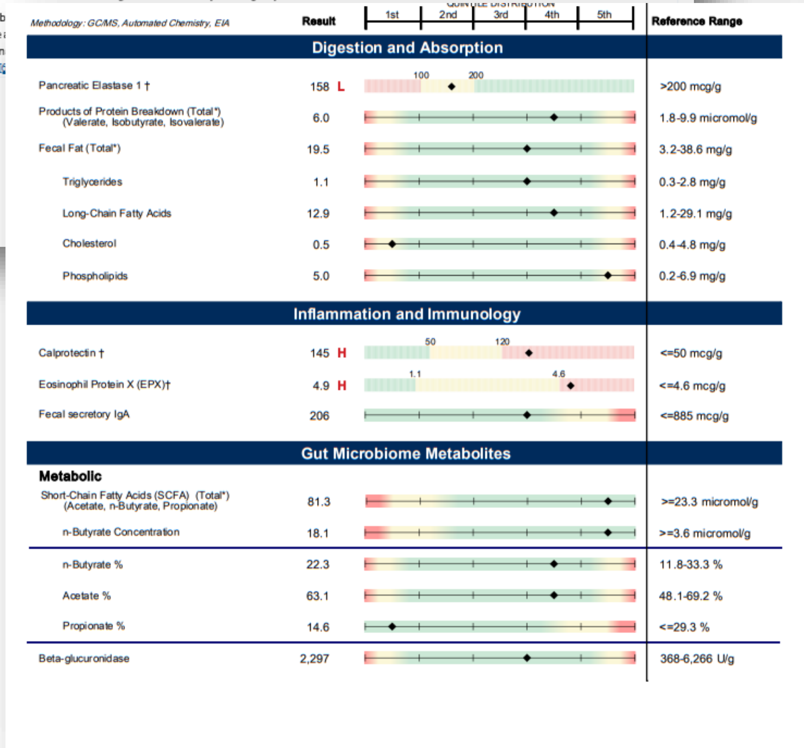
The abundance of this metabolite is higher than the comparison group.

Lactate, or lactic acid, is a beneficial substance produced by our gut bacteria. It can reduce inflammation, help maintain the gut cell barrier, and protect from gut infections by lowering the pH in the gut. Lactate can also be converted by some bacterial species to beneficial short chain fatty acids. Lactate or lactic-acid producing bacteria have a long tradition of being used to produce fermented foods such as yoghurt, kefir, sauerkraut and kimchi.
[1] [2]

		ND	LOW	AVERAGE	HIGH
Propionate production	9.24%				You

The abundance of this metabolite is higher than the comparison group.

Propionate is a b
control appetite
increase propion
[1] [2] [3] [4] [5] [6



Methodology: GC/MS, Automated Chemistry, EIA

	Result	Quintile Distribution (1st, 2nd, 3rd, 4th, 5th)	Reference Range
Digestion and Absorption			
Pancreatic Elastase 1 †	158 L	100 / 200	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	6.0		1.8-9.9 micromol/g
Fecal Fat (Total*)	19.5		3.2-38.6 mg/g
Triglycerides	1.1		0.3-2.8 mg/g
Long-Chain Fatty Acids	12.9		1.2-29.1 mg/g
Cholesterol	0.5		0.4-4.8 mg/g
Phospholipids	5.0		0.2-6.9 mg/g
Inflammation and Immunology			
Calprotectin †	145 H	50 / 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	4.9 H	1.1 / 4.6	<=4.6 mcg/g
Fecal secretory IgA	206		<=885 mcg/g
Gut Microbiome Metabolites			
Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	81.3		>=23.3 micromol/g
n-Butyrate Concentration	18.1		>=3.6 micromol/g
n-Butyrate %	22.3		11.8-33.3 %
Acetate %	63.1		48.1-69.2 %
Propionate %	14.6		<=29.3 %
Beta-glucuronidase	2,297		368-6,266 U/g

GENOVA DIAGNOSTICS

The Microbiome's Adaptability

- The presence of genes related to the production and or consumption of various metabolites, neurotransmitters, and vitamins are in constant flux.

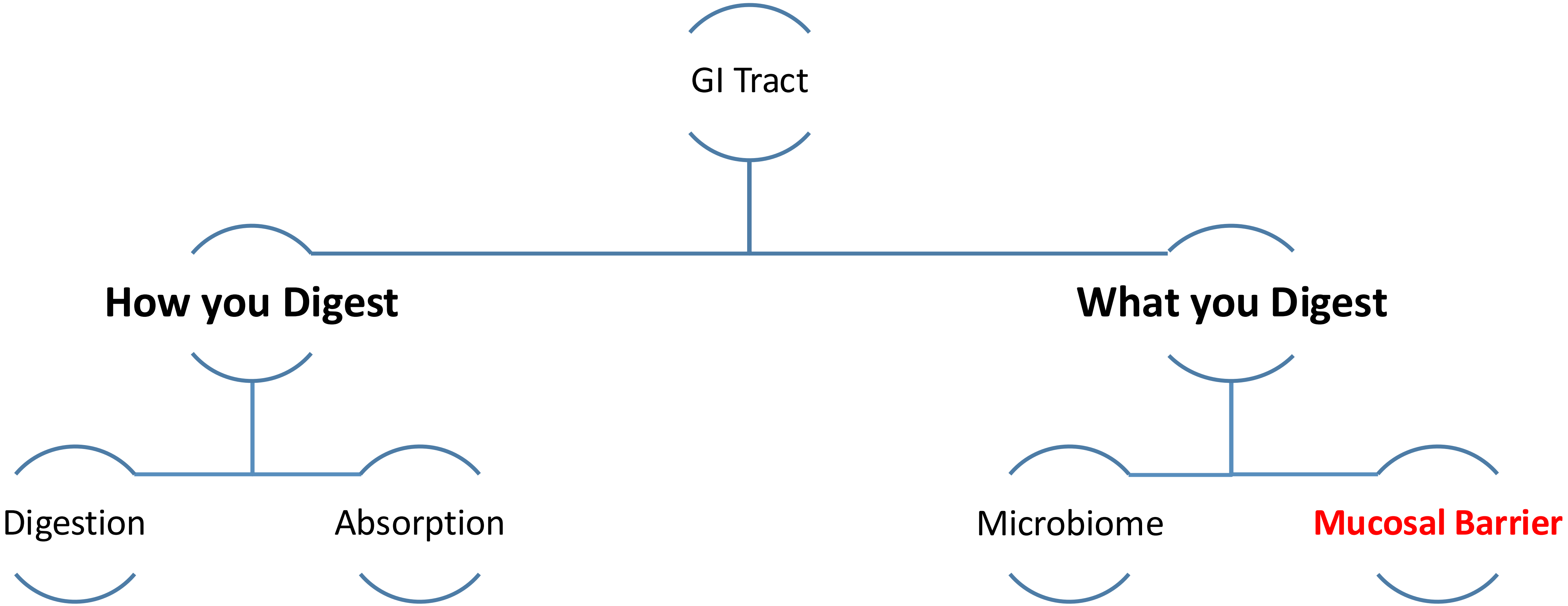
- The abundance of one of these genes in the microbiome is generally a good indication into what the microbiome is creating/consuming
  - Example: High-protein diet
    - The microbiome populations rapidly shift:
      - Promotion of bacteria that use protein substrates as fuel
      - Increase relative abundance of DNA from those bacteria
    - Increase in the metagenomic finding for protein degradation

Integrating Multi-omic Clinical Information

- Example:
  - n-Butyrate
    - Produced by gut microbiota
    - Passively absorbed in the GI tract
    - Excess is excreted in stool

- What if?
  - A patient's test shows a low potential to produce butyrate, AND
  - A high value of n-butyrate in the stool?

  - What does this mean? Is it a discrepancy?


GI Tract
How you Digest
What you Digest
Digestion
Absorption
Microbiome
Mucosal Barrier

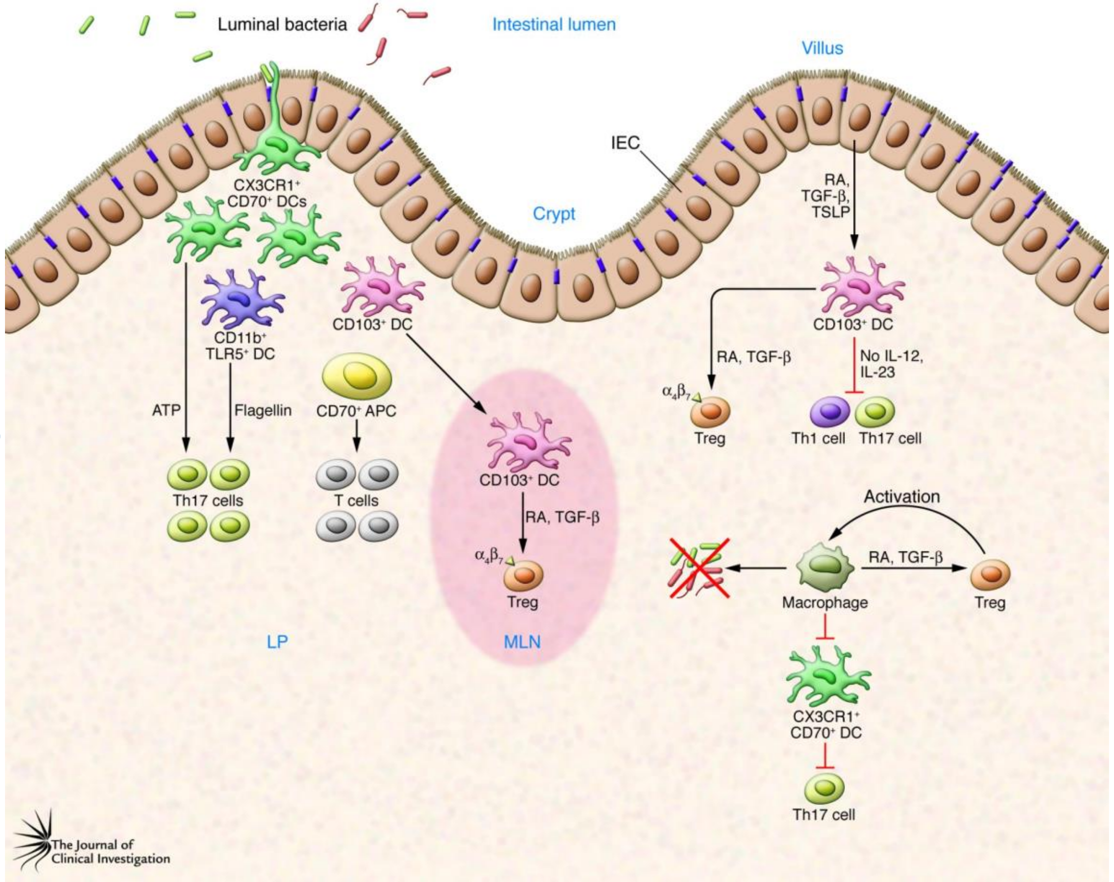
Mucosal Barrier Function & Assessment

- Main player :: The Immune System
  - It is responsible for keeping in check what you just ate to make sure it’s okay
    - Pathogens
    - Toxins/Poisons

- How it does this:
  - Assessment
  - Tolerance/Activation

Mucosal Assessment

- Let's hear it for the dendritic cells!!!
- Serve as the watchdogs for the gut
  - Constant sampling of the microbiome populatio as well food products
  - Critical in immune homeostasis for the gastrointestinal tract
- Recent evidence suggests that Vitamin A is critical for their proper functioning



GENOVA DIAGNOSTICS

Tolerance

- Primary tolerance developed early in childhood development
- Continued tolerance is critical to reduce unnecessary inflammatory reactions to safe stimuli, such as new foods and increased microbe diversity

- The literature is starting to suggest that immune tolerance *loss* in the GI tract is potentially due to the result of increased intestinal permeability and inflammation.

Intestinal Permeability

- Factors that increase permeability:
  - Reduced microbial butyrate production
  - Increased serotonin
  - Impairment in mucus production
  - Pathogens and pathobionts that directly can stimulate tight junctions
  - Gluten
  - Diet (indirectly through influence on microbiome)
  - Strenuous Exercise

Bischoff, S.C., Barbara, G., Buurman, W. *et al*. *BMC Gastroenterol* 14, 189 (2014).

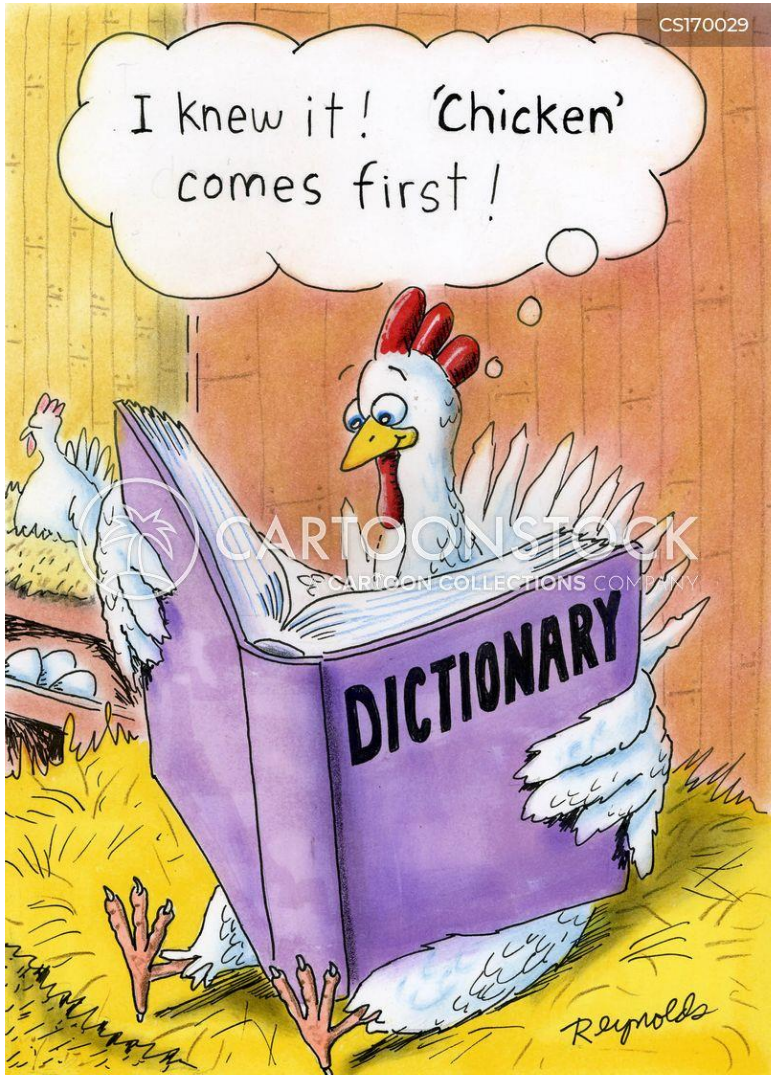
Permeability

What comes first?

The Inflammation

Or

The Permeability



GENOVA DIAGNOSTICS

Let’s Talk Food Sensitivities & Leaky Gut

IgG Food Antibody Results

Dairy

Casein	VL
Cheddar cheese	VL
Cottage cheese	VL
Cow's milk	1+
Goat's milk	0
Lactalbumin	VL
Yogurt	1+

Fruits

Apple	0
Apricot	0
Banana	0
Blueberry	0
Cranberry	0
Grape	VL
Grapefruit	0
Lemon	0
Orange	0
Papaya	0
Peach	VL
Pear	0
Pineapple	1+
Plum	0
Raspberry	0
Strawberry	0

Vegetables

Alfalfa	0
Asparagus	VL
Avocado	0
Beets	0
Broccoli	VL
Cabbage	0
Carrot	0
Celery	0
Cucumber	0
Garlic	1+
Green Pepper	0
Lettuce	0
Mushroom	0
Olive	0
Onion	0
Pea	0
Potato, sweet	0
Potato, white	VL
Spinach	VL
String bean	VL
Tomato	VL
Zucchini	0

Fish/Shellfish

Clam	0
Cod	0
Crab	0
Lobster	0
Oyster	0
Red snapper	0
Salmon	0
Sardine	0
Shrimp	0
Sole	0
Trout	0
Tuna	0

Poultry/Meats

Beef	0
Chicken	0
Egg white	0
Egg yolk	0
Lamb	0
Pork	0
Turkey	0

Nuts and Grains

Almond	VL
Buckwheat	VL
Corn	0
Corn gluten	1+
Gluten	3+
Kidney bean	VL
Lentil	0
Lima bean	0
Oat	0
Peanut	0
Pecan	0
Pinto bean	0
Rice	VL
Rye	0
Sesame	3+
Soy	VL
Sunflower seed	3+
Walnut	VL
Wheat	3+

Miscellaneous

Yeast	VL
Cane sugar	VL
Chocolate	VL
Coffee	3+

Total IgE

	Inside	Outside	Reference Range
Total IgE ◆	9.2		<=87.0 IU/mL

0 None Detected | VL Very Low | 1+ Low | 2+ Moderate | 3+ High

Clinical Assessment of Permeability

- Lactulose/Mannitol Test
- Plasma L-citrulline

Biomarkers of epithelial cell integrity

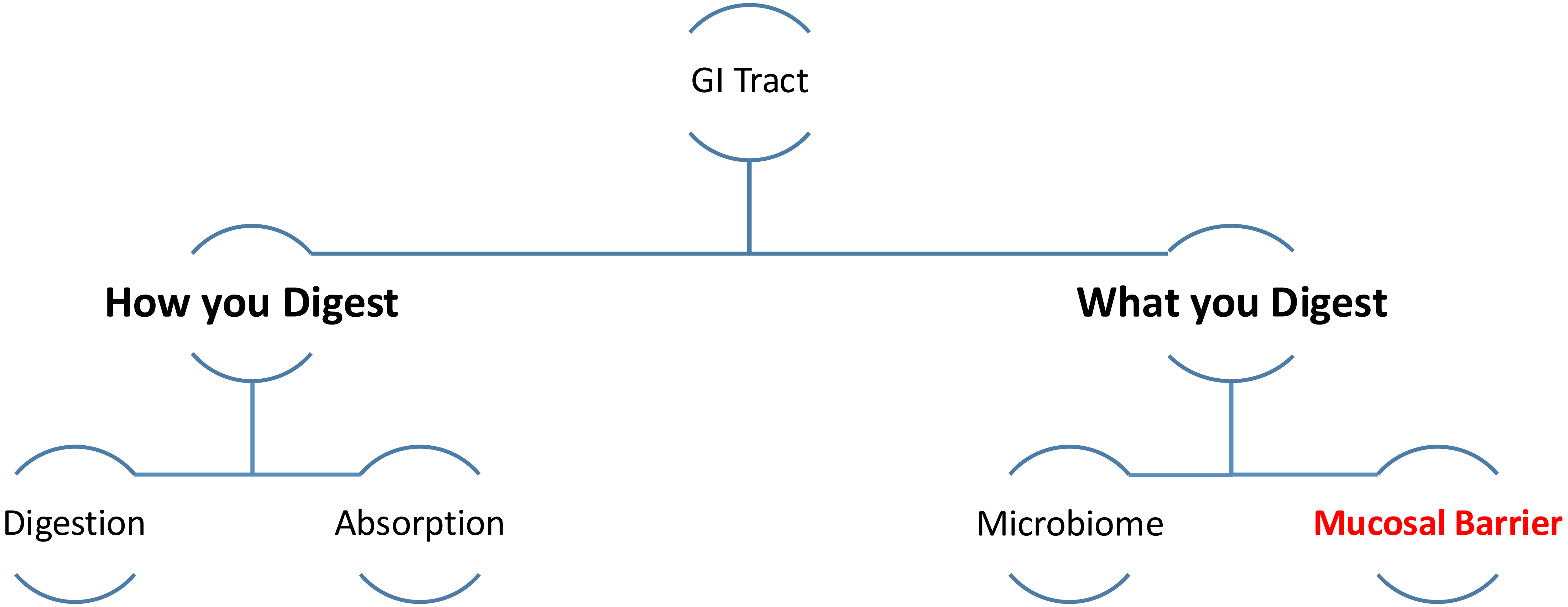
Plasma levels of citrulline, an amino acid not incorporated into proteins, but produced by small intestinal enterocytes from glutamine have been proposed as a marker of functional enterocyte mass. Loss of small bowel epithelial cell mass results in impaired intestinal permeability and in declined circulating levels of citrulline, as is shown in haemopoietic stem cell transplant recipients suffering from severe oral and gastrointestinal mucositis following intensive myeloablative therapy [158]. More recently, citrulline was established as a valuable marker for chemotherapy-induced mucosal barrier injury in pediatric patients [159]. Most interestingly, sensitivity and specificity seem to be better for the citrulline assay compared with sugar permeability tests [160].

GENOVA DIAGNOSTICS

Bischoff, S.C., Barbara, G., Buurman, W. *et al. BMC Gastroenterol* 14, 189 (2014).

Approaches to Improve Barrier Function

- T-Regulatory and Tolerance Support
  - Vitamin A
  - Supplemental IgG
- Mucus Support
  - Aloe
  - Slippery Elm
  - Probiotic *Akkermansia*
- Activation Support
  - Antimicrobials for infection
  - Quercetin, Fiber, L-Glutamine for mucosal repair


GI Tract
How you Digest
What you Digest
Digestion
Absorption
Microbiome
Mucosal Barrier

The GI tract's role in the system is to maximize assimilation of essential nutrition at the highest efficiency…

While…

Limiting risks of exposure to external dangers.

Presenter:
Michael Chapman, ND

Thank You!!!

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