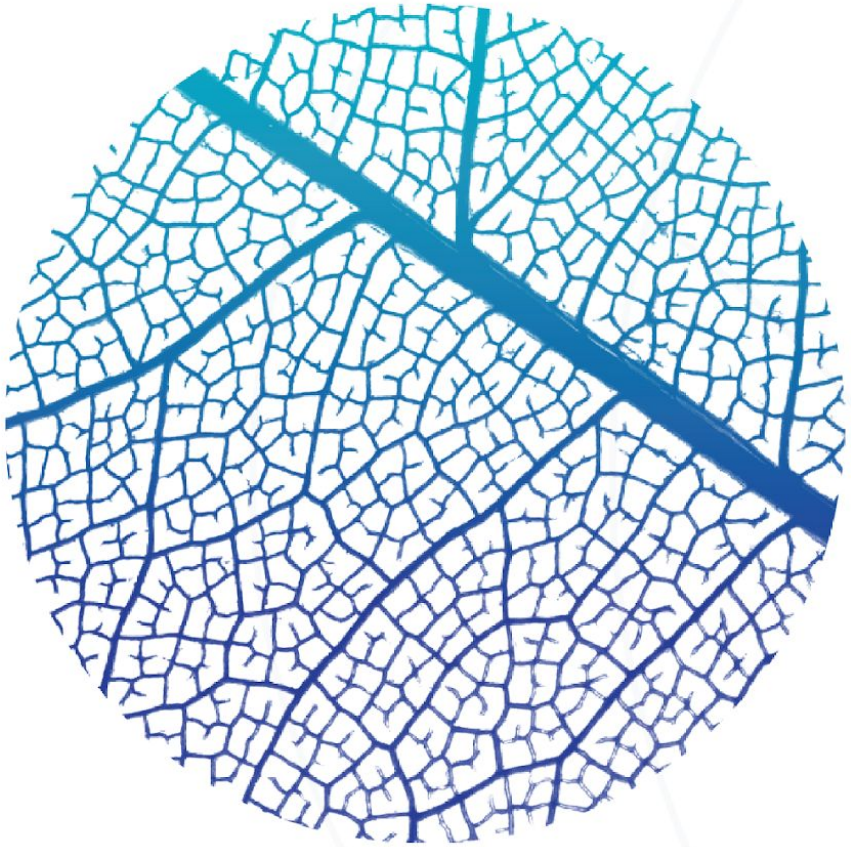
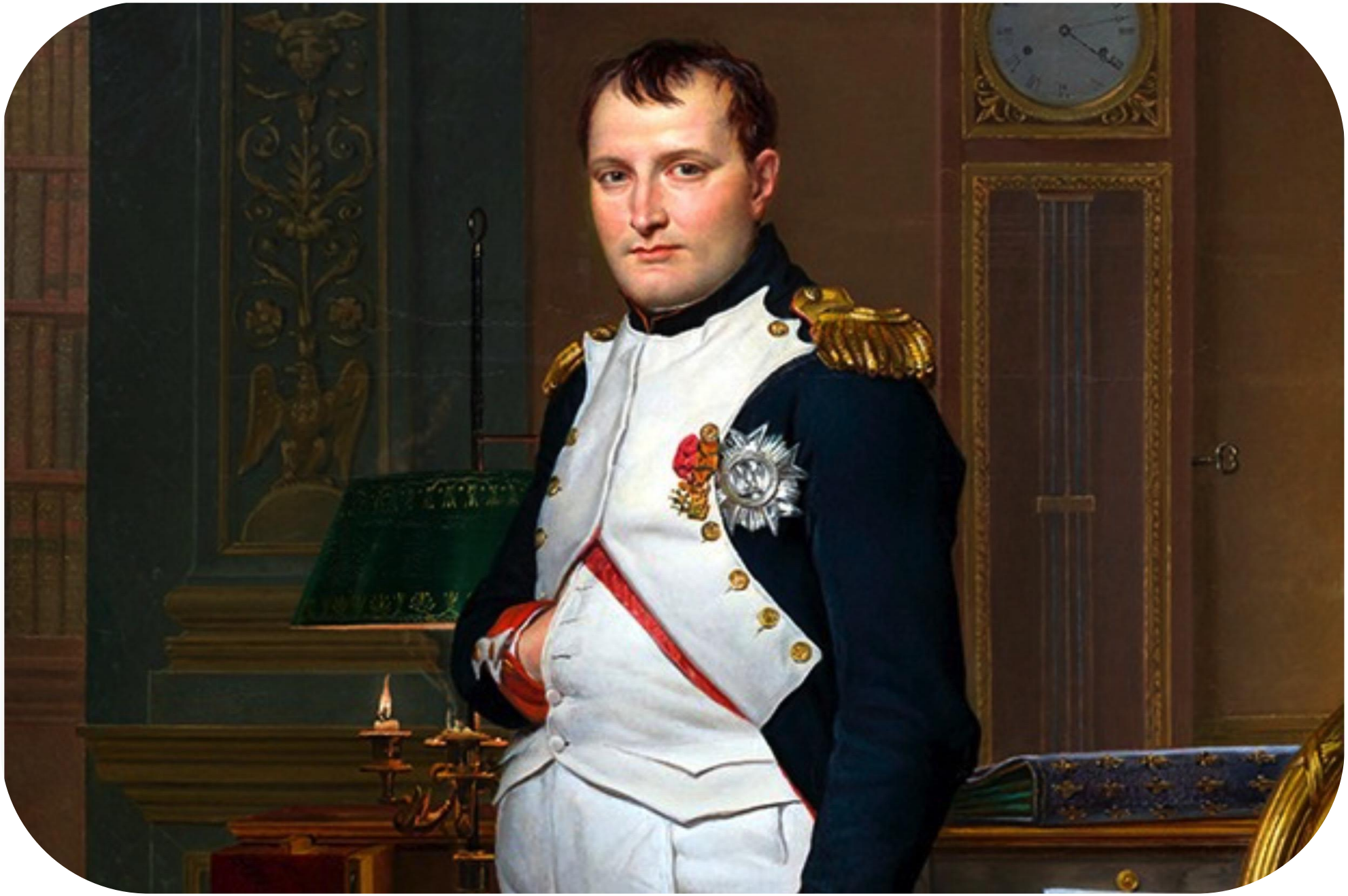


THE ROLE OF THE INTESTINAL MICROBIOME IN IRRITABLE BOWEL SYNDROME

ROBERT ROUNTREE, MD

Biocidin Botanicals

"All disease begins in the gut,"
Hippocrates



THE GASTROINTESTINAL TRACT WITH ITS LUMINAL CONTENTS…

- Contains more microbial cells (39+ trillion) than the total amount of somatic cells in the body
- Has a collective genome 100 – 1000x larger than the human genome (millions of genes -- in constant flux/transfer)
- Contains 70% of the body's immune cells & tissue
- Produces 80% of the body's neurotransmitters
- Has a metabolic activity greater than the liver

ALL THIS COMPLEXITY IN THE GI TRACT CREATES MANY OPPORTUNITIES FOR IMBALANCE AND DYSFUNCTION

“FUNCTIONAL” GASTROINTESTINAL DISORDERS

TheRomeFoundation.org

GI symptoms related to any combination of

- Altered central nervous system processing
  - Visceral hypersensitivity (discomfort)
  - Motility disturbance
- Altered mucosal and immune function
- Altered gut microbiota

IRRITABLE BOWEL SYNDROME

- The most commonly diagnosed functional gastrointestinal disorder (FGID) – worldwide prevalence of 10–20%
- Predominantly affects women (female/male = 2/1)
- Highest prevalence: South America (21%)
- Lowest prevalence: South Asia (7%)

Molecular and Cellular Pediatrics (2016) 3:7

IRRITABLE BOWEL SYNDROME
SYMPTOMS AND SIGNS

- Not a single disease, but a constellation of clinical symptoms, including abdominal pain and/or discomfort, bloating, and distension (meteorism)
- Accompanied by altered bowel function, ranging from diarrhea-predominant (IBS-D) to constipation-predominant (IBS-C) or mixed (IBS-M)
- With the "absence of identifiable structural, biochemical, or metabolic abnormalities" (???)

Gut Microbes, 2016, VOL. 7, NO. 5, 397–413

"IT HAS BEEN ESTIMATED THAT PATIENTS WOULD GIVE UP 10 TO 15 YEARS OF LIFE EXPECTANCY FOR AN INSTANT CURE OF THE DISEASE."

Irritable Bowel Syndrome (review) N Engl J Med 2017;376:2566-78

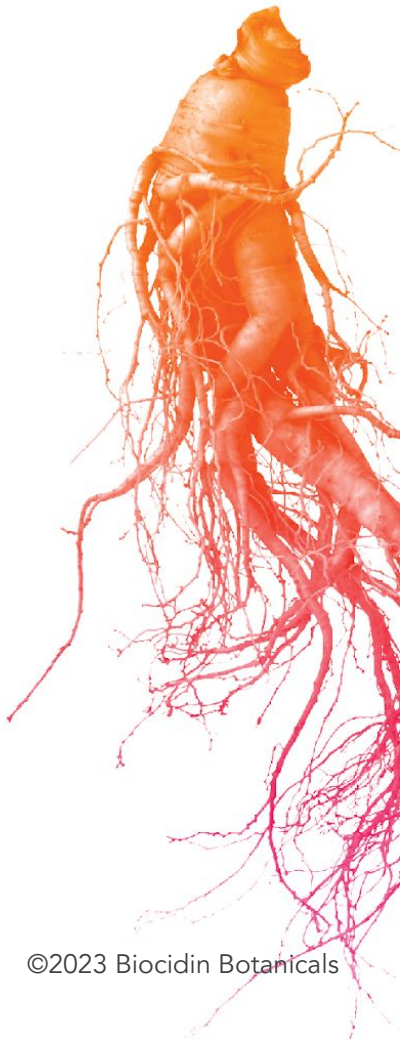
IS IBS PREDOMINANTLY A DISORDER
OF THE BRAIN & NERVOUS SYSTEM….

OR IS DOES IT ORIGINATE
IN THE GI TRACT?

IRRITABLE BOWEL SYNDROME
GI-SPECIFIC CAUSES

- Maldigestion (pancreatic and/or brush border enzyme deficiency) with excess fermentation
- Bile acid malabsorption
- Dysbiosis (with disrupted metabolomics)
- Hyperpermeability (leaky gut)
- Subacute inflammation / immune activation (e.g., food-mediated; mast cell activation)

https://f1000research.com/articles/7-1029/v1



A systematic review and meta-analysis on the prevalence of non-malignant, organic gastrointestinal disorders misdiagnosed as irritable bowel syndrome

The prevalence of five gastrointestinal conditions: bile acid diarrhea (BAD), carbohydrate malabsorption (CM), microscopic colitis (MC), pancreatic exocrine insufficiency (PEI) and small intestinal bacterial overgrowth (SIBO) was systematically assessed from studies including consecutive patients meeting diagnostic criteria for IBS

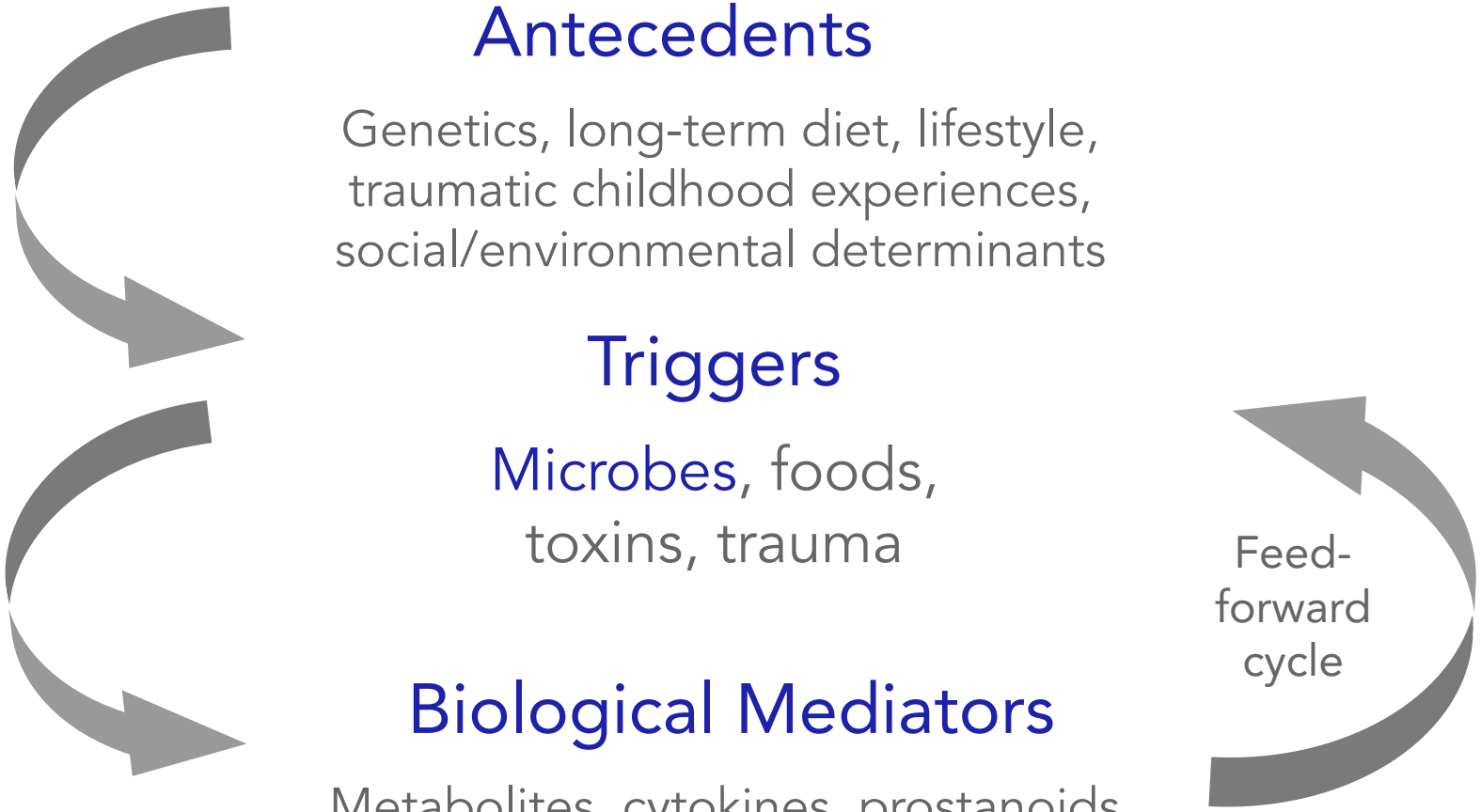
A significant proportion of patients presenting to secondary care with IBS have an organic condition which may account for their symptoms.

"Failure to exclude such conditions will deny patients effective treatment"

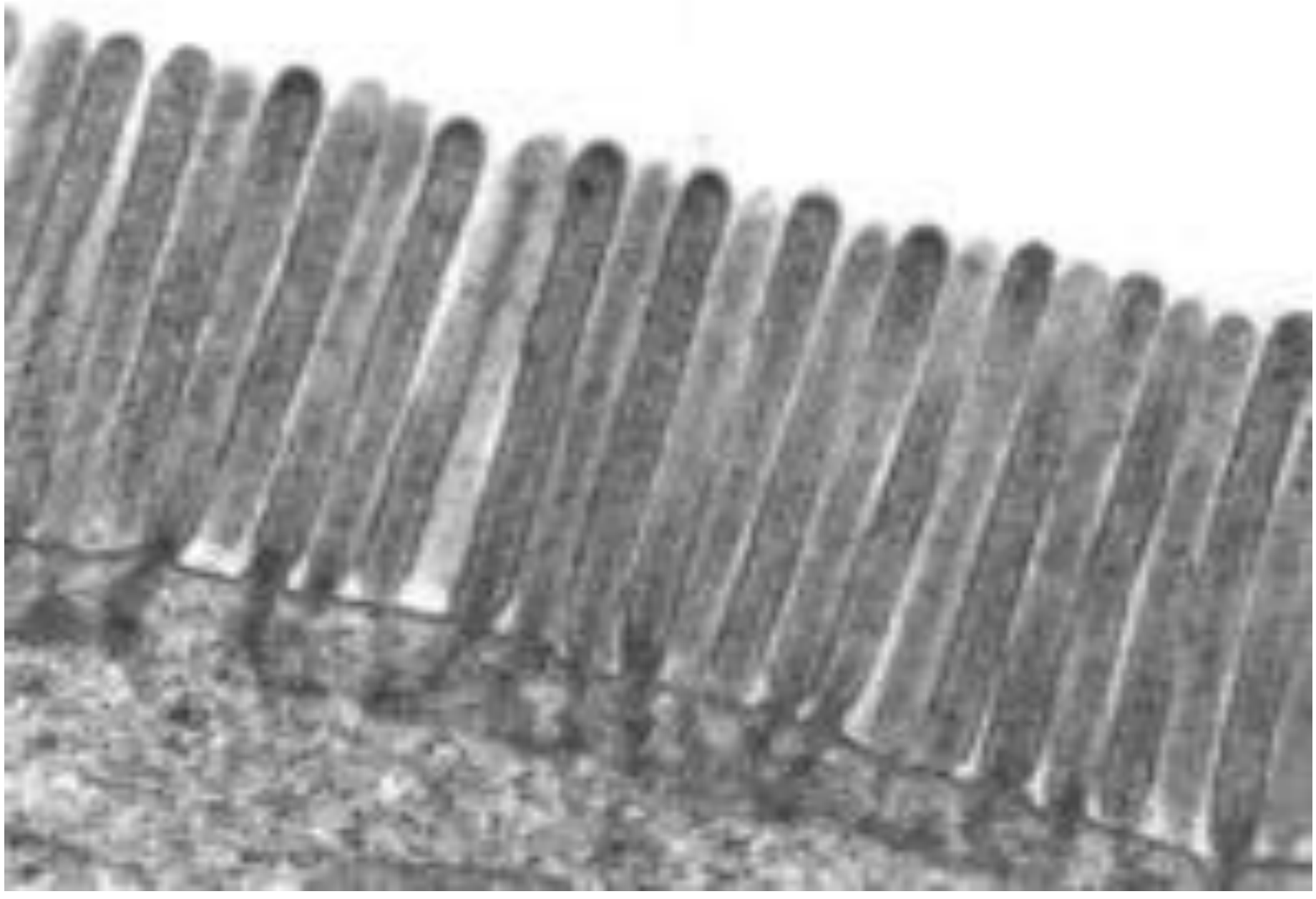
Sci Rep. 2022 Feb 4;12(1):1949.

IBS

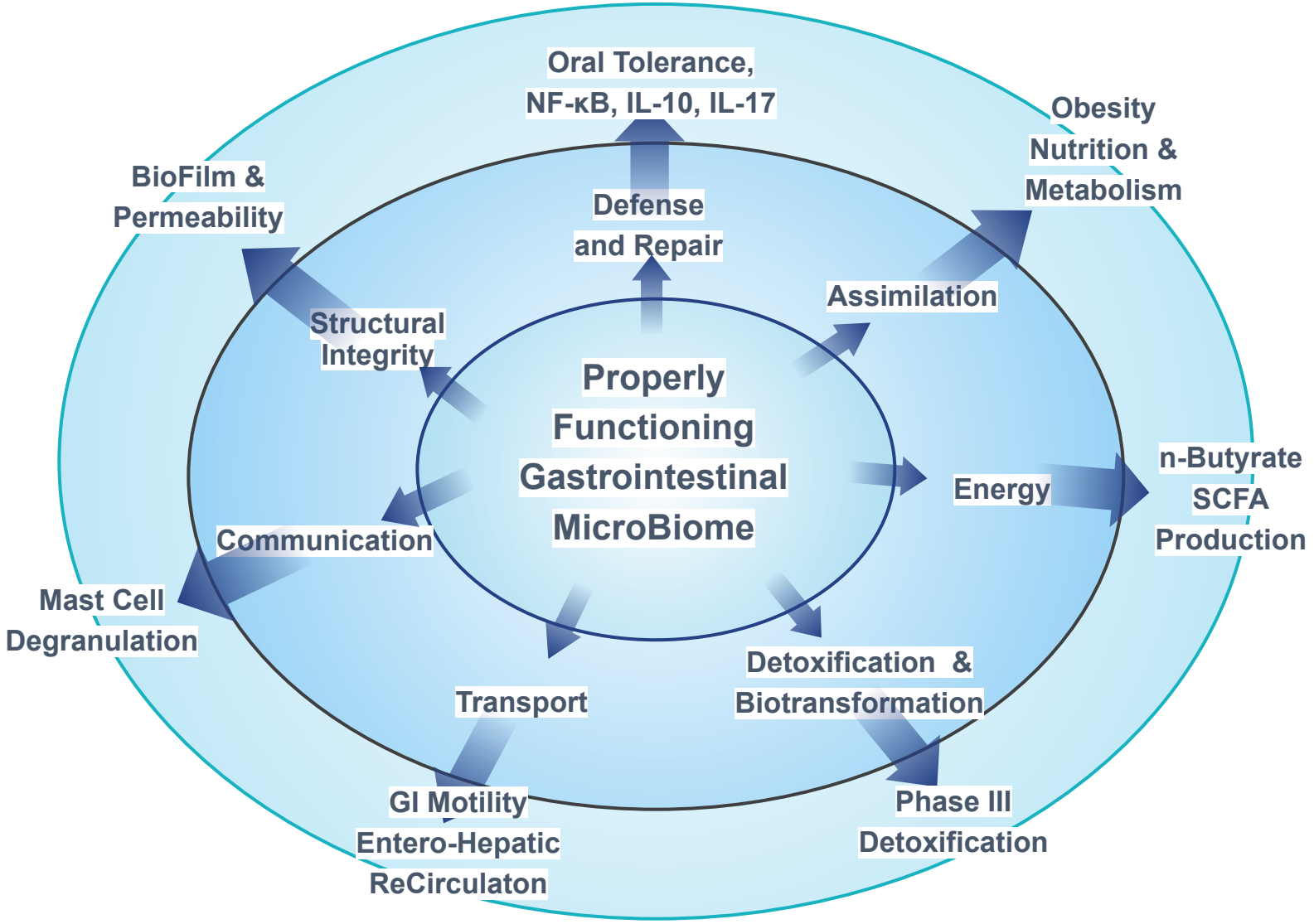
A FUNCTIONAL MEDICINE/ SYSTEMS BIOLOGY MODEL



SMALL INTESTINAL MICROVILLI: THE BRUSH BORDER



Bacteria on Intestinal Villus



Source: Institute for Functional Medicine

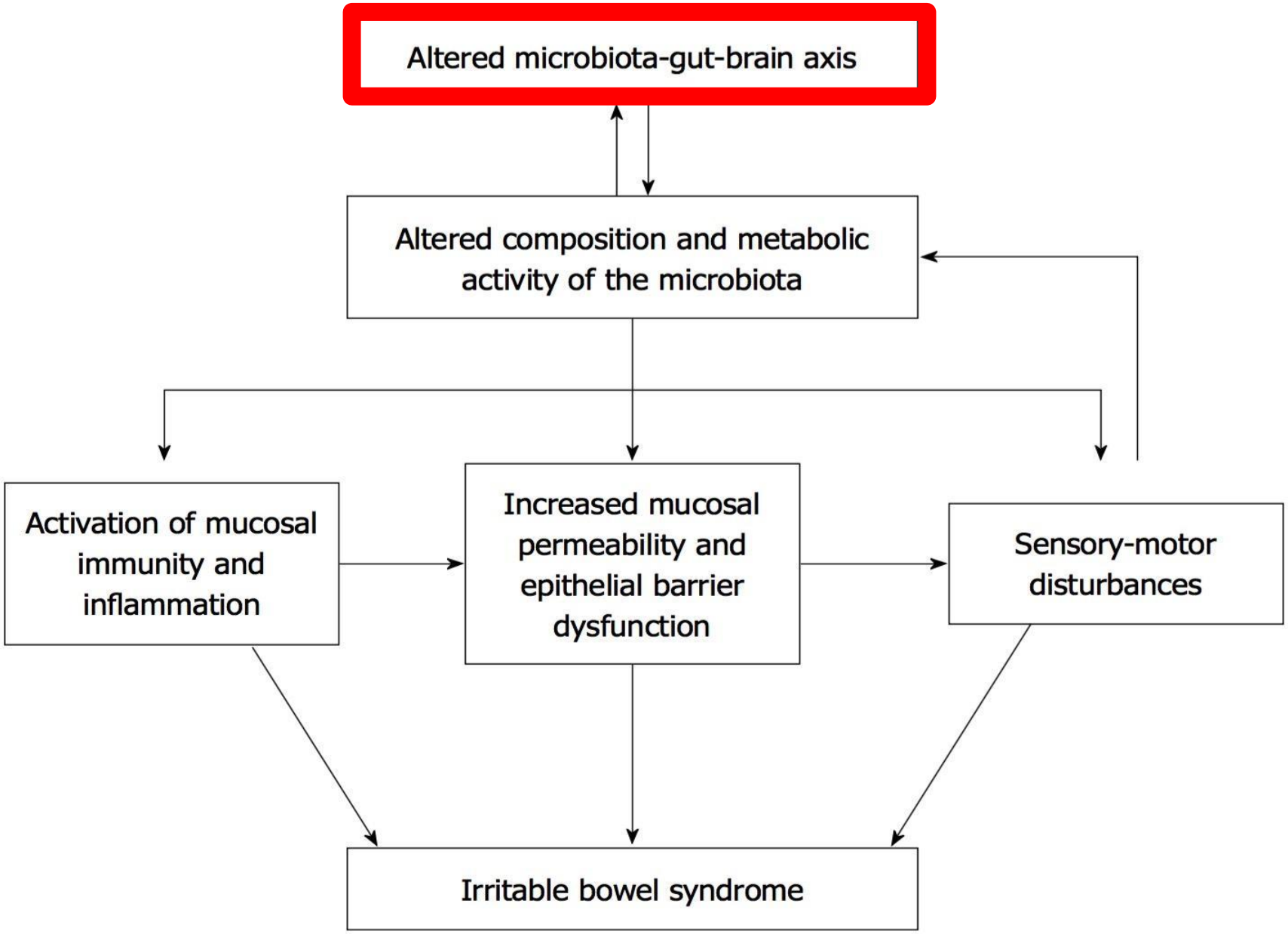
MICROBIOME TERMINOLOGY

- Alpha diversity: A function of richness and relative abundance of species (Richness = number of species or genes observed in a single sample).
- Low microbial alpha diversity indicates decreased variability and/or uneven distribution of species.
- Beta diversity measures variation between samples, or variation from average population.
- Dysbiosis implies a decrease in beneficial species with overabundance of potentially detrimental species

THE GUT MICROBIOME AND IRRITABLE BOWEL SYNDROME

- The prevailing hypothesis is that an imbalance (loss of diversity) in gut bacterial communities, or "dysbiosis," leads to activation of the gut immune system and potential low-grade inflammation.
- Furthermore, the alpha diversity, richness, and stability of gut microbiota may be reduced in patients with IBS.
- Recent data suggest that the community of fungi or "mycobiome" is also altered in patients with IBS and may be associated with the development of visceral hypersensitivity

F1000Research 2018, 7: 1029

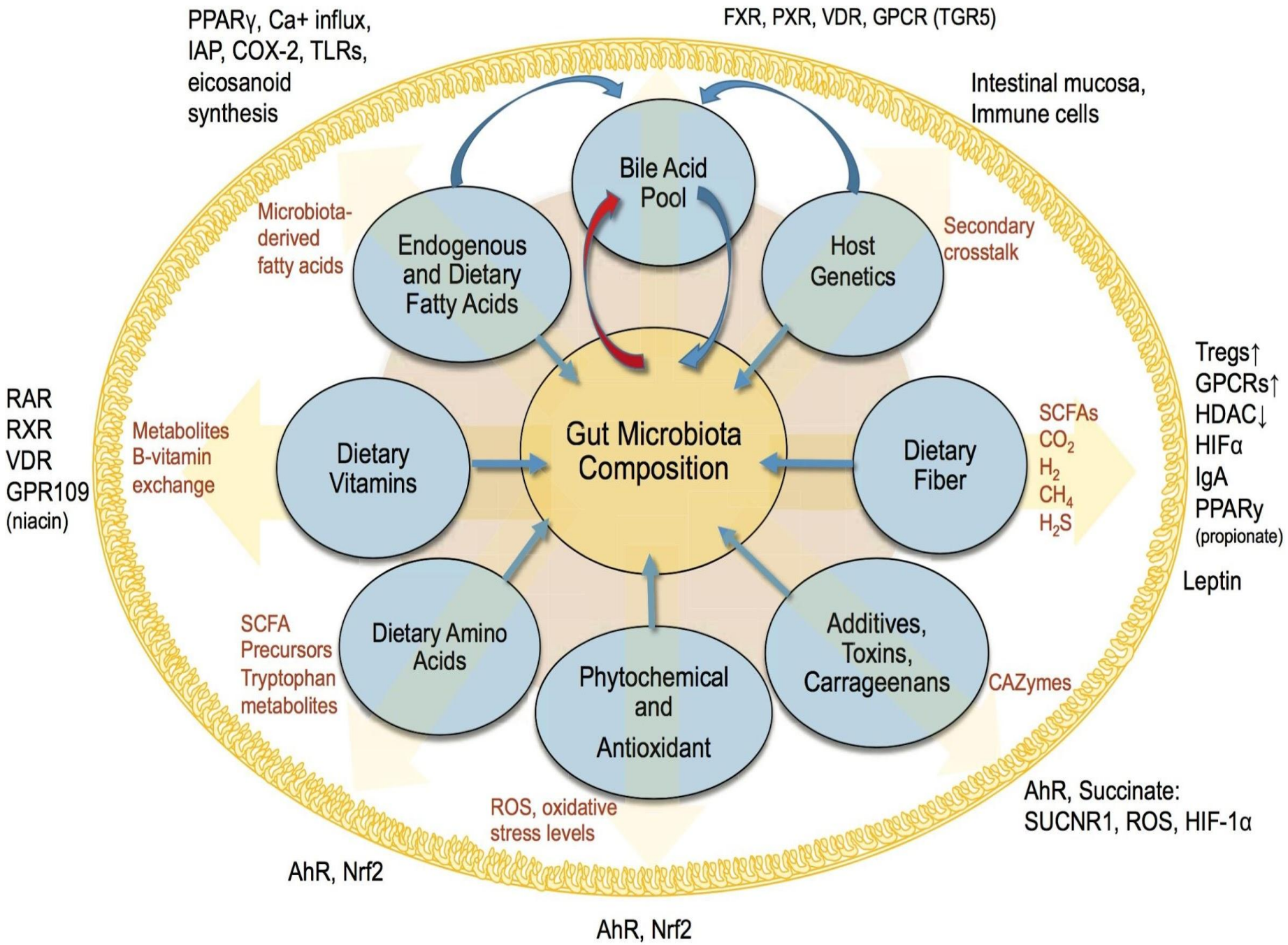


World J Gastroenterol 2014 July 21; 20(27): 8886-8897

THE GUT MICROBIOME AND IRRITABLE BOWEL SYNDROME

- Recent research suggests that environmental factors such as diet, drugs, and lifestyle exert a greater influence on the gut microbiome than genetics.
- Furthermore, the gut microbiome may possess a greater ability to predict clinical phenotype and metabolic variables than genetics

F1000Research 2018, 7: 1029


PPARγ, Ca+ influx,
IAP, COX-2, TLRs,
eicosanoid
synthesis
FXR, PXR, VDR, GPCR (TGR5)
Intestinal mucosa,
Immune cells
Bile Acid
Pool
Microbiota-
derived
fatty acids
Endogenous
and Dietary
Fatty Acids
Host
Genetics
Secondary
crosstalk
RAR
RXR
VDR
GPR109
(niacin)
Metabolites
B-vitamin
exchange
Dietary
Vitamins
Gut Microbiota
Composition
Dietary
Fiber
SCFAs
CO_2
H_2
CH_4
H_2S
Tregs↑
GPCRs↑
HDAC↓
HIFα
IgA
PPARγ
(propionate)
Leptin
SCFA
Precursors
Tryptophan
metabolites
Dietary Amino
Acids
Phytochemical
and
Antioxidant
Additives,
Toxins,
Carrageenans
CAZymes
ROS, oxidative
stress levels
AhR, Nrf2
AhR, Succinate:
SUCNR1, ROS, HIF-1α
AhR, Nrf2

POST-INFECTIOUS IRRITABLE BOWEL SYNDROME AND DYSBIOSIS

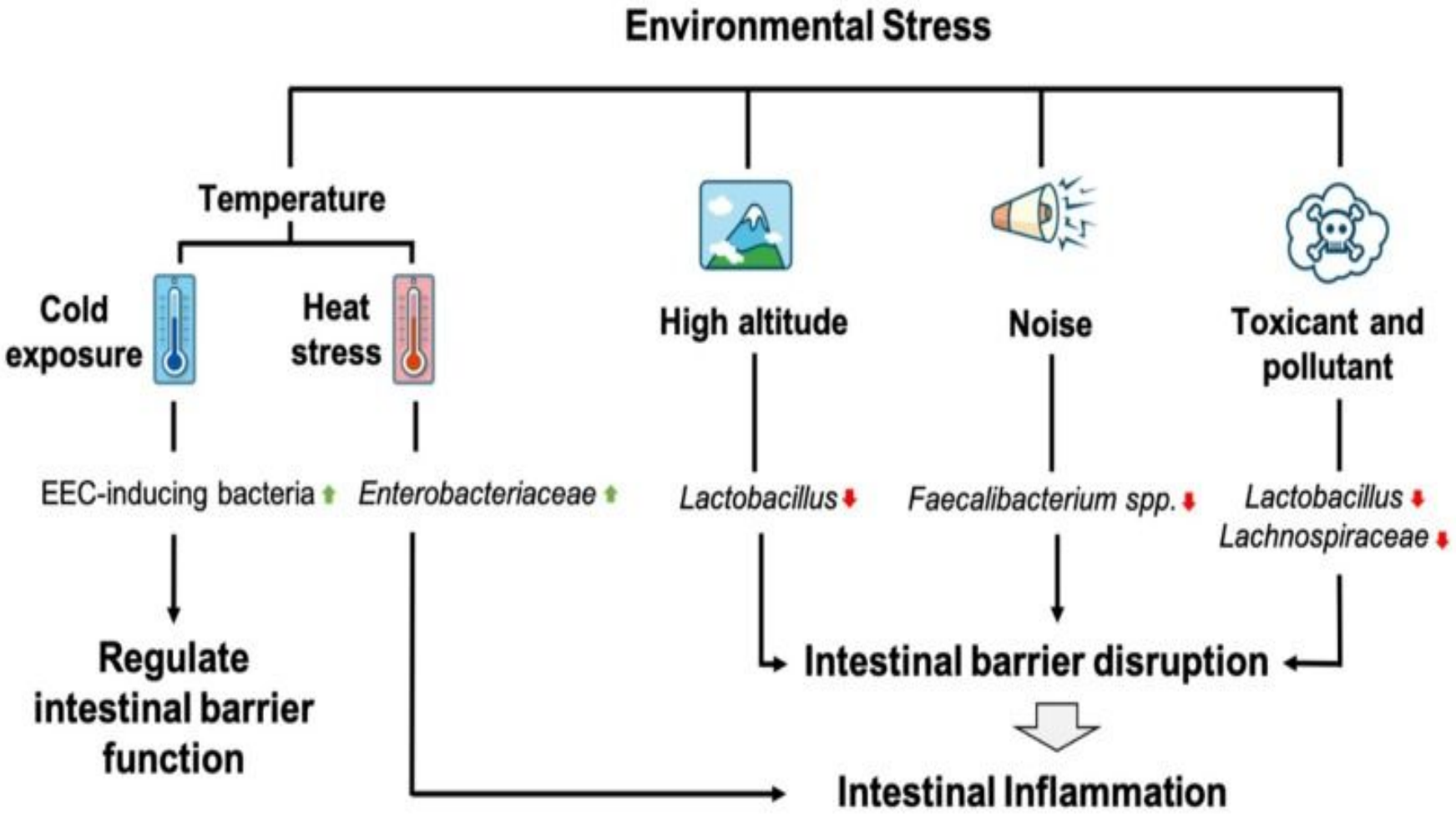
- A key argument supporting the dysbiosis hypothesis is the dramatically increased risk of developing IBS after acute gastroenteritis
- The increased risk of developing so-called "post-infection" IBS is agnostic to the type of infection (bacteria, viruses, or parasites)
- Etiology of post-infectious IBS: disrupted microbiome and/or chronic inflammatory-autoimmune disorder

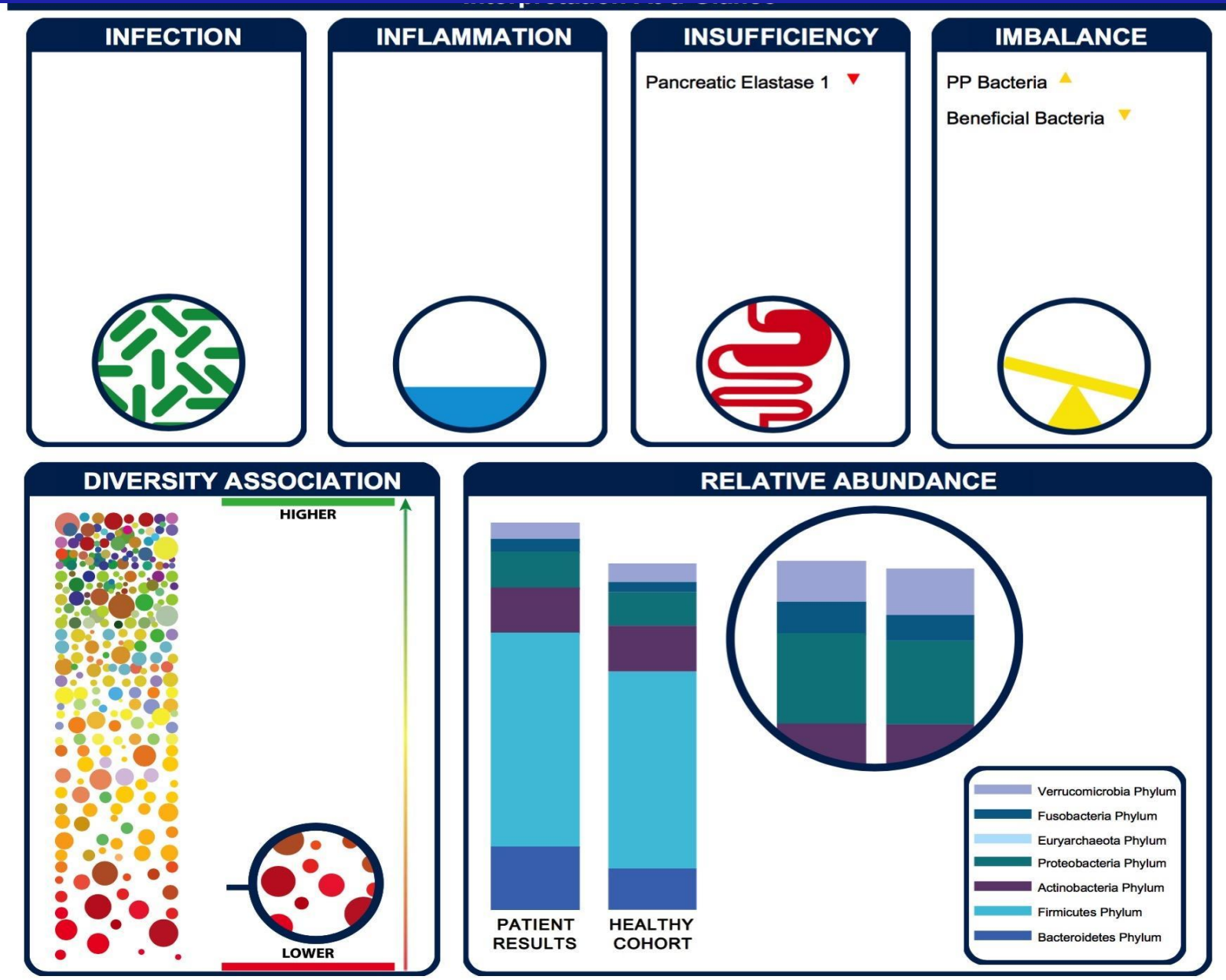
F1000Research 2018, 7: 1029

THE ROLE OF GUT MICROBIOTA IN INTESTINAL INFLAMMATION WITH RESPECT TO DIET & EXTRINSIC STRESSORS

- The gut microbiota maintains a symbiotic relationship with the host and regulates several important functions including host metabolism, immunity, and intestinal barrier function.
- Intestinal inflammation and inflammatory bowel disease (IBD) are commonly associated with dysbiosis of the gut microbiota. Alterations in the gut microbiota and associated changes in metabolites as well as disruptions in the intestinal barrier are evidence of the relationship between the gut microbiota and intestinal inflammation.
- Recent studies have found that many factors may alter the gut microbiota, with the effects of diet being commonly-studied.

MICROORGANISMS. 2019 AUG; 7(8): 271.


Environmental Stress
Temperature
Cold exposure
Heat stress
High altitude
Noise
Toxicant and pollutant
EEC-inducing bacteria
Enterobacteriaceae
Lactobacillus
Faecalibacterium spp.
Lactobacillus
Lachnospiraceae
Regulate intestinal barrier function
Intestinal barrier disruption
Intestinal Inflammation



72-yr-old female with multi-year Hx of IBS-D

GUT MICROBIOME TESTING: QUESTIONS TO ASK

- Population (census): Which microorganisms are present or absent?

- Quantity and diversity: What is the relative percentage of each microbe? How do these percentages compare to the "normal" population?

- Function (economic status): What metabolic tasks is the individual microbiome capable of performing?

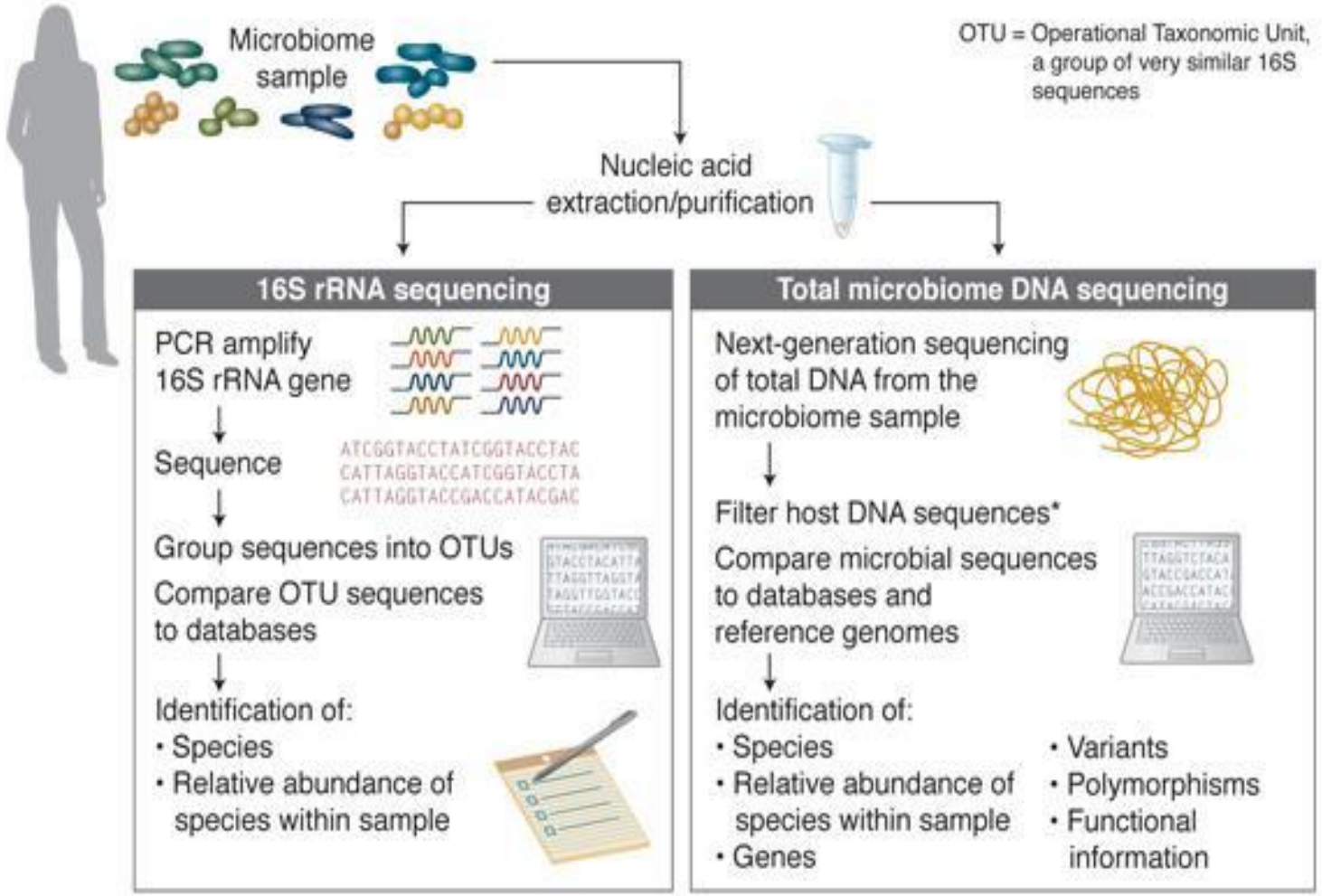
REDUCTION OF BUTYRATE & METHANE-PRODUCING MICROORGANISMS IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

- Deep sequencing of 16S rRNA gene from 113 IBS patients and 60 controls, including some repeats
- Patients with IBS showed a significantly lower microbial diversity
- IBS-M and IBS-D patients were characterized by
  - reduction of butyrate-producing bacteria, known to improve intestinal barrier function, and
  - a reduction of methane-producing microorganisms, a major mechanism of hydrogen disposal in the human colon, which could explain excess of abdominal gas in IBS

Nature Scientific Reports volume 5, Article number: 12693 (2015)

STOOL ANALYSIS

16S rRNA SEQUENCING VS SHOTGUN METAGENOMICS



While 16S analysis is fast and inexpensive, it provides little information regarding function. More detailed information can be obtained through shotgun metagenomics sequencing, particularly once host DNA is removed.

A REVIEW OF MICROBIOTA & IRRITABLE BOWEL SYNDROME

FUTURE IN THERAPIES

There is good evidence that the microbiota is a predominant factor in IBS pathophysiology.

Data suggests a relative abundance of proinflammatory bacterial species including the family *Enterobacteriaceae (*e.g., *E coli).*

A decreased percentage of *Lactobacillus* and *Bifidobacterium* genera has also been described in the IBS microbiota.

Adv Ther (2018) 35:289–310

A REVIEW OF MICROBIOTA & IRRITABLE BOWEL SYNDROME

FUTURE IN THERAPIES

Several species of *Lactobacillus* and *Bifidobacterium* genera can secrete bacteriocins, compounds that exert, in vitro, a bactericidal effect against pathogens.

Faecalibacterium prausnitzi, the *Bifidobacterium* genus, *Clostridiales* order, *Ruminococcaceae* and *Erysipelotrichaceae* families, all short-chain fatty acids (SCFAs) producers, have been found in lower proportions in IBS patients.

Adv Ther (2018) 35:289–310

Table 1 Summary of dysbiosis findings in IBS

Taxon	Percentage in IBS	Citations
Enterobacteriaceae	Higher	[38]
Lactobacillus	Lower	[22–24]
Lactobacillus genus or *Lactobacillales* order	Higher	[33–35]
Bifidobacterium	Lower	[23, 25–28]
Firmicutes/Bacteroides	Higher	[26, 33, 39, 40]
Firmicutes/Bacteroides	Lower	[31, 41]
Clostridiales		[31]
Ruminococcaceae or *Ruminococcus*	Higher	[23, 26, 31, 36, 37]
Erysipelotrichaceae		[31]
Methanogens	Lower	[39, 45]
Veillonella	Higher	[23, 33, 34]
Faecalibacterium	Lower	[26, 38]

"[Numerous] observations all support a causative role for gut dysbiosis in IBS. However, there are conflicting reports in studies about the composition of gut microbiota in IBS and there has not been a specific microbial signature identified in IBS to date."

Gastroenterol Res Pract. 2018; 2018: 6961783.

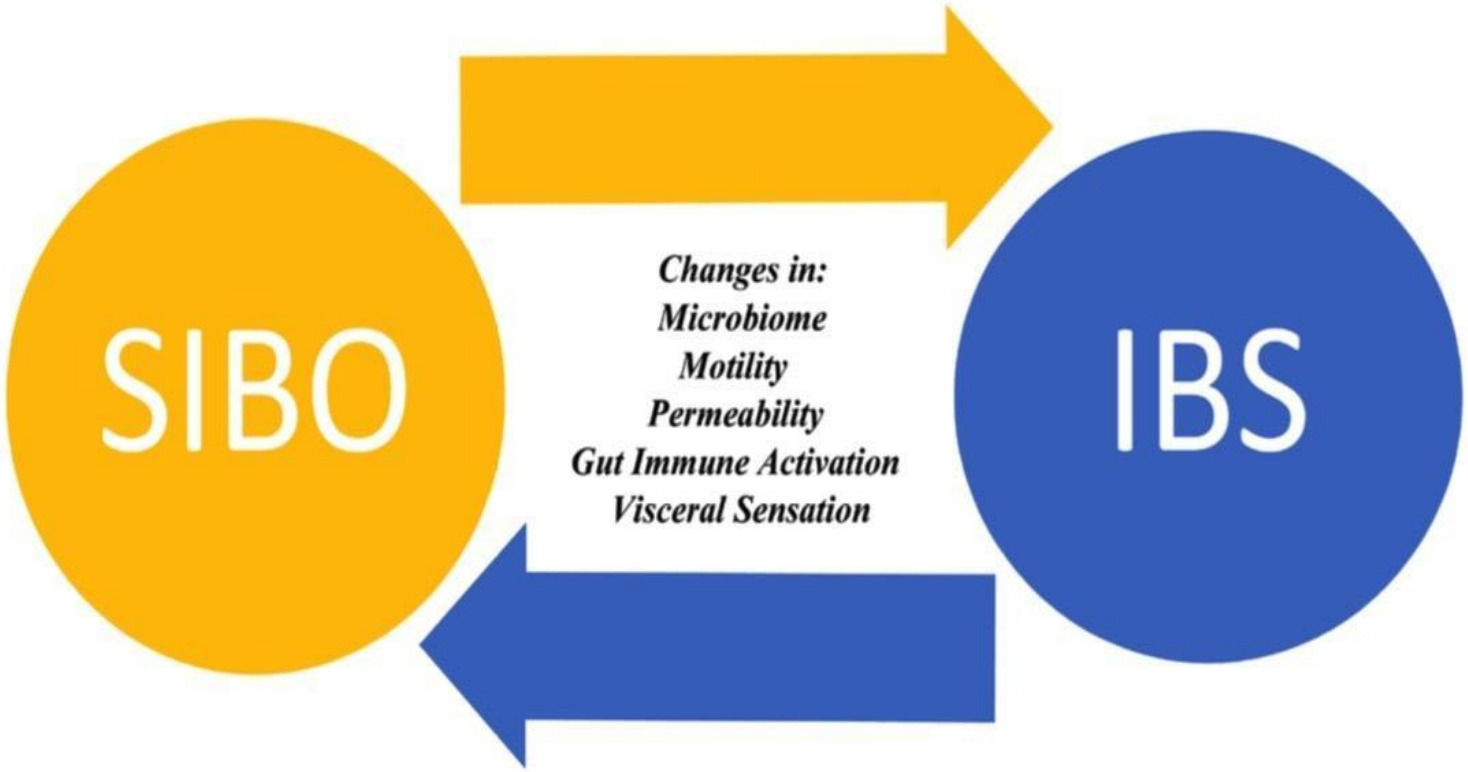


Figure 2. Small intestinal bacterial overgrowth: the chicken or the egg? IBS, irritable bowel syndrome; SIBO, small intestinal bacterial overgrowth.

F1000Res. 2018 Jul 9;7. pii: F1000 Faculty Rev-1029

SIBO & IRRITABLE BOWEL SYNDROME

Patients with IBS are more likely than healthy volunteers to have an abnormal breath test for SIBO.

It is biologically plausible to suggest that SIBO can cause IBS symptoms in some but that, in others, alterations in motility, gut immune function, or microbiome predispose to the development of SIBO.

If this is true, it is not difficult to imagine how one hand would feed the other, leading to a vicious cycle.

F1000Research 2018, 7: 1029

PREVALENCE OF OVERGROWTH BY AEROBIC BACTERIA IN THE SMALL INTESTINE BY SMALL BOWEL CULTURE

RELATIONSHIP WITH IRRITABLE BOWEL SYNDROME

Small intestinal bacterial overgrowth (SIBO) is traditionally characterized by excessive bacteria in the small intestine.

The results of our study demonstrate that overall, 37.5% of IBS subjects have overgrowth from aerobe colonic type bacteria in the small intestine.

That is even greater at 60% among patients with IBS-D

Dig Dis Sci, 2012 May;57(5):1321-9

SIBO: What does Methane Have to Do with It?

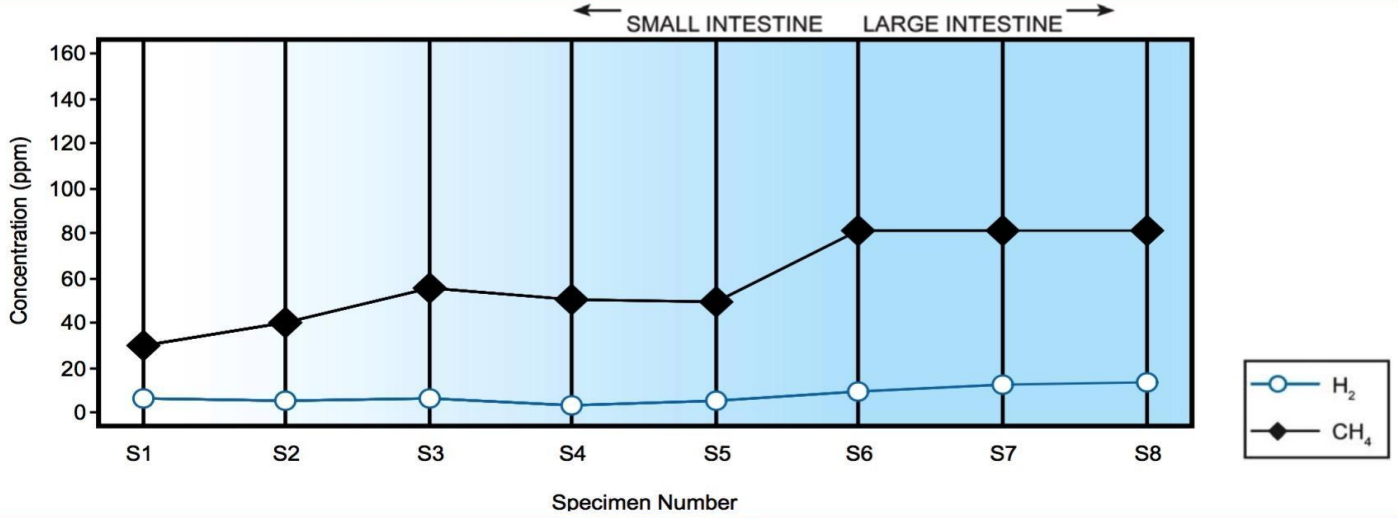
- Methane is detected in 30%-50% of the healthy adult population worldwide
- Its production has been epidemiologically and clinically associated with constipation related diseases, like constipation predominant irritable bowel syndrome (IBS-C) and chronic constipation (CC).
- While a causative relation is not proven yet, there is strong evidence from animal studies that methane delays intestinal transit, possibly acting as a neuromuscular transmitter.
- Antibiotic reduction of methanogens (as evidenced by reduced methane production) predicts the clinical response in terms of symptomatic improvement in patients with IBS-C.

Triantafyllou K, Chang C, Pimentel M. Methanogens, methane and gastrointestinal motility. J Neurogastroenterol Motil. 2014 Jan;20(1):31-40.

2337 Small Intestinal Bacterial Overgrowth (SIBO) 3 Hour - Breath

Methodology: GC-TDC/SSS

Hydrogen (H_2) and Methane (CH_4) Breath Gases



Hydrogen (H_2), Methane (CH_4) and Carbon Dioxide (CO_2) (ppm)	Baseline 0 min (S1)	20 min (S2)	40 min (S3)	60 min (S4)	90 min (S5)	120 min (S6)	150 min (S7)	180 min (S8)
H_2	6	5	6	3	5	9	12	13
CH_4	29	40	55	50	49	81	>81	>81
H_2 + CH_4	35	45	61	53	54	90	NR	NR
CO_2 **	✓	✓	✓	✓	✓	✓	✓	✓
Actual Collection Times								
Actual Time	8:30	8:50	9:10	9:30	10:00	10:30	11:00	11:30
Actual Interval	0 min	20 min	40 min	60 min	90 min	120 min	150 min	180 min

**CO_2 is measured for quality assurance. ✓ indicates the CO_2 level is acceptable. X indicates room air contamination exceeding acceptable limits.

Evaluation for Hydrogen (H_2)

Hydrogen increase over baseline by 90 minutes

	Result	Expected Value
Change in H_2	0	<20 ppm

A rise of ≥ 20 ppm from baseline in hydrogen by 90 min should be considered a positive test to suggest the presence of SIBO.

Evaluation for Methane (CH_4)

Peak methane level at any point

	Result		Expected Value
CH_4 Peak	>81	H	<10 ppm

A peak methane level ≥ 10 ppm at any point is indicative of a methane-positive result.

20 yo female with bloating/IBS-C

METHANOGENS (*ARCHAEA*) IN THE GI TRACT

BENEFICIAL OR HARMFUL OR NEITHER?

Should we strive to eliminate methanogens or simply to support an “appropriate” level of growth?

IBS TRIGGERS & MEDIATORS

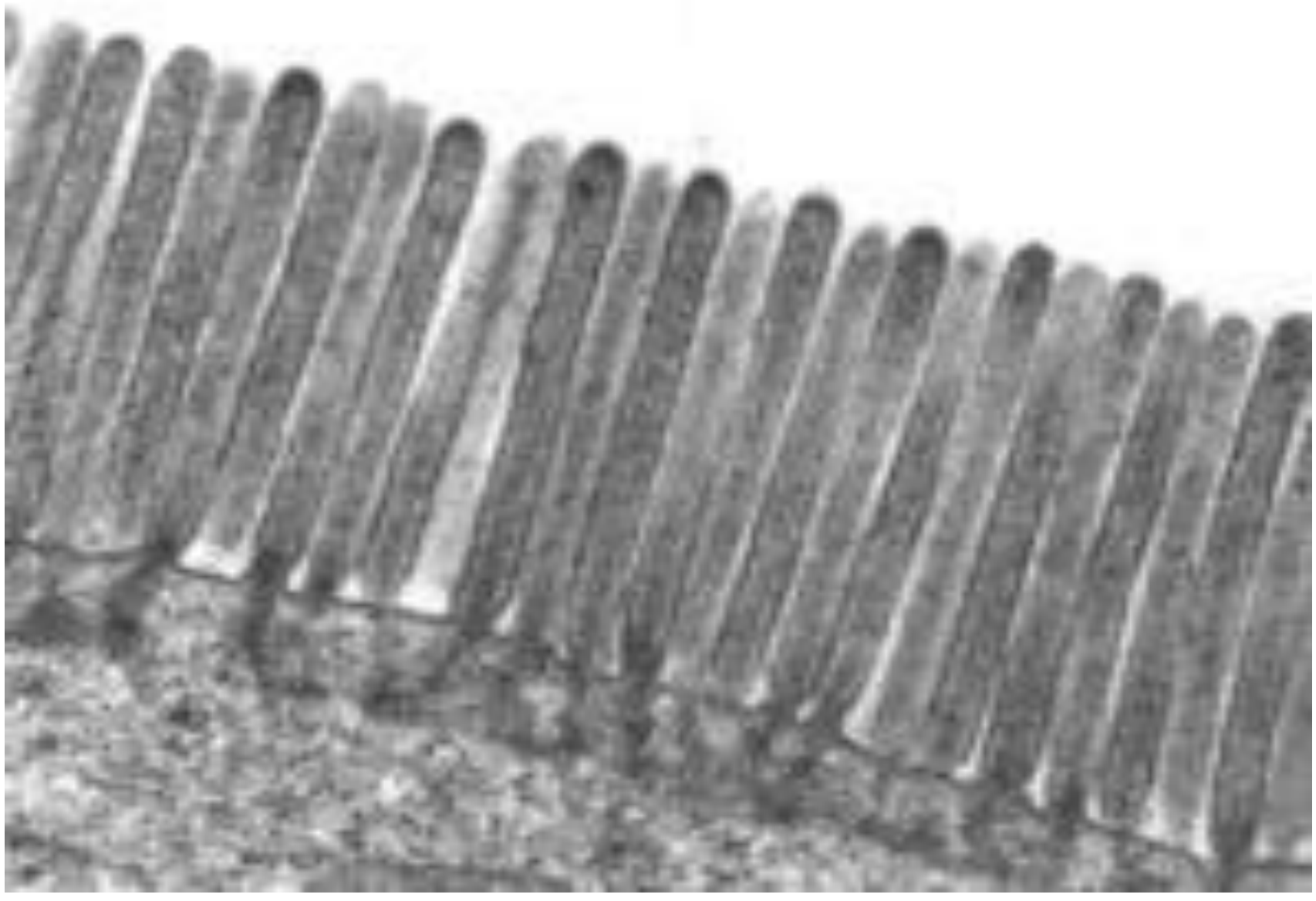
Biocidin Botanicals™

“...most IBS patients report that meals induce or exacerbate symptoms…”

Dietary Interventions and Irritable Bowel Syndrome – What Really Works? Curr Opin Gastroenterol. 2021;37(2):152-157

SMALL INTESTINAL MICROVILLI

THE BRUSH BORDER



BRUSH BORDER DISSACCHARIDASES

Lactase (beta-galactosidase)

Sucrase-isomaltase (aka palatinase)

Maltase-glucoamylase

Trehalase

BRUSH BORDER ENZYME DEFICIENCIES:
MORE COMMON THAN YOU THINK!

Genetic (variable severity)

- Lactase deficiency (rarely congenital)
  - up to 70% of world's population >5 y/o
  - result of epigenetic modification (gene silencing via DNA methylation)
  - "lactase persistence" = lactose tolerance: multiple SNPs in lactase genes confer resistance to methylation
  - prebiotics (pure GOS) and probiotics may improve lactose tolerance by inducing growth of lactic acid bacteria (convert lactose into lactic acid)
- Sucrase-isomaltase deficiency: 2nd most common deficiency; *autosomal recessive* trait; can also be acquired (?transient)

A double-blind, 377-subject randomized study identifies *Ruminococcus*, *Coprococcus*, *Christensenella*, and *Collinsella* as long-term potential key players in the modulation of the gut microbiome of lactose intolerant individuals by galacto-oligosaccharides

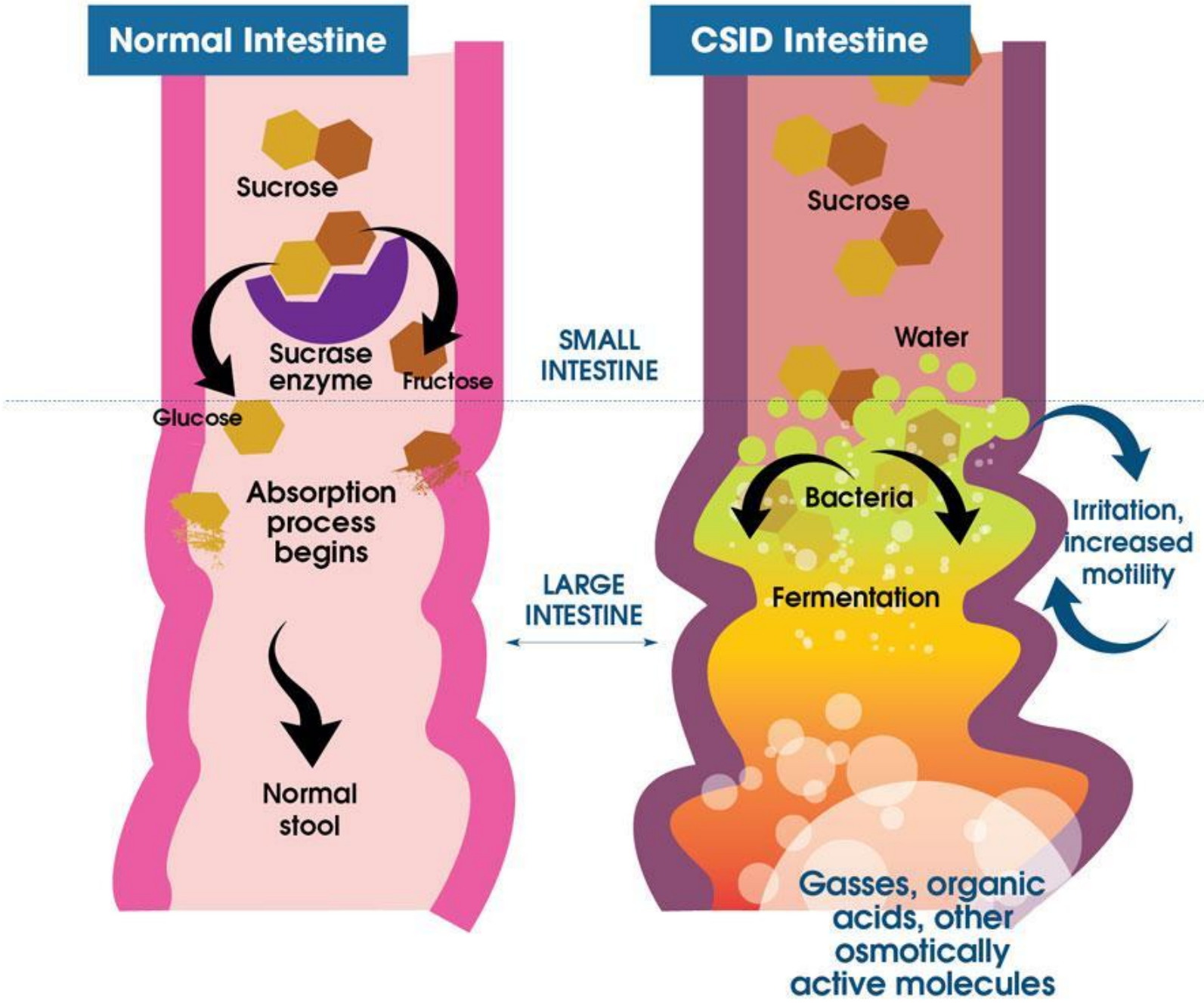
30 day consumption of pure GOS, significantly reduced symptoms and altered the fecal microbiome in patients with lactose intolerance

Taxa impacted by treatment and subsequent dairy consumption included lactose-fermenting species of *Bifidobacterium, Lactobacillus, Lactococcus*, and *Streptococcus*.

Increased secondary fermentation microorganisms included *Coprococcus* and *Ruminococcus* species, *Blautia producta*, and *Methanobrevibacterium*.

Tertiary fermenters that use acetate to generate butyrate were also increased, including *Faecalibacterium prausnitzii, Roseburia faecis*, and *C. eutactus*.

Gut Microbes, 2021 Jan-Dec;13(1):1957536.



CSID: Congenital Sucrase-Isomaltase Deficiency

BRUSH BORDER ENZYME DEFICIENCIES:
MORE COMMON THAN YOU THINK!

Acquired – mucosal damage

- Celiac disease
- Infection/overgrowth: *Vibrio cholerae*, *Giardia lamblia*, rotavirus infection, SIBO (nonspecific)
- Treatment induced: antibiotics, chemotherapy, radiation therapy
- Inflammatory: Crohn's Disease, eosinophilic gastroenteritis

"In spite of being a critical source of energy, the CAZyme repertoire encoded by the human genome is minimal (approximately 17 enzymes). Most of the carbohydrate degradation is performed by the resident gut microbiome. *The capacity for carbohydrate degradation by the gut flora is immense.*"

Global Profiling of Carbohydrate Active Enzymes in Human Gut Microbiome; PLoS One. 2015; 10(11): e0142038

ALPHA GALACTOSIDASE, CHO DIGESTION, AND GAS

Found in lysosomes (intracellular) but not secreted by brush border enterocytes -- only produced by lactic acid bacteria (LAB), *Ruminococcus gnavus, Bacillus coagulans*, and other gut microbes (primarily in colon)

Breaks down non-absorbable galactooligosaccharides (fermentable CHO), eg stachyose and raffinose family GOS, found in grains, legumes, root vegetables, and brassica (broccoli, cabbage)

Oral enzyme preparations (from *Aspergillus niger*) show that 1200 IU per meal decreases intestinal gas production and bloating - works best combined with invertase (sucrase)

BMC Gastroenterology 2013, 13:142

BILE ACID MALABSORPTION: A MAJOR CAUSE OF CHRONIC DIARRHEA

Excessive bile acids entering the colon

- Impaired ileal reabsorption – SNPs in transporter genes
- Excess synthesis – SNPs in **β**-Klotho (Klb) gene (regulates BA synthesis)

Known association with chronic diarrhea for over 50 years

Mayo Clinic Study: reported in 25% to 33% of patients presenting with chronic diarrhea

BAM found in 35% of those diagnosed with microscopic colitis

Lab Dx: 48 hr fecal fractionated bile acids; serum 7alphaC4

www.mayoclinic.org/medical-professionals/digestive-diseases/news/identifying-diarrhea-caused-by-bile-acid-malabsorption/mac-20430098

DIETARY TRIGGERS IN IRRITABLE BOWEL SYNDROME: IS THERE A ROLE FOR GLUTEN?

Non-celiac gluten sensitivity (NCGS) or non-celiac wheat sensitivity (NCWS) is now well established in clinical practice.

Patients with NCGS/NCWS have symptoms that mimic those present in IBS

Patients with IBS often report worsening of symptoms by a wheat containing diet.

Journal of Neurogastroenterology and Motility 2016; 22(4): 547-557

DIETARY TRIGGERS IN IRRITABLE BOWEL SYNDROME: IS THERE A ROLE FOR GLUTEN?

Gluten sensitization causes altered smooth muscle contractility and altered barrier function in animal studies—this may result in immune activation

Other proteins in wheat, such as **α**-amylase/trypsin inhibitors (ATIs) and wheat lectin agglutinin, have been shown to induce innate immune pathways (TLR4)

Wheat contains fructans, a poorly absorbed CHO (FODMAP) which may cause excessive excessive bacterial fermentation and gas production

Journal of Neurogastroenterology and Motility 2016; 22(4): 547-557

DIETARY TRIGGERS IN IRRITABLE BOWEL SYNDROME: IS THERE A ROLE FOR GLUTEN?

Some studies have suggested that IBS patients who carry celiac susceptibility genes (HLA-DQA1, HLA-DQB1) are more likely to respond to the gluten-free diet.

Those with celiac susceptibility genes may be more prone to develop mild immune responses and gut dysfunction to gluten.

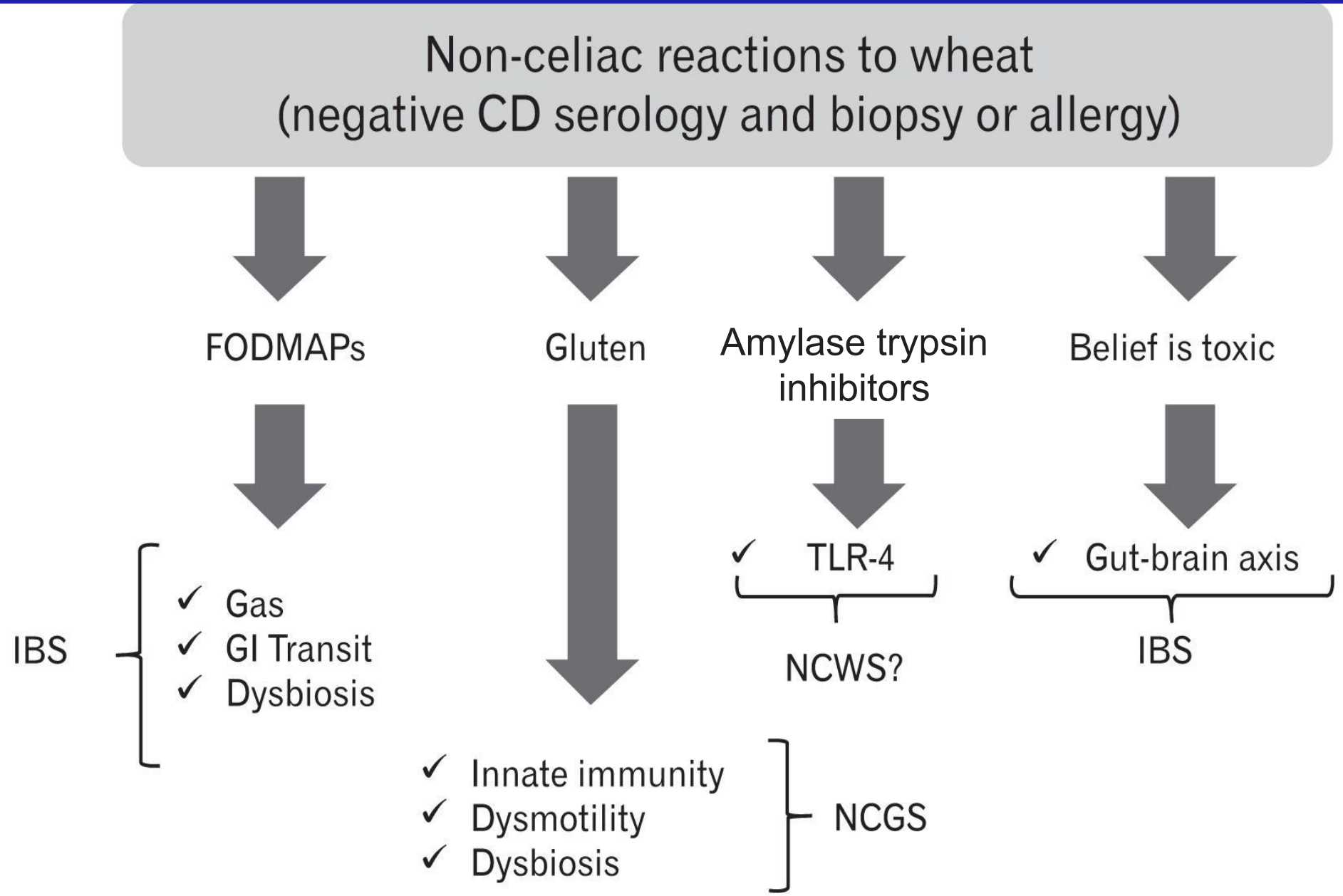
Journal of Neurogastroenterology and Motility 2016; 22(4): 547-557

DIETARY TRIGGERS IN IRRITABLE BOWEL SYNDROME: IS THERE A ROLE FOR GLUTEN?

Recent data reported a high prevalence of serum autoantibodies (antinuclear antibodies, ANA) and a frequent association with autoimmune disorders (ie, Hashimoto's thyroiditis) in patients with NCGS/NCWS.

Up to 20% of NCGS/NCWS show mild laboratory abnormalities, such as low levels of ferritin, folic acid, vitamin D and B12, most likely related to a minimal inflammatory state in the intestinal mucosa.

Journal of Neurogastroenterology and Motility 2016; 22(4): 547-557

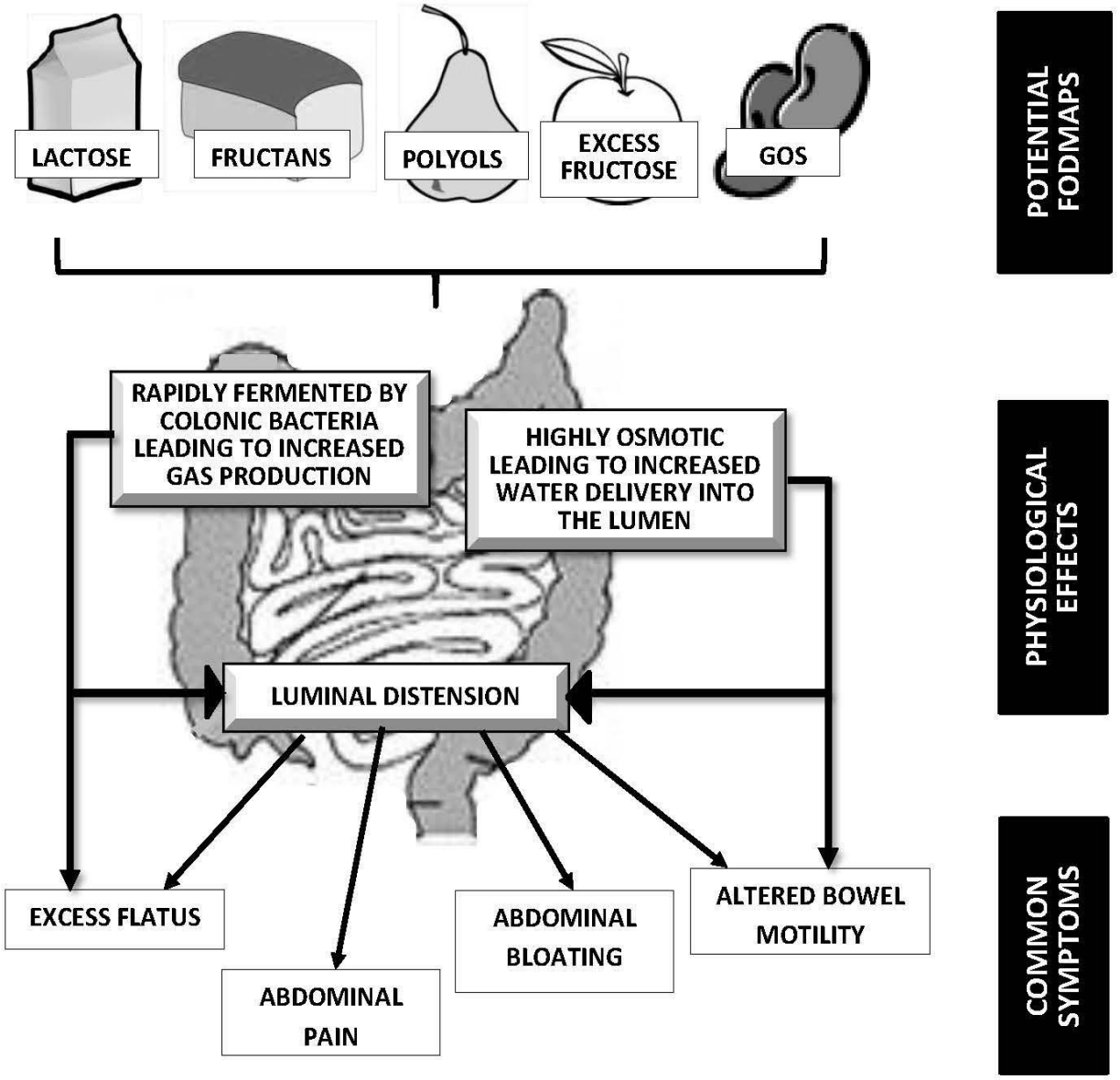


Journal of Neurogastroenterology and Motility 2016; 22(4): 547-557

FODMAPS & IBS

- Fermentable oligosaccharides (fructans/FOS, galactans/galacto-oligosaccharides), disaccharides (lactose), monosaccharides (fructose), and polyols
  (Peter Gibson, Monash University)
- Studies suggest that >50% of patients with IBS report symptomatic benefit from low-FODMAP diets:
  Gut. 2017; 66(8): 1517–27
- Symptom relief can occur in 48 hours

FODMAPS AND IBS: PATHOPHYSIOLOGY



Randomised Clinical Trial: Gut Microbiome Biomarkers are Associated with Clinical Response to a Low FODMAP Diet in Children with Irritable Bowel Syndrome

Responders to a low FODMAP diet were enriched at baseline in taxa with known greater saccharolytic metabolic capacity (e.g., *Bacteroides*, *Ruminococcaceae*, *Faecalibacterium prausnitzii*) and 3 KEGG orthologs, of which two relate to carbohydrate metabolism.

Breath hydrogen production was lower during the low FODMAP diet versus typical American childhood diet. However, breath hydrogen production did not correlate with abdominal pain frequency during either dietary intervention period

Methane production did not differ between the two dietary periods

Aliment Pharmacol Ther. 2015 Aug; 42(4): 418–427.

Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With Irritable Bowel Syndrome

Fecal metabolomic patterns of volatile organic compounds (VOCs) determined by GS-MS were able to separate patients with IBS, inflammatory bowel disease, and healthy controls with excellent accuracy.

This validates the potential for VOC as a diagnostic biomarker for IBS and supports the contention that IBS is associated with abnormal microbial metabolism

Clin Gastroenterol Hepatol. 2018; **16**(3): 385–391. e1.

Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With Irritable Bowel Syndrome

95 patients with IBS, placed on low FODMAP or sham diet, with or without probiotics

Fecal VOC patterns showed clear separation between responders and non-responders to both LFD and probiotic at both baseline and end-of-treatment.

***Clin Gastroenterol Hepatol.* 2018; 16(3): 385–391. e1.**

Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With IBS

Eating a “normal”diet, naturally high in FODMAPs, in the presence of IBS-associated dysbiosis may generate specific products of fermentation that give rise to symptoms.

Once the normal diet has been replaced by a LFD, there is less substrate for bacterial metabolism; consequently, gas production and the associated symptoms are reduced

On the basis of this hypothesis, only people with the saccharolytic-rich, IBS-associated dysbiosis eating a high FODMAP diet may exhibit the specific VOC pattern predictive of response

***Clin Gastroenterol Hepatol.* 2018; 16(3): 385–391. e1.**

FODMAPS

GOOD OR BAD FOR IBS?

Biocidin Botanicals™

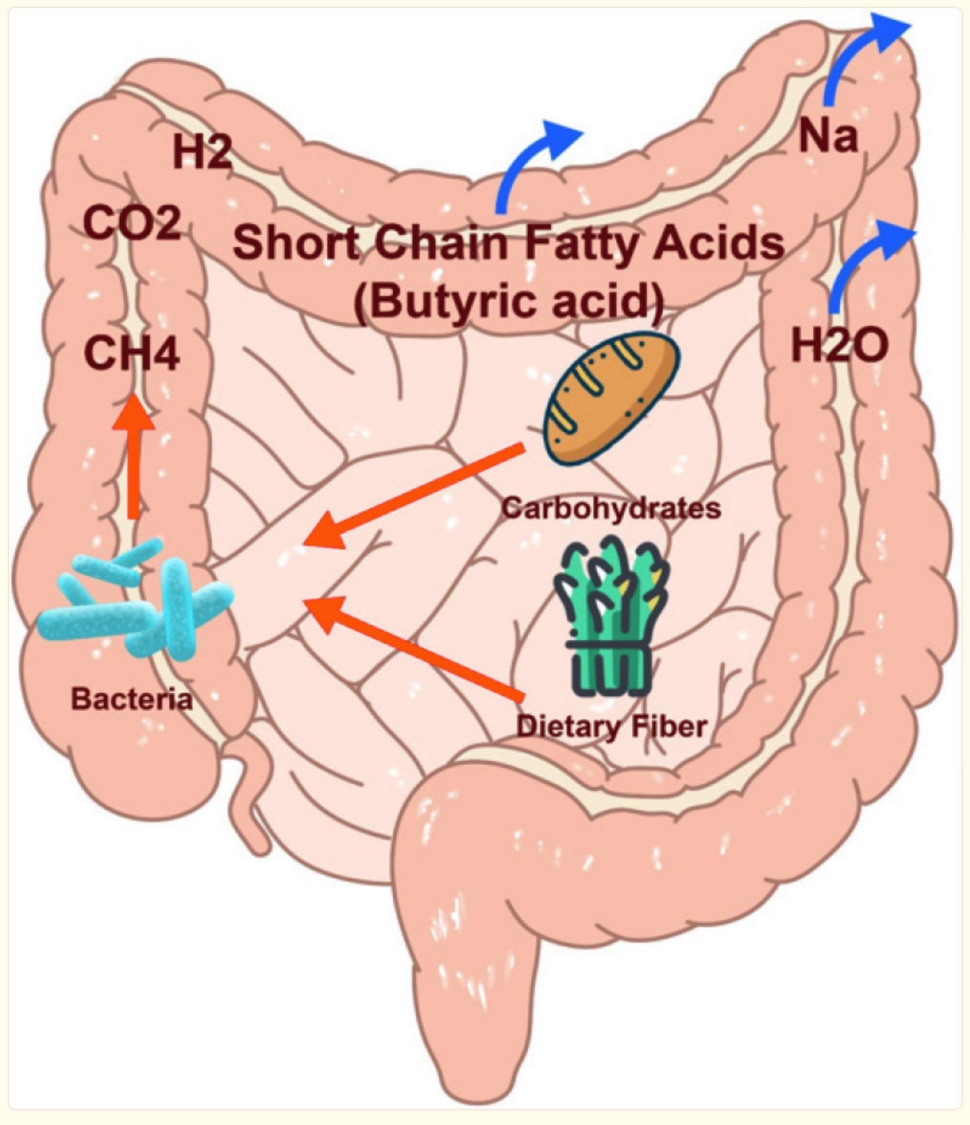
IBS AND LOW FODMAP DIETS

Recent studies have demonstrated reductions in potentially beneficial fecal bifidobacteria and butyrate levels in IBS patients on a low-FODMAP diet: *Gut.* 2015; 64(1): 93–100

This effect may be mitigated by administration of probiotics (esp. *Bifidobacteria* spp): *Am J Gastroenterol.* 2014; 109(10): 1547–61

After a 2-6 week exclusion period, responders should be instructed to reintroduce foods containing individual FODMAPs to determine their sensitivities and allow diversification of their diet to minimize detrimental effects on the microbiome.

α-galactosidase supplements may allow some patients with IBS to tolerate galacto-oligosaccharides (eg D-raffinose): *Am J Gastroenterol.* 2018; 113(1): 124–34



The prebiotic paradox

Bacterial fermentation is essential for butyrate production!

World J Clin Pediatr. 2021 Sep 9; 10(5): 84–92

Butyrate (butanoic acid) & IBS

Highly beneficial short chain fatty acid

Found in butter, ghee, raw milk, animal fats, plant oils

Increased in GI tract after fermentation of dietary fiber (prebiotics, eg. certain FODMAPs)

Preferred fuel for enteric cells

- Supplies up to 80% of energy needs
- Trophic properties – maintains healthy gut barrier
- Regulates motility

Low butyrate levels associated with

- Dysbiosis (butyrate suppresses pathogens)
- Irritable bowel syndrome (butyrate decreases visceral sensitivity and pain)
- Inflammatory bowel disease (butyrate is anti-inflammatory)
- Colon cancer

Butyric acid in irritable bowel syndrome, Prz Gastroenterol 2013; 8 (6): 350–353

DIFFERENTIAL EFFECTS OF WESTERN AND MEDITERRANEAN-TYPE DIETS ON GUT MICROBIOTA:
A METAGENOMICS AND METABOLOMICS APPROACH

Compared to those on a Western diet, 2 weeks of consuming the fiber-enriched MD (rich in fruits, vegetables and legumes) resulted in a

- higher number of anal gas evacuations
- sensation of flatulence and borborygmi
- larger volume of gas after the meal
- larger colonic content

This was also manifested by increases in the relative abundance of specific butyrate-producing taxa, a higher abundance of a number of microbial metabolic pathways, and a distinct urinary pattern of microbial metabolites.

Nutrients 2021, *13*, 2638

EFFECTS OF PREBIOTICS VS A DIET LOW IN FODMAPS IN PATIENTS WITH FUNCTIONAL GUT DISORDERS

- 44 patients with IBS randomized to receive prebiotic plus Mediterranean Diet (21) or low FODMAP diet (23) plus placebo (xylose)
- Prebiotic = 2.8 g per day of Bimuno containing 1.8 g beta-galactooligosaccharide
- After 4 weeks both groups had statistically significant reduction in all symptom scores except no reduction in flatulence and borborygmi in the prebiotic group
- *Bilophila wadsworthia* decreased with the prebiotic and increased with LFD
- *Bifidobacteria* spp. increased with the prebiotic and decreased with LFD
- The decrease in symptoms with the LFD disappeared soon after the diet was discontinued
- The prebiotic group experienced symptom improvement for 2 weeks after stopping the supplement

Gastroenterology 2018;155:1004–1007

FODMAPS & IBS
TREATMENT STRATEGY

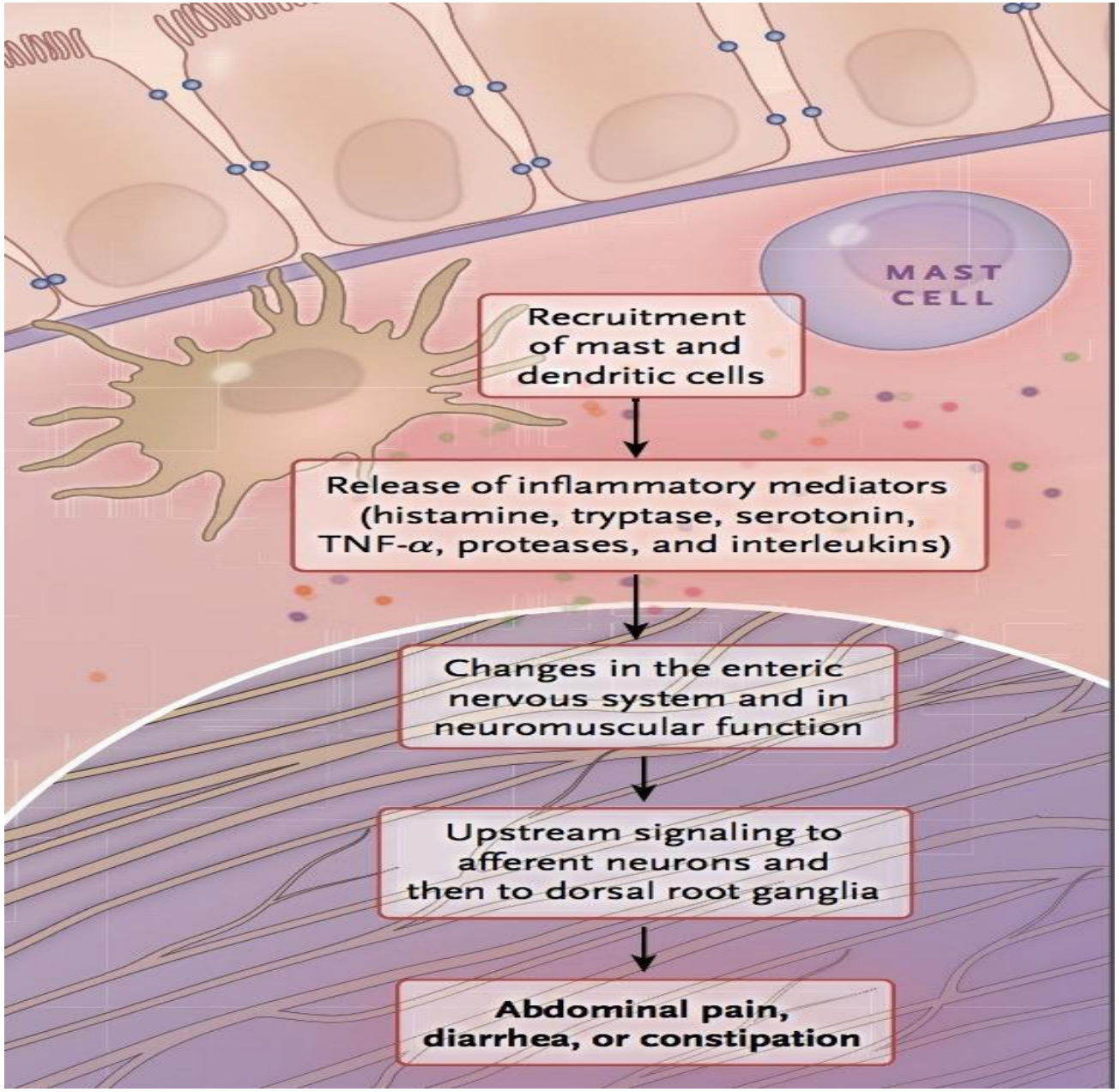
- Short-term avoidance can be very helpful for diagnostic purposes (identifying problematic foods)
- Reintroduction should (eventually) be attempted to restore healthy microbial flora and SCFA production
- Gut microbiome analysis may guide choice of prebiotics
- Persistent intolerances may indicate enzyme deficiency (brush border or microbial)

IBS and Inflammation

- For many years, IBS was considered an exclusively "functional" disorder *(Dx based on symptoms and exclusion criteria for more serious conditions)*
- Recent studies: low-grade inflammation found in a subset of patients with IBS
- In 2009, Cremon et al confirmed low-grade inflammation in the lamina propria and mucosa in 72% of pts with IBS but to a lesser extent than in microscopic colitis or ulcerative colitis

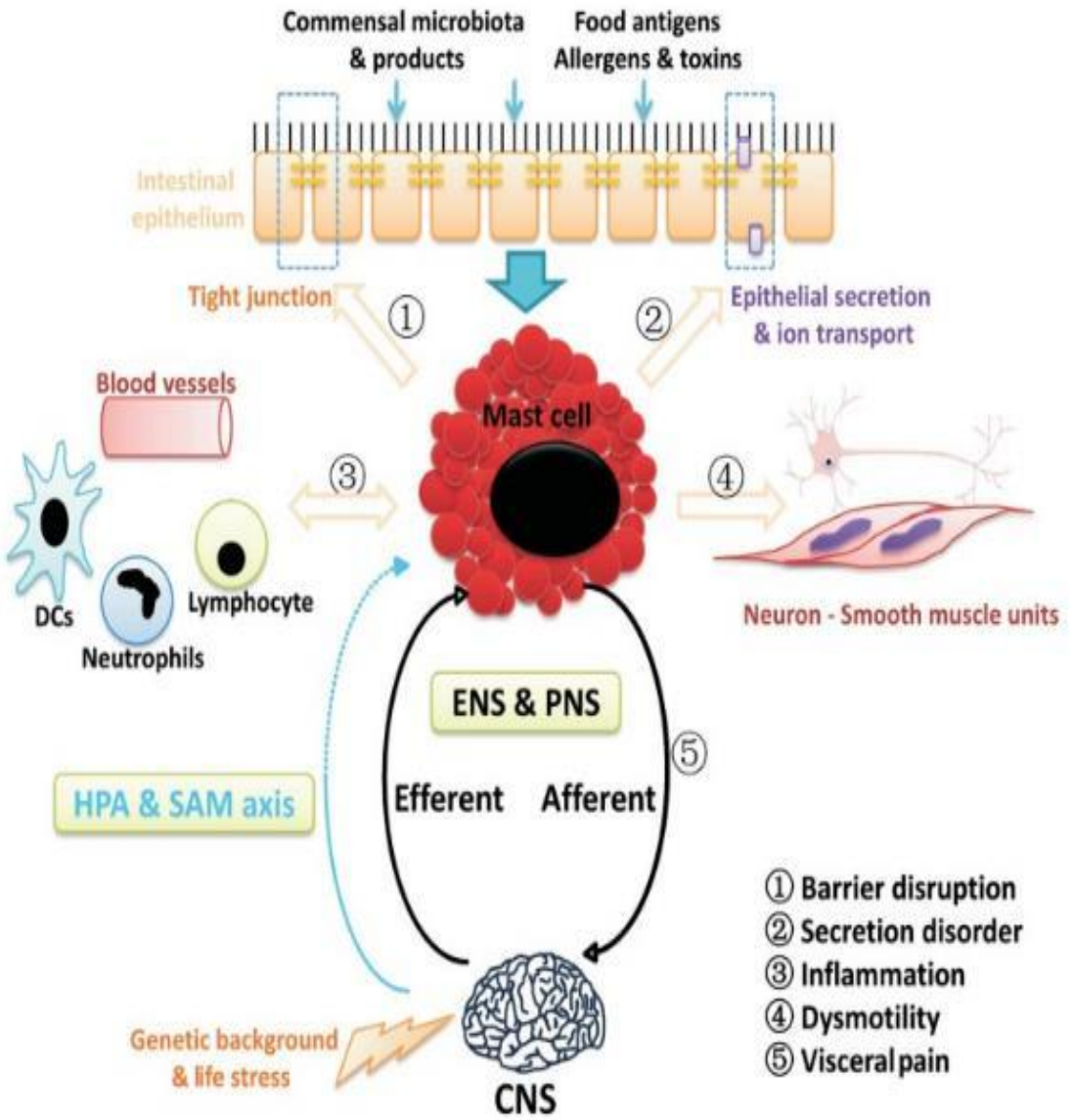
Plavsic, I. et al. (2015). Diagnosis of Irritable Bowel Syndrome: Role of Potential Biomarkers. *Gastroenterology Research & Practice*, Vol. 2015, Article ID 490183.

Inflammation and IBS



NEJM, 2017; 376:2566-78.

Inflammation, Mast Cells, and IBS



Journal of Neurogastroenterology and Motility. 2016;22(2):181-192.

IBS and Inflammation

- Besides mast cells in the mucosa of IBS pts, other kinds of inflammatory cells can be found, including T-lymphocytes
- Another finding that supports immune activation in IBS is the increased number of pro-inflammatory cytokines in the serum of IBS pts *(IL-6 and IL-8 in certain IBS subgroups)*
- One etiology in 10% of pts: post-infectious low-grade inflammation related to genetic polymorphism

Plavsic, I. et al. (2015). Diagnosis of Irritable Bowel Syndrome: Role of Potential Biomarkers. Gastroenterology Research & Practice, Vol. 2015, Article ID 490183.

IBS
INITIAL WORKUP

- CBC, CRP, celiac serology
- Fecal occult blood, calprotectin
- Breath test (lactulose) for hydrogen/methane/hydrogen sulfide
- Lactulose/mannitol test
- Gut microbiome by DNA sequencing (shotgun metagenomic profiling)

IBS: Potential Biomarkers

- Fecal short-chain fatty acids (butyrate, acetate, propionate)
- Fecal pancreatic elastase (marker for insufficiency)
- Antibodies to
  - Cytolethal distending toxin B factor) (pathogenic bacterial virulence
  - Vinculin (cross reaction with CdtB Ab): nerve cell protein
- Zonulin: fecal vs serum zonulin antibodies ??

Assessment of Anti-vinculin and Anti-cytolethal Distending Toxin B Antibodies in Subtypes of Irritable Bowel Syndrome, Dig Dis Sci, 2017 Jun;62(6):1480-1485

53 year old female with persistent loose stools/diarrhea x several years (normal colonoscopy)

Antibody	Reference Range	Patient Value
	Optical Density*	
Cytolethal distending toxin B (CdtB)	0-2.80	**2.018**
Vinculin	0-1.68	**2.742**

*Optical Density (OD) values are corrected for nonspecific binding and cross-reactivity within the patient sample.

IFM'S FIVE "R" STRATEGY FOR HEALING IBS

- **Remove:** Potential pathogens, triggering foods (lactose, wheat/gluten, FODMAPs), excess bile
- **Replace:** Enzymes (pancreatic and plant-based), betaine HCL/pepsin, butyrate
- **Reinoculate:** Prebiotics, probiotics, fecal transplants
- **Repair:** Gut lining/reduce hyperpermeability, decrease inflammation
- **Rebalance:** Liberalize diet; stress management

Improved gastrointestinal health for irritable bowel syndrome with metagenome-guided interventions

88 individuals with IBS (multiple subtypes)

Obtained baseline gut metagenomic sequencing

Targeted interventions included dietary, supplement, prebiotic/probiotic, and lifestyle recommendations for a 30-day period

Follow-up gut microbiome sequencing performed

***Meydan, et al, Precision Clinical Medicine*, 3(2), 2020, 136–146**

Improved gastrointestinal health for irritable bowel syndrome with metagenome-guided interventions

Average baseline IBS symptom score: 160

Average endpoint IBS symptom score: 100.9

Mixed IBS subtype showed most significant reduction in symptom scores across different subtypes

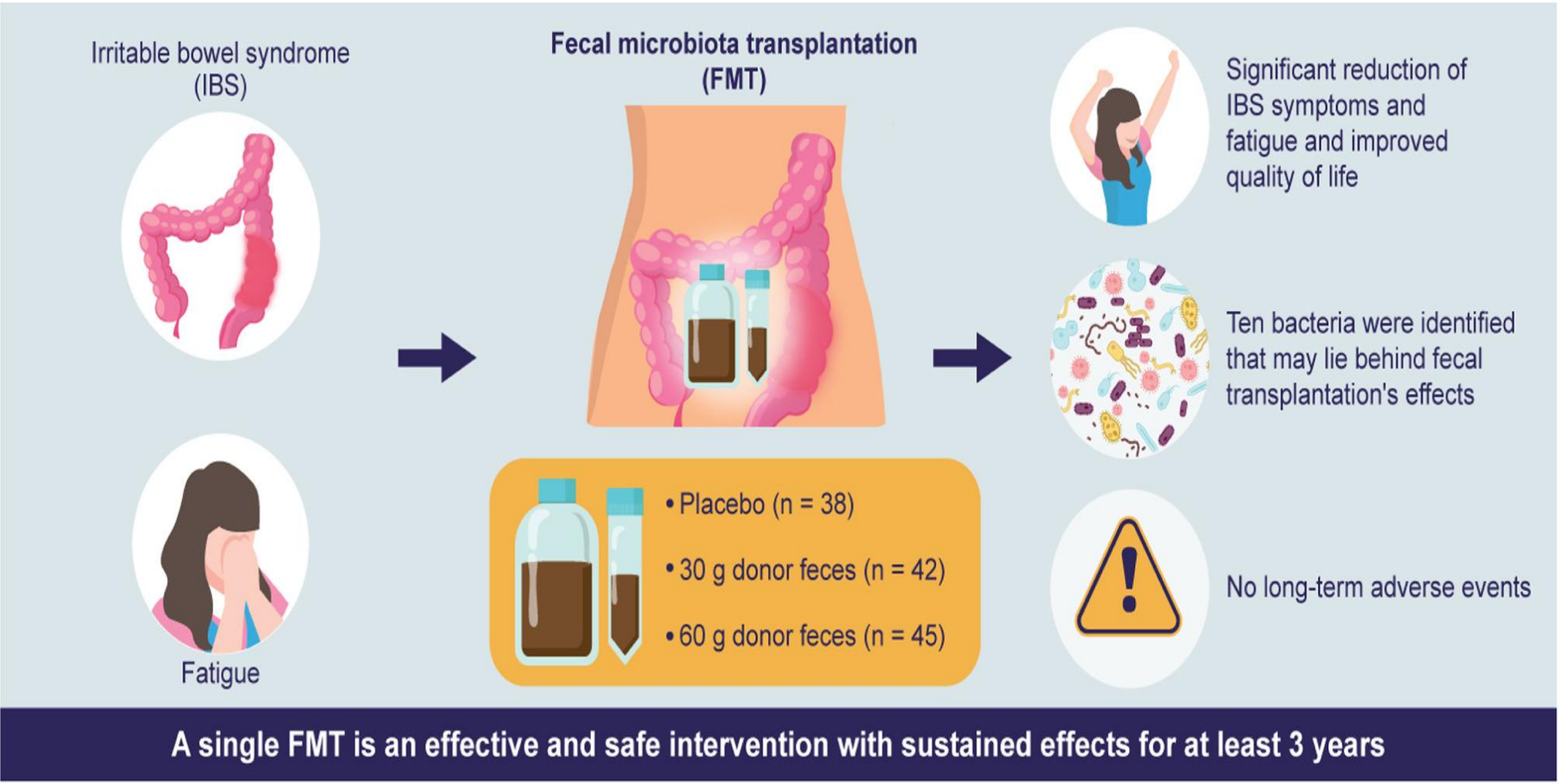
Metagenomics analysis revealed post-intervention shifts in the microbiome that have been cross-validated with the literature as being associated with improvement of IBS symptoms

***Meydan, et al, Precision Clinical Medicine*, 3(2), 2020, 136–146**

EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION FOR PATIENTS WITH IRRITABLE BOWEL SYNDROME AT 3 YEARS AFTER TRANSPLANTATION

- 125 patients with IBS (IBS-SSS >175)
- 38 in a placebo group (own feces), 42 who received (single duodenal dose) 30 g of donor feces, and 45 who received 60 g of donor feces
- Superdonor: 36 y/o male with high microbial diversity
- Response rates
  - 2 yrs post FMT: 26.3%, 69.1%, and 77.8% in the placebo, 30-g, and 60-g groups, respectively
  - 3 yrs post PMT: 27.0%, 64.9%, and 71.8%, respectively

El-Salhy, et al, Gastroenterology 2022;163:982–994



Gastroenterology

Gastroenterology 2022 163982-994.e14DOI: (10.1053/j.gastro.2022.06.020)

EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION FOR PATIENTS WITH IRRITABLE BOWEL SYNDROME AT 3 YEARS AFTER TRANSPLANTATION

- Increases in 6 bacteria: *Alistipes, Bacteroides* spp, *Prevotella* spp, *Parabacteroides johnsonii*, Firmicutes, *Eubacterium biforme*, and *Faecalibacterium prausnitzii*
- Decreases in 3 bacteria: *Coprobacillus cateniformis*, *Streptococcus salivarius* spp *thermophilus*, and *Enterobacteriaceae*

El-Salhy, et al, Gastroenterology 2022;163:982–994

"It seems that bacteria in the small intestine play a more central role in IBS, as well as its associated fatigue, than bacteria in the large intestine… Until now, we've been targeting the wrong part of the intestine."

Magdy El-Salhy, MD,
University of Bergen, Bergen, Norway

Quote from Medscape Medical News, Oct 14, 2022

BACILLUS COAGULANS MTCC 5856 SUPPLEMENTATION IN THE MANAGEMENT OF DIARRHEA PREDOMINANT IRRITABLE BOWEL SYNDROME

A DOUBLE-BLIND RANDOMIZED PLACEBO CONTROLLED PILOT CLINICAL STUDY

- 18 patients with IBS-D received *B coagulans*, 2 billion CFUs, for 90 days (vs 18 in placebo group)
- There was a significant decrease in the clinical symptoms like bloating, vomiting, diarrhea, abdominal pain and stool frequency in a patient group receiving *B. coagulans* MTCC 5856 when compared to placebo group ($p < 0.01$)
- Similarly, disease severity also decreased and the quality of life increased in the patient group receiving *B. coagulans*

Nutrition Journal (2016) 15:21

EFFICACY OF PROBIOTICS FOR IRRITABLE BOWEL SYNDROME: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

- Studies regarding the treatment of IBS with probiotics have not yielded consistent results, and the best probiotics has not yet been confirmed. Forty-three RCTs, with 5,531 IBS patients, were included in this analysis.
- *Bacillus coagulans* exhibited the highest probability to be the optimal probiotic species in improving IBS symptom relief rate, as well as global symptom, abdominal pain, bloating, and straining scores. The efficacy of *B.coagulans* for 8 weeks ranked first both in improving the abdominal pain and straining scores. Additionally, *B. coagulans* still had significant efficacy compared to different types of probiotic combinations in this study
- *Lactobacillus plantarum* ranked first in ameliorating the QOL of IBS patients, but without any significant differences compared with other probiotic species in standardized mean differences (SMD) estimates.
- Patients receiving *L. acidophilus* had lowest incidence of adverse events

Front Cell Infect Microbiol, 2022 Apr 1;12:859967

IBS
SUMMARY

- IBS is a multifactorial group of disorders that can be united by a systems biology approach
- Genetic testing for IBS predisposition holds promise; interpretation remains complex (a role for AI)
- Stool microbiome testing via shotgun metagenomics has high potential for ruling out inflammatory pathogens, identifying low diversity/ dysbiosis, and presence of fermenting organisms
- Future horizon: analysis of mycobiome & virome
- Breath testing: results guide interventions (e.g., low FODMAPs)

IBS
SUMMARY

- Low-FODMAP diets may be helpful for identifying problematic foods in >50% of individuals; umbrella approach to avoidance should not be used long term
- Wheat and dairy free diets should be attempted in all clients with IBS
- Brush border enzyme deficiencies may be more common than realized: digestive enzymes, esp. containing alpha-galactosidase, etc. are viable options
- *B coagulans, L plantarum*, and other probiotics are worthwhile interventions

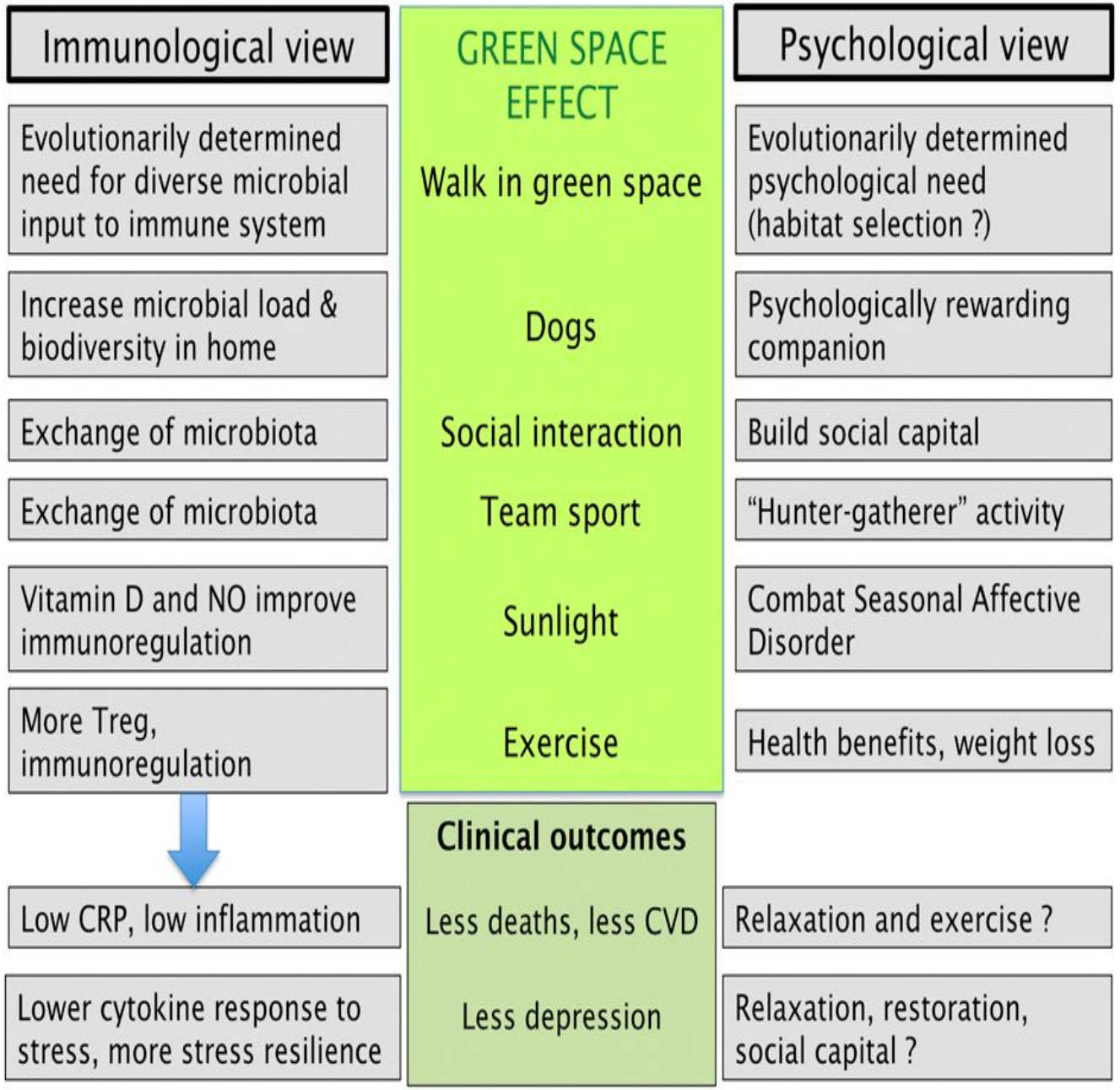
IBS: Summary

With IBS-D, consider testing and/or treating for bile acid malabsorption

Metabolomic testing has potential especially for alterations in SCFA, VOCs

Permeability testing: may clarify pathophysiology

L-glutamine and partially hydrolyzed guar gum are evidence-based interventions



Rook GA. PNAS. 2013;110(46):18360–18367