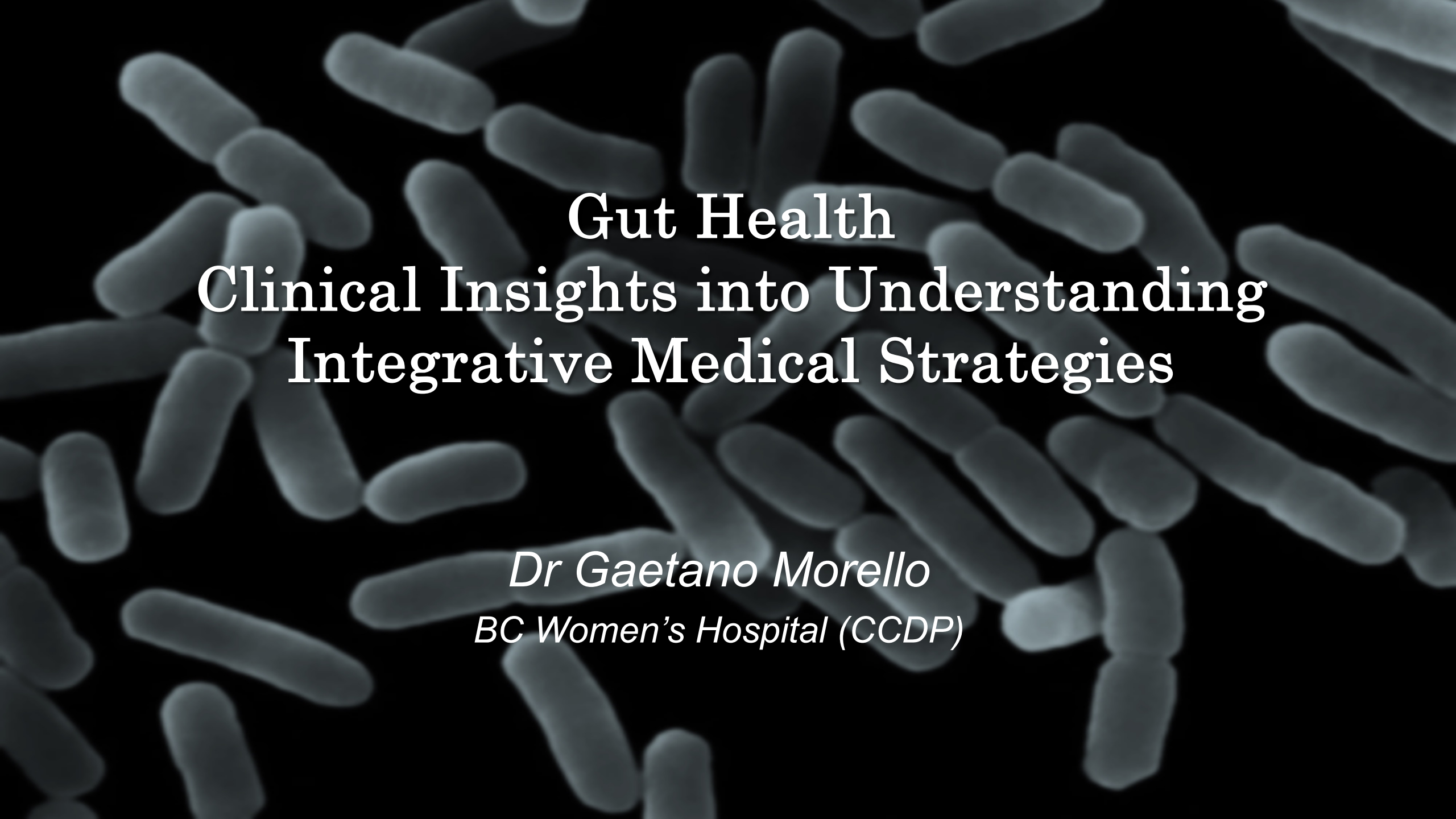


Gut Health
Clinical Insights into Understanding Integrative Medical Strategies

Dr Gaetano Morello

BC Women's Hospital (CCDP)

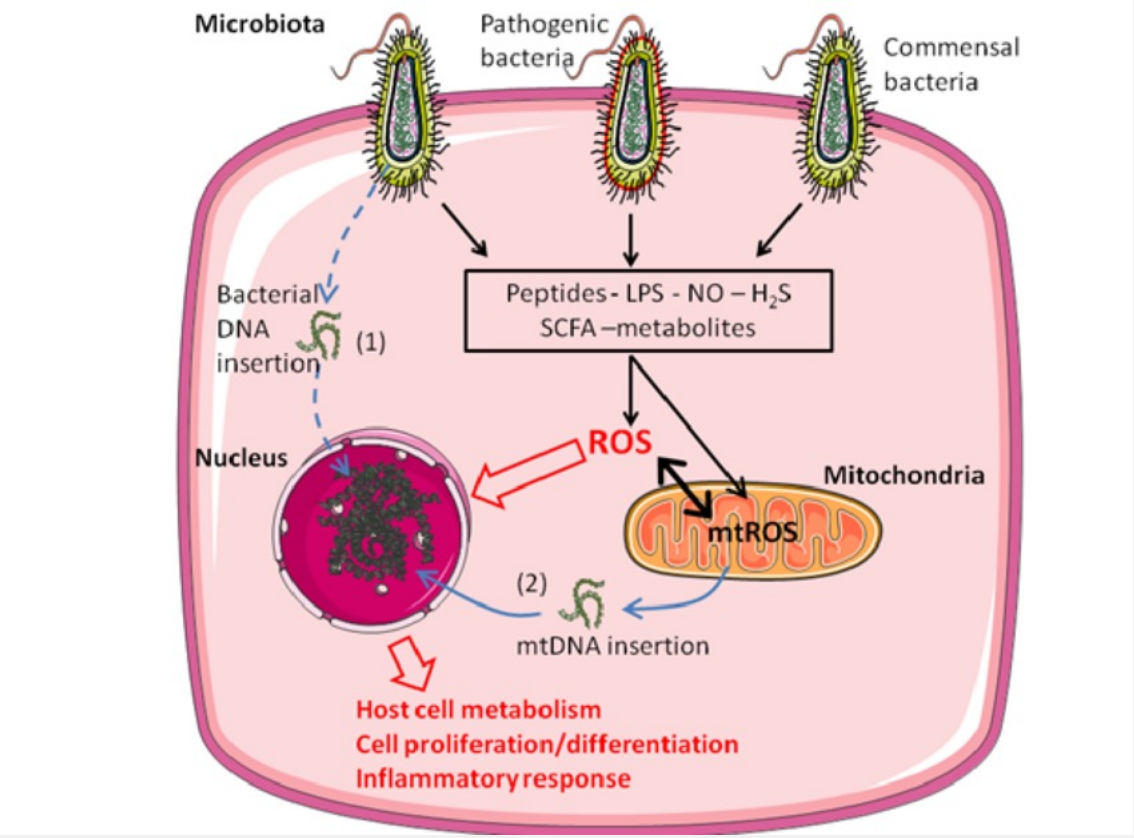
DR. GAETANO MORELLO
BACKGROUND

- Complex Chronic Disease Program at BC Women's Hospital and Health Center in Vancouver
- University of British Columbia, BSc in Cell Biology, Bastyr University graduate 1991
- ME/CFS, FM, Chronic Lyme, Long COVID
- Referral system
- We do direct clinical care and involved in the facilitation of research
- We also have an ongoing educational resource for patients
- What we've been seeing is lots of correlations with Post COVID-19 symptomology.
- I'll be sharing with you my clinical experience

THE GUT IS NOT VEGAS

What happens in the gut, absolutely does NOT stay in the gut!

PRELUDE



Microbiota -mitochondria intertalk : Commensal and pathogenic bacteria release factors that promote or decrease the mitochondrial activity and the subsequent cellular ROS concentration. High ROS production, due to unbalanced release of microbiotal factors, is able to trigger cell proliferation or differentiation, as well as an inflammatory response. Moreover, it can also promote mitochondrial biogenesis in case of mitochondrial fragmentation. Furthermore, microbiota can trigger mitochondrial and bacterial DNA insertion in the nuclear genome leading to alteration of cellular gene expression. (1) Arrow 1: Bacterial DNA insertion into the nucleus. (2) Arrow 2: mitochondrial DNA insertion into the nucleus.

MITOCHONDRIAL STRUCTURAL ALTERATIONS IN FIBROMYALGIA - APILOT ELECTRON

MICROSCOPY STUDY

Linoy Israel, Victoria Furer, Atan Gross, Jacob Ablin

Weizmann Institute of Science &

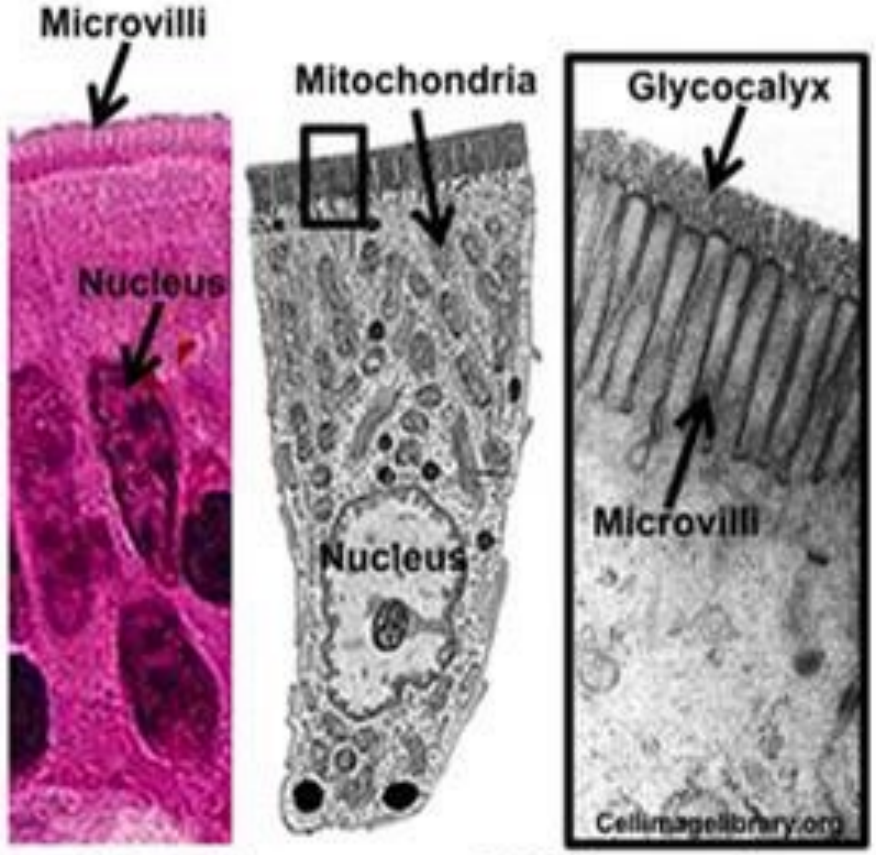
Tel Aviv Sourasky Medical Center

Israel

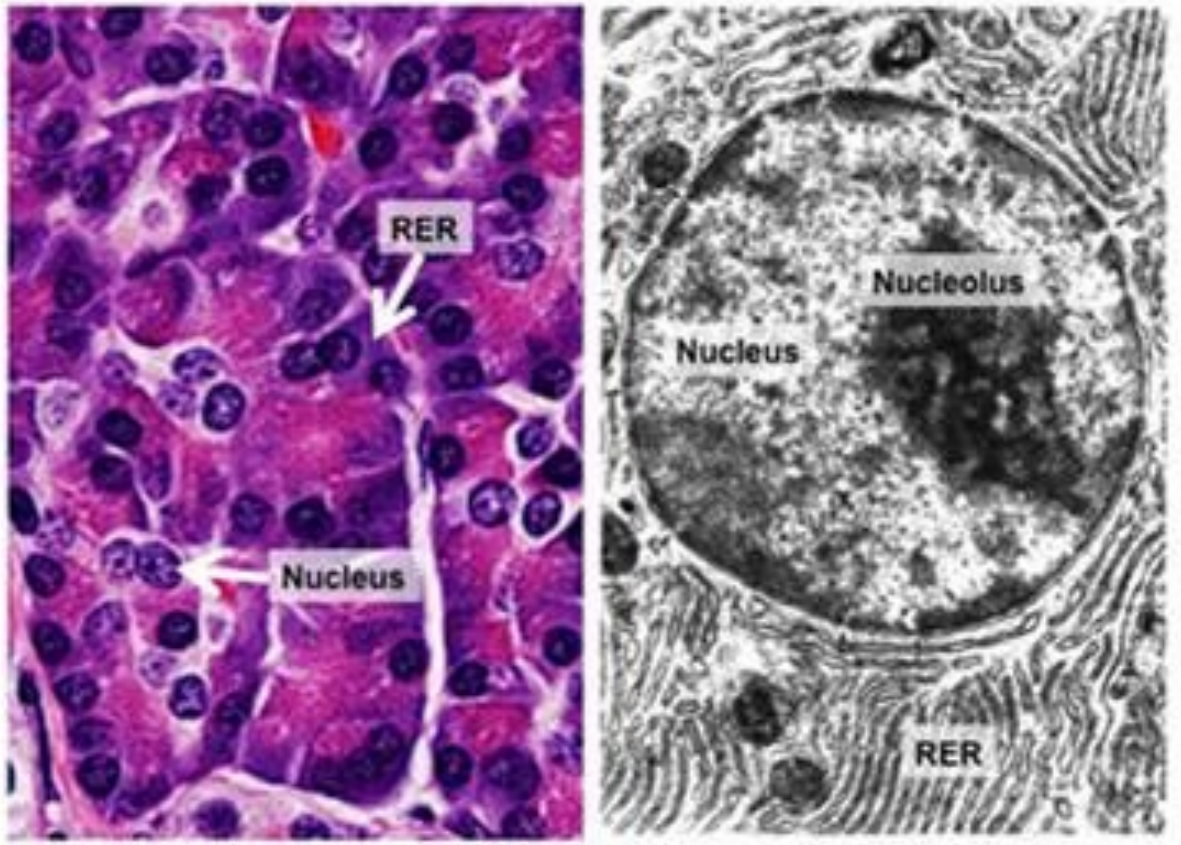
TEM

Transmission Electron Microscopy

- **Definition:** Transmission Electron Microscopy (TEM) - a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image
- Allows visualization of structures at the **atomic** or **molecular** level, offering much higher resolution than light microscopy
- **How It Works:** An electron gun emits a high-energy beam of electrons that passes through an ultra-thin specimen
- **Electromagnetic lenses** focus and magnify the beam
- The interaction of the electrons with the specimen creates an **image** that is projected onto a detector or photographic plate

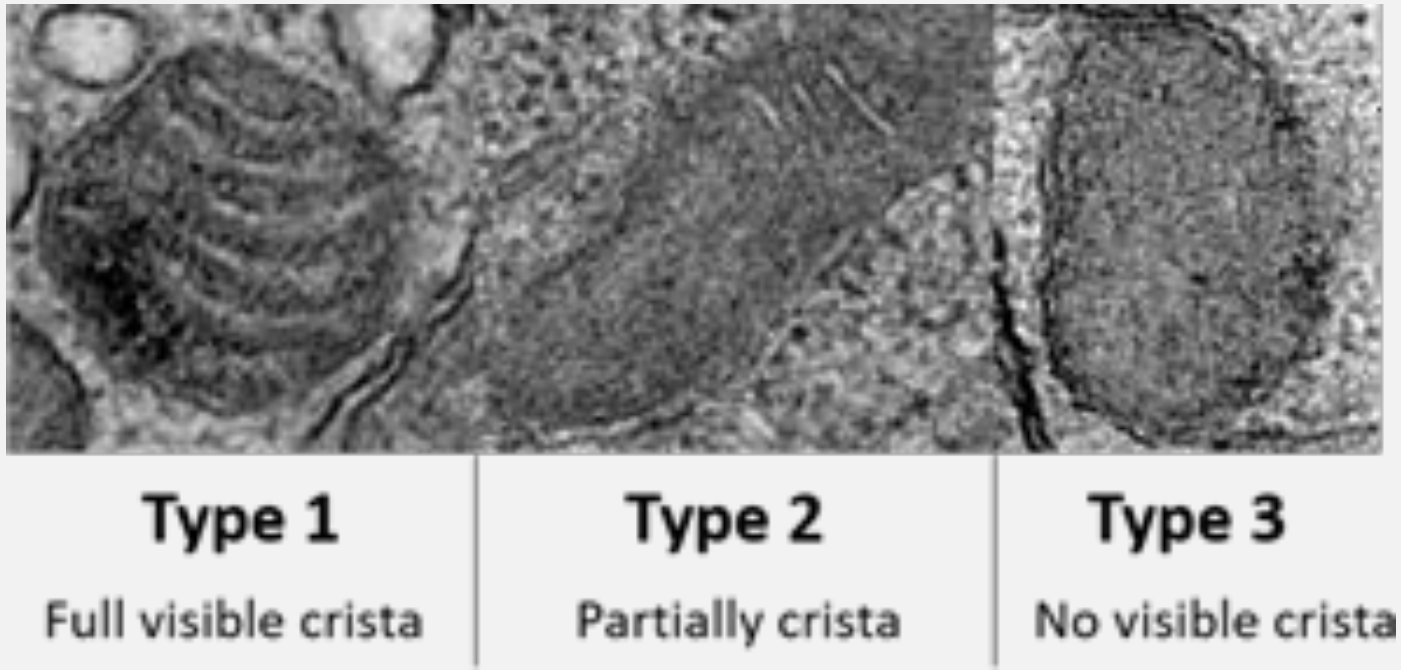


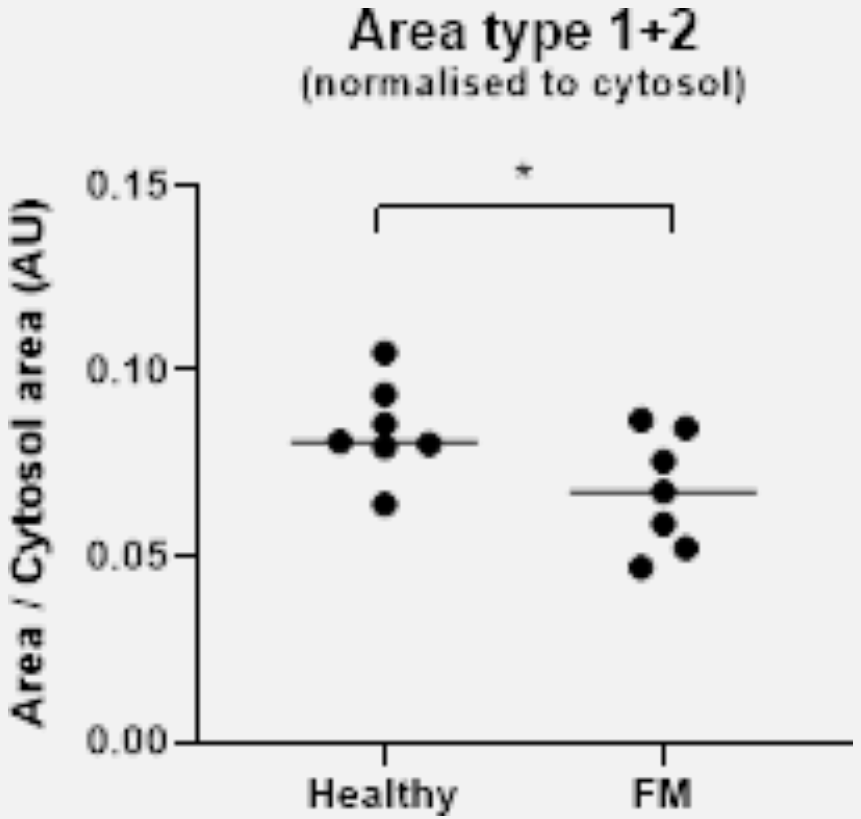
Light microscope vs. TEM images of intestinal epithelial cells



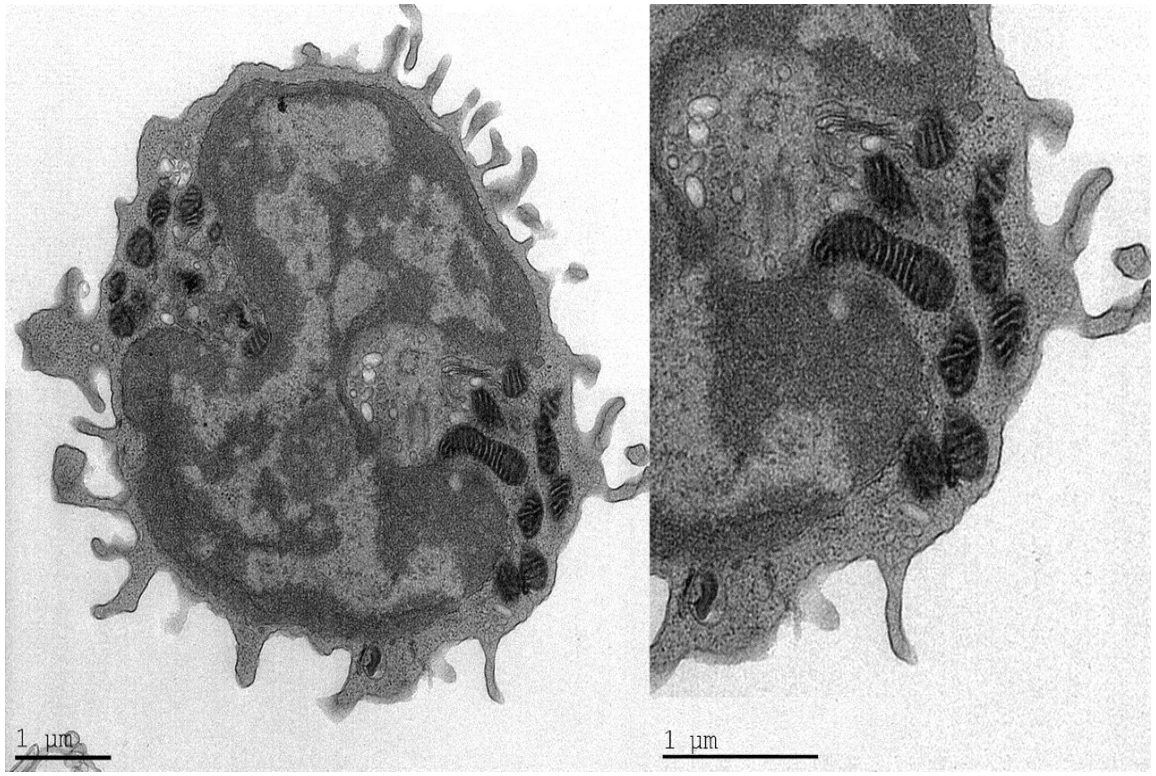
Light microscope vs. TEM images of pancreatic epithelial cells

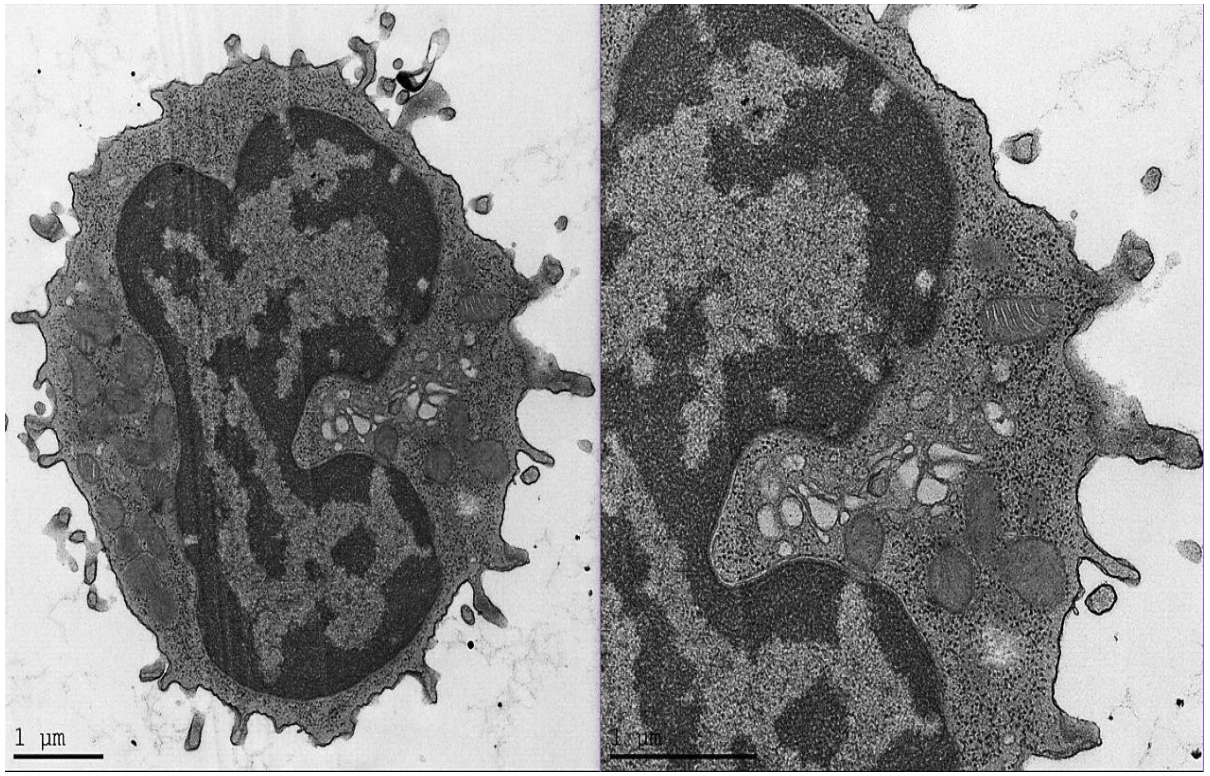
TEM analysis of structural changes in PBMCs (peripheral blood mononuclear cells) mitochondria from FMS patients versus healthy controls





PBMCS FOR HEALTHY CONTROLS DEMONSTRATING NORMAL CRISTA MORPHOLOGY VS FM PATIENTS' MITOCHONDRIA



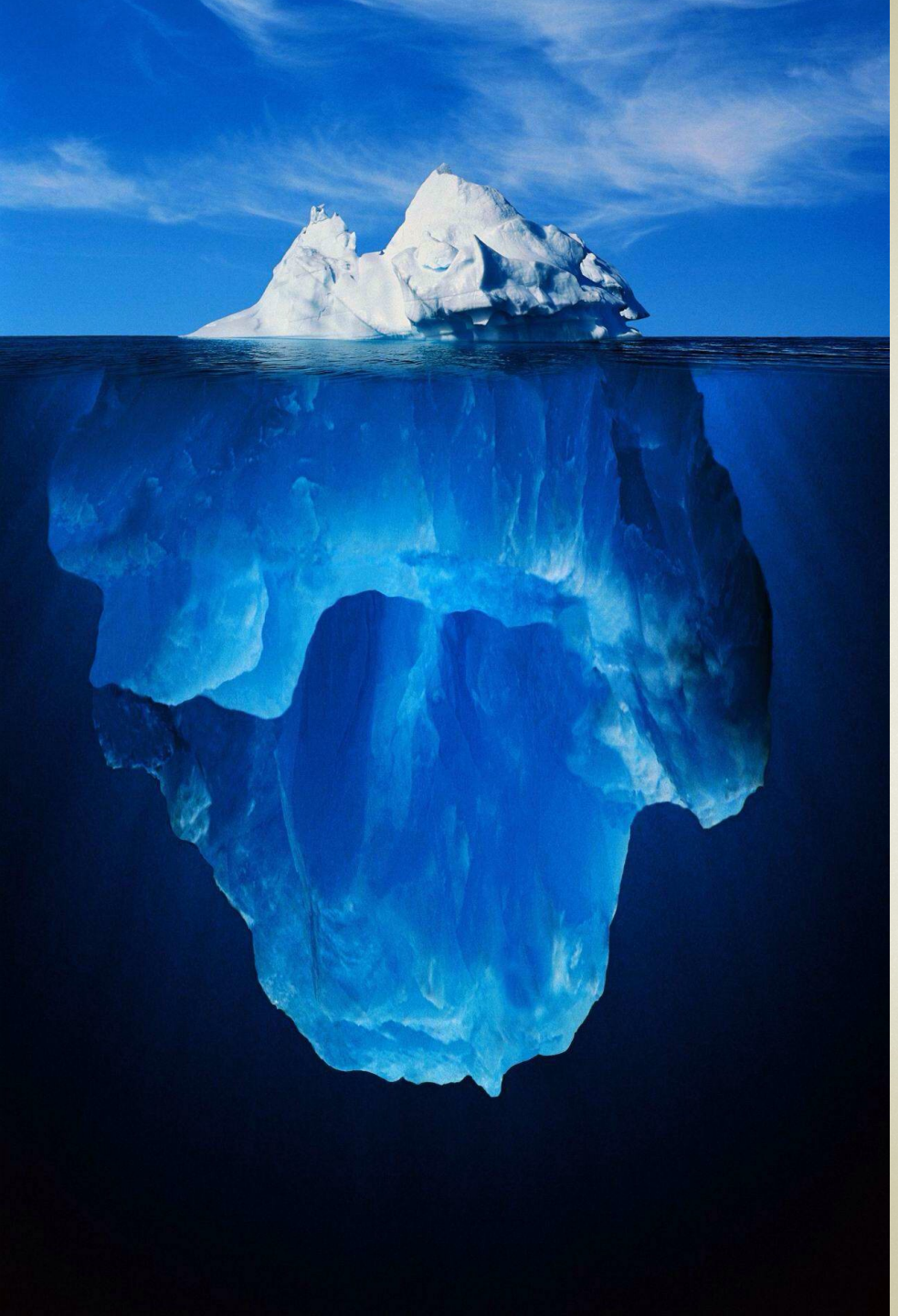


NEW HOPE FOR FM PATIENTS WITH FTM

- January 16, 2024 – Groundbreaking research led by Dr. Amir Minerbi, FM patients MD/PhD, treatment-resistant severe fibromyalgia participated in this novel study. The microbiomes of healthy research subjects were given to fibromyalgia patients via an ingestible capsule. Eleven study participants reported less pain, fatigue, and memory disturbances, surpassing the effects of prior treatments. This improvement continued for months, and the patients returned to work, studies, and their usual lifestyles.

PRESENTATION

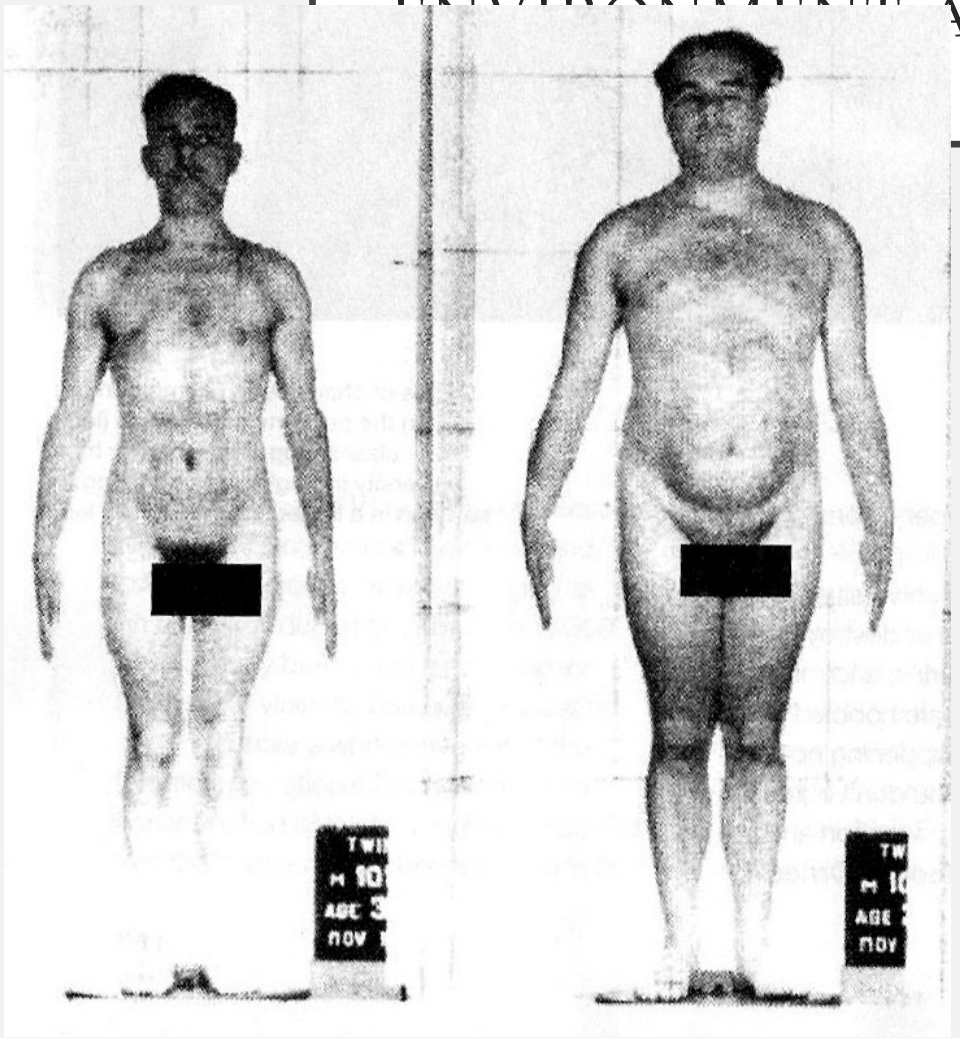
1. Background Microbiome Influence
2. The Factors
3. The gut what you may not know
4. Relationship between gut and human health
5. Histamine and the gut
6. Hormesis
7. Understanding SIBO
8. Therapeutic Approach
9. Probiotics a different view
10. Dietary considerations
11. Questions

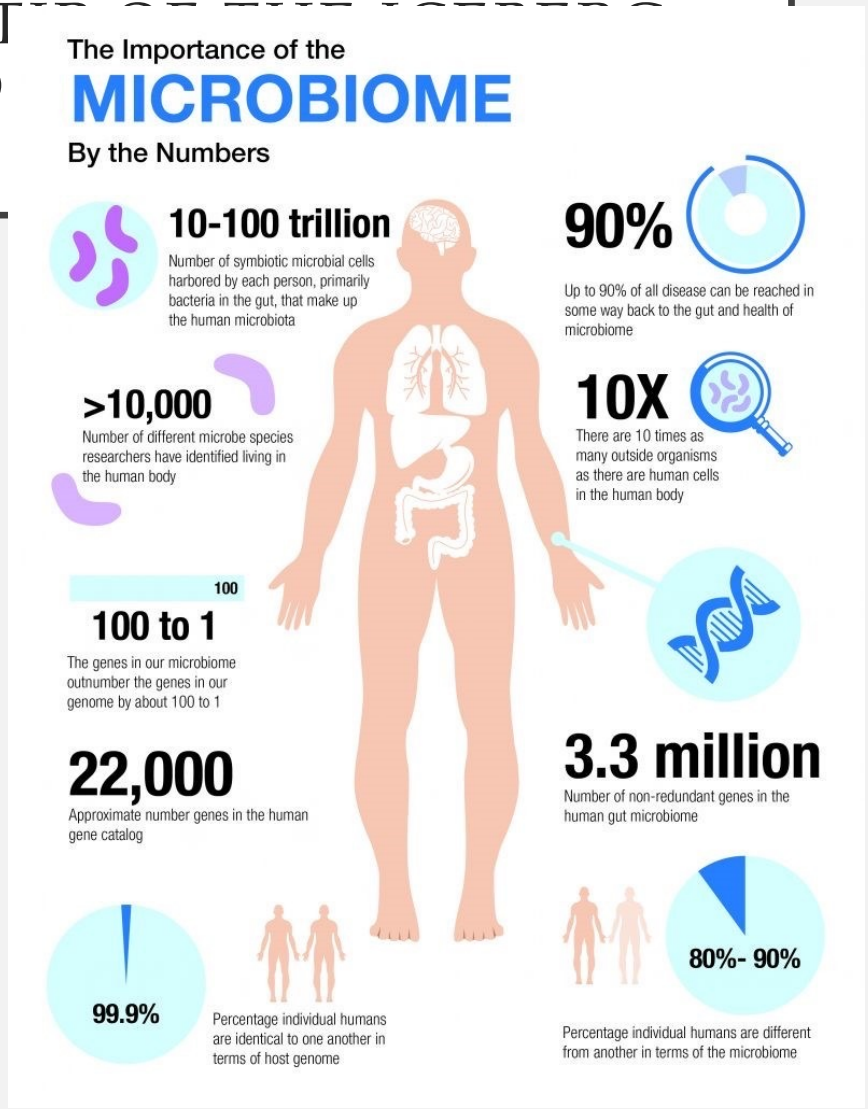


BACKGROUND

- The human body has an estimated 100 trillion bacteria
- Known as the microbiome
- Bacteria outnumber our cell count, for every one of our cells there may be 10 bacteria
- SYMBIOTIC relationship keeps less desirable bacteria at bay
- VITAL role in body – without them we could not survive
- Bioavailability may not be as important as we once thought?
- Signaling pathways and new discoveries monthly

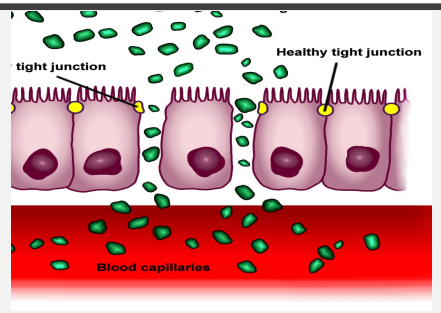
UNDERSTANDING THE T
ENVIRONMENT AND D





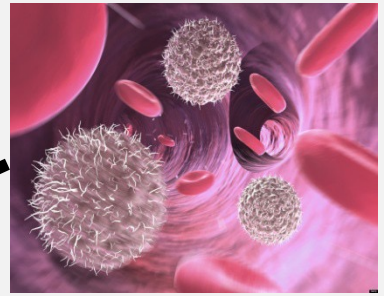
AN OVERVIEW OF FACTORS

TO CHRONIC INFLAMMATORY DISEASES

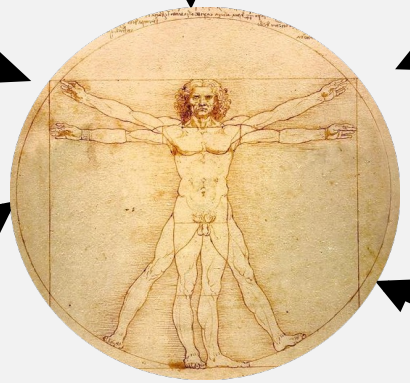


Increased Gut Permeability

Human Genome

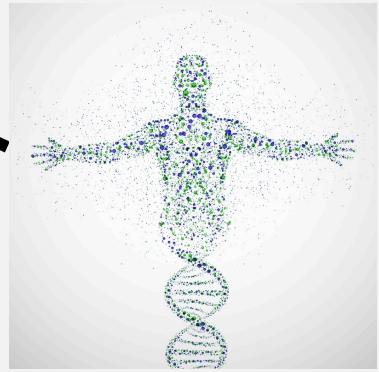


Immune Response



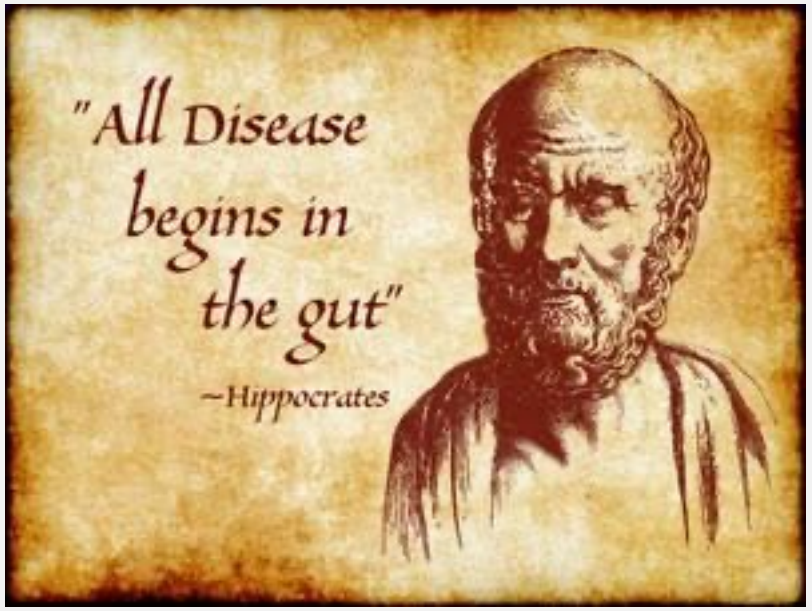
Environment /Stress (Epigonome)

Clinical Outcome



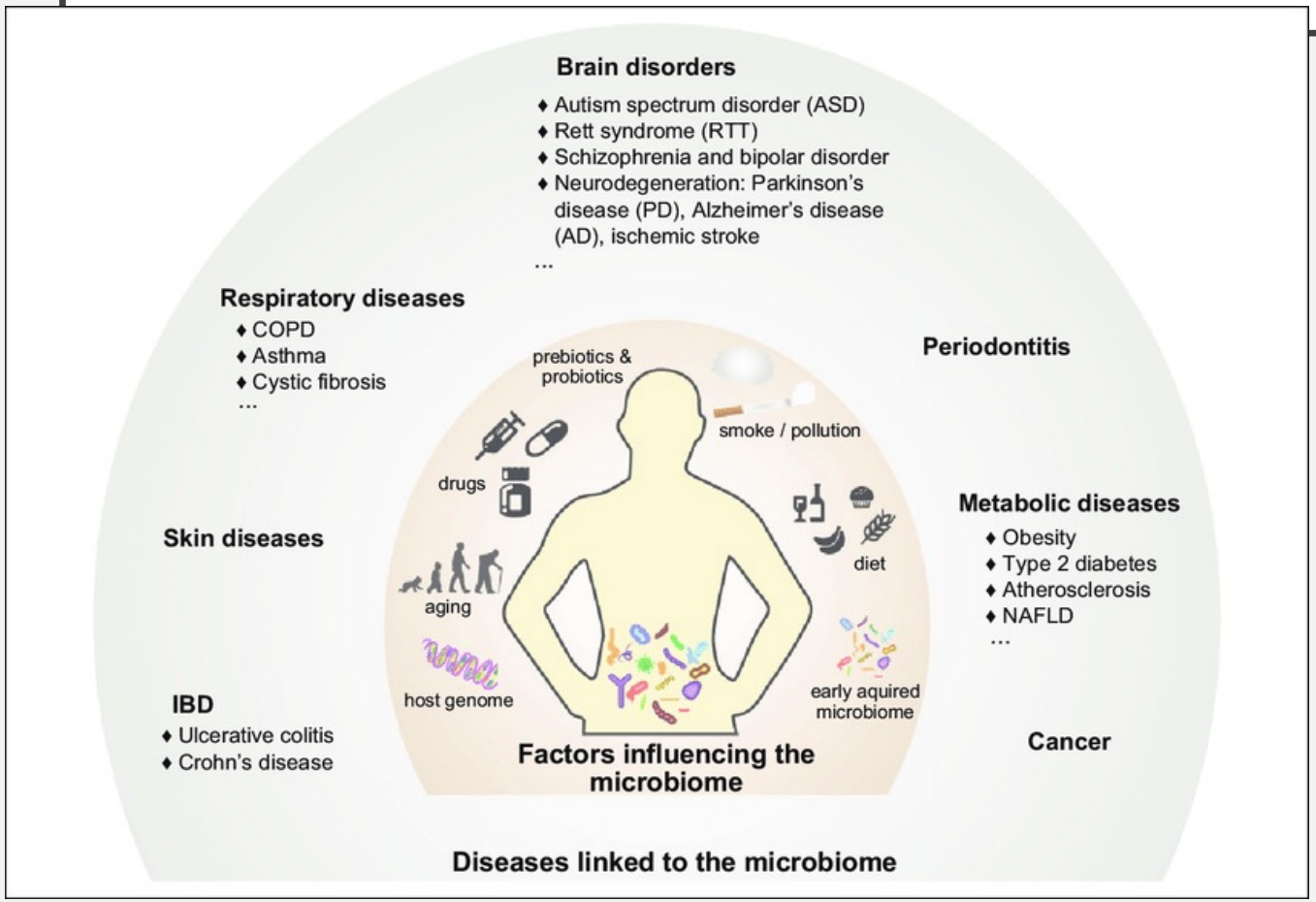
Microbiome

STARTED LONG AGO



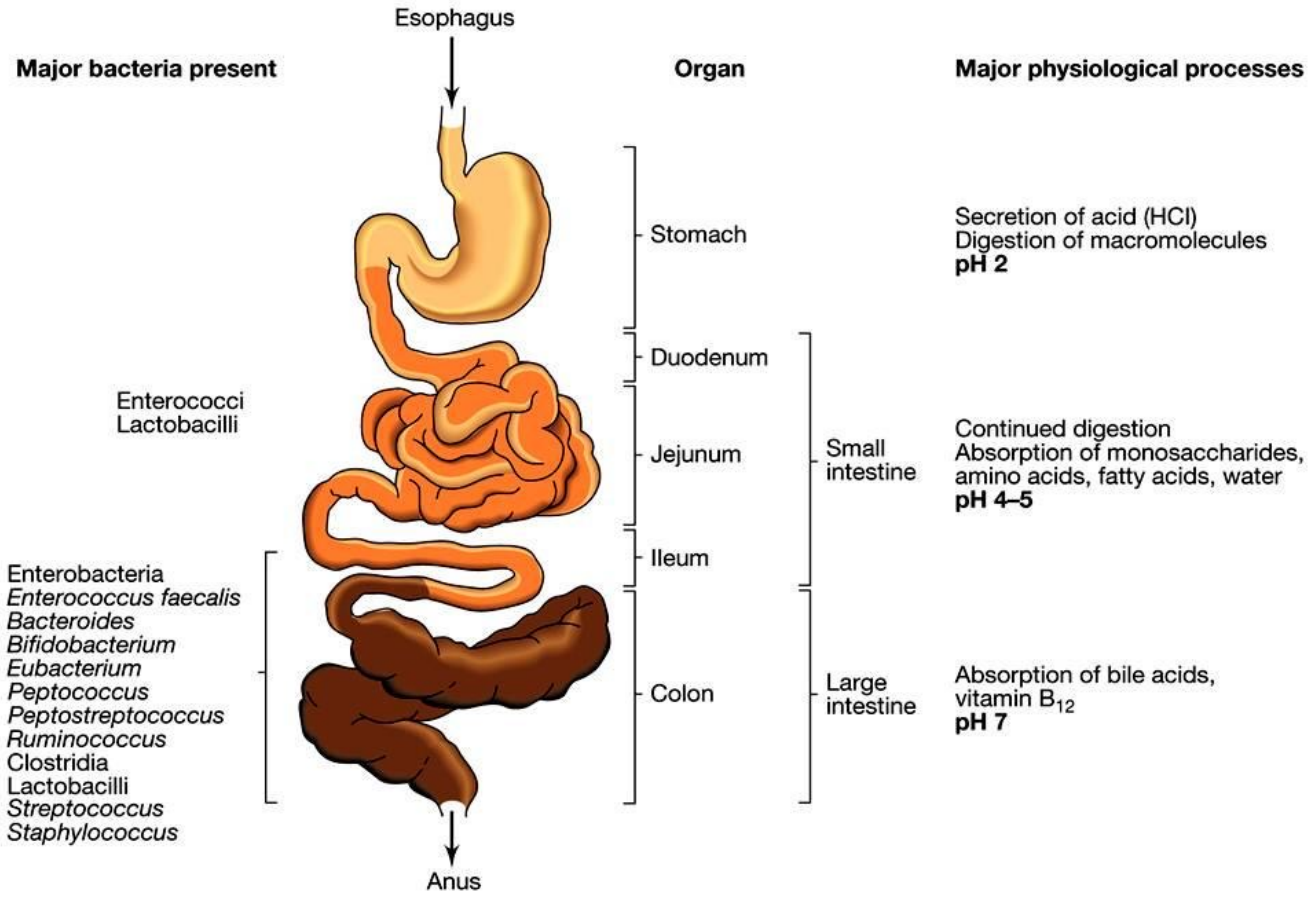
Today we can almost say
ALL HEALTH BEGINS IN THE GUT

DISEASES LINKED TO THE MICROBIOME

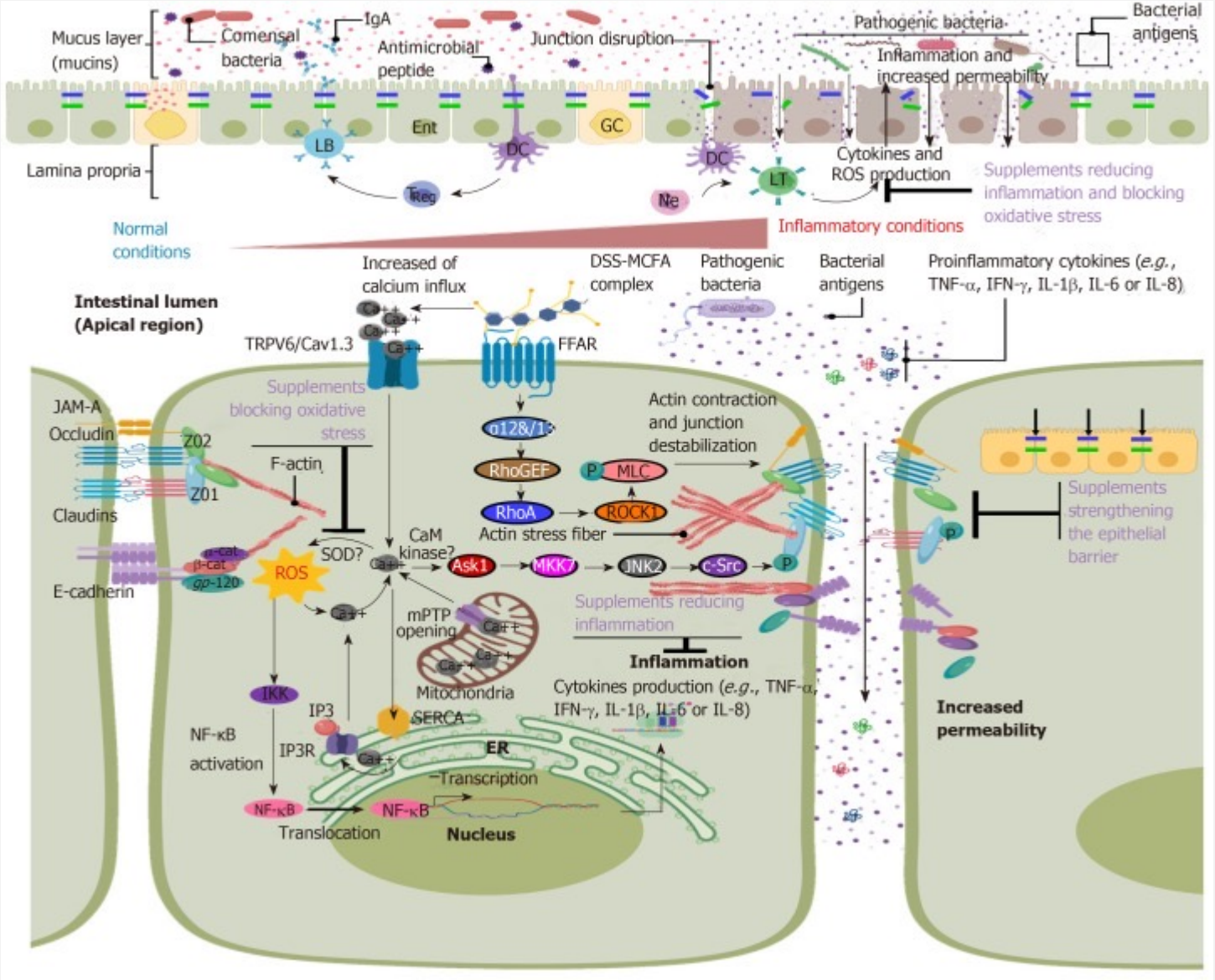


THE GASTROINTESTINAL TRACT

A TRIP DOWN THE GI TRACT



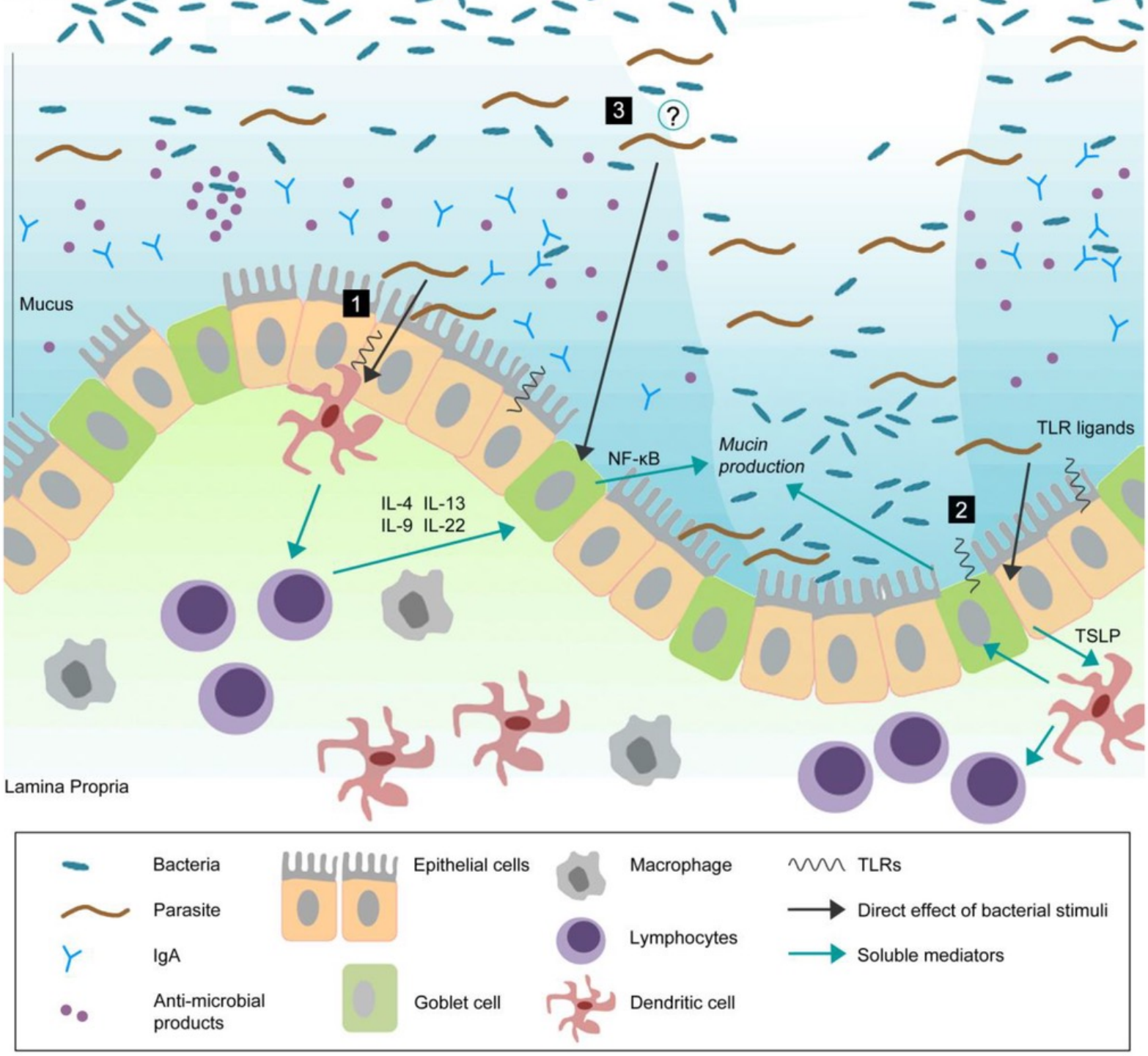
One Cell Paradox-Loss of Mucosal Immune Homeostasis



Mechanisms causing intestinal epithelial barrier dysfunction that can be counteracted by nutritional supplements. The epithelial barrier is comprised of a mucus layer, an epithelial monolayer and the mucosa containing resident immune cells. It is a stable, tightly regulated barrier under basal conditions (top, left). Under inflammatory conditions, this barrier becomes compromised with a diminished mucus layer, disrupted epithelial monolayer and recruitment of many immune cells including neutrophils (top, right). The mechanisms causing loss of barrier integrity are summarized below. The apical junctional complex, built by tight and adherens junctions, controls epithelial permeability and maintains intestinal homeostasis. During inflammation, as seen in inflammatory bowel diseases or induced in rodents by dextran sulphate sodium or 2,4,6-trinitrobenzene sulfonic acid treatment, junctions are disrupted. The depicted inflammatory pathways ultimately lead to the disruption of tight and adherens junctions allowing for bacterial translocation that further triggers inflammation. For example, oxidative stress promotes activation and nuclear translocation of the transcription factor nuclear factor-κB and increased expression of proinflammatory cytokines. Dextran sulphate sodium treatment also promotes the activation of the RhoA pathway leading to actomyosin contraction, which contributes to junction destabilization, opening of the paracellular space and thus hyperpermeability. Nutritional supplements can alleviate colitis signs including inflammation, oxidative stress and junction disruption as indicated (compare Tables Tables11 and and2) 2). DC: Dendritic cells; Ne: Neutrophils; LT: T-lymphocytes; LB: B-lymphocytes; DSS: Dextran sulphate sodium; NF-κB: Nuclear factor-κB; TNF-α: Tumor necrosis factor-α; JAM-A: Junctional adhesion molecule-A; ZO-1: Zonula occludens-1; ROS: Reactive oxygen species; IFN-γ: Interferon-γ; ER: Endoplasmic reticulum.

GOBLET CELLS

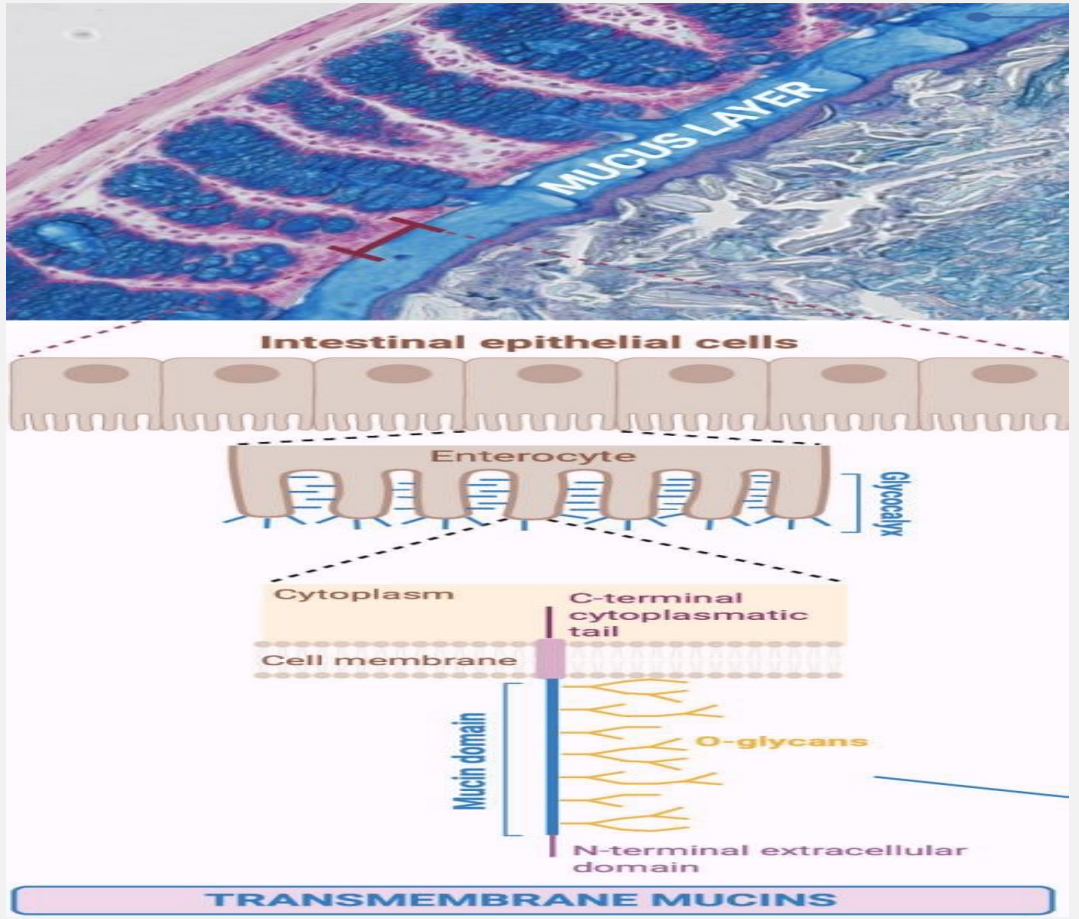
- Goblet cells reside throughout the gastrointestinal (GI) tract and are responsible for the production and preservation of a protective mucus layer by synthesizing and secreting mucins.



Mucus barrier, mucins and gut microbiota: the expected slimy partners?

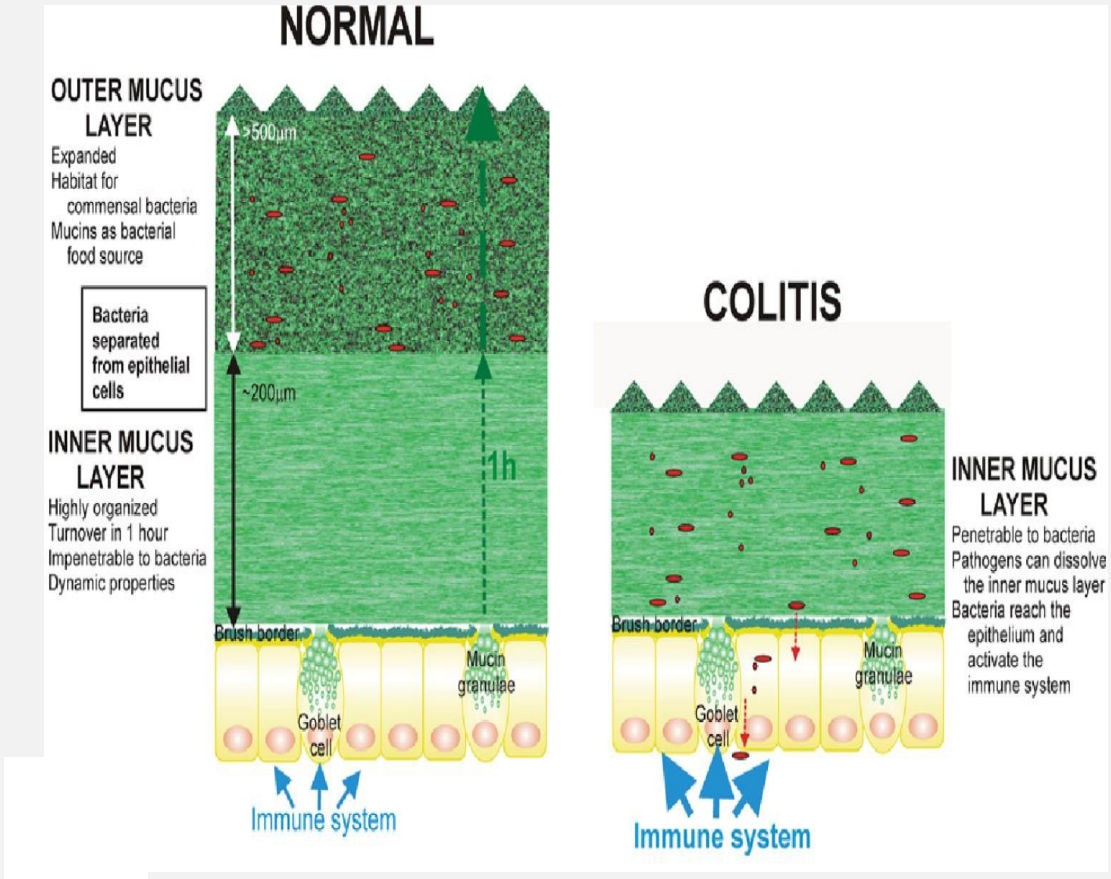
Key messages

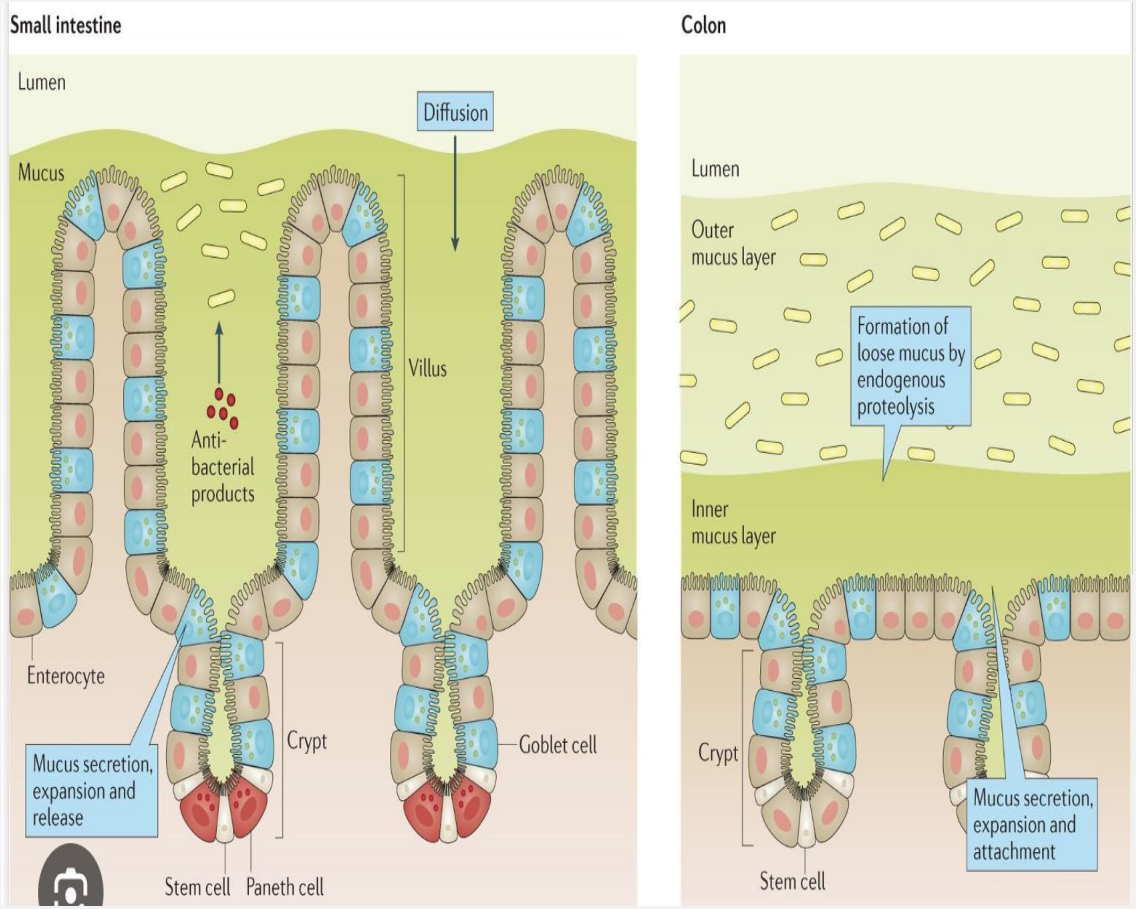
- The gut mucus layer is vital for maintaining intestinal health.
- The regulation of the intestinal mucus barrier and glycobiology are very complex and dynamic systems still poorly understood.
- There is a complex bidirectional interaction between host glycans and gut microbes.
- Gut microbiota composition is an important factor contributing to the regulation of the intestinal mucus barrier function.
- Specific nutrients or potential next-generation beneficial bacteria can be used to prevent, improve or maintain a protective mucus layer.



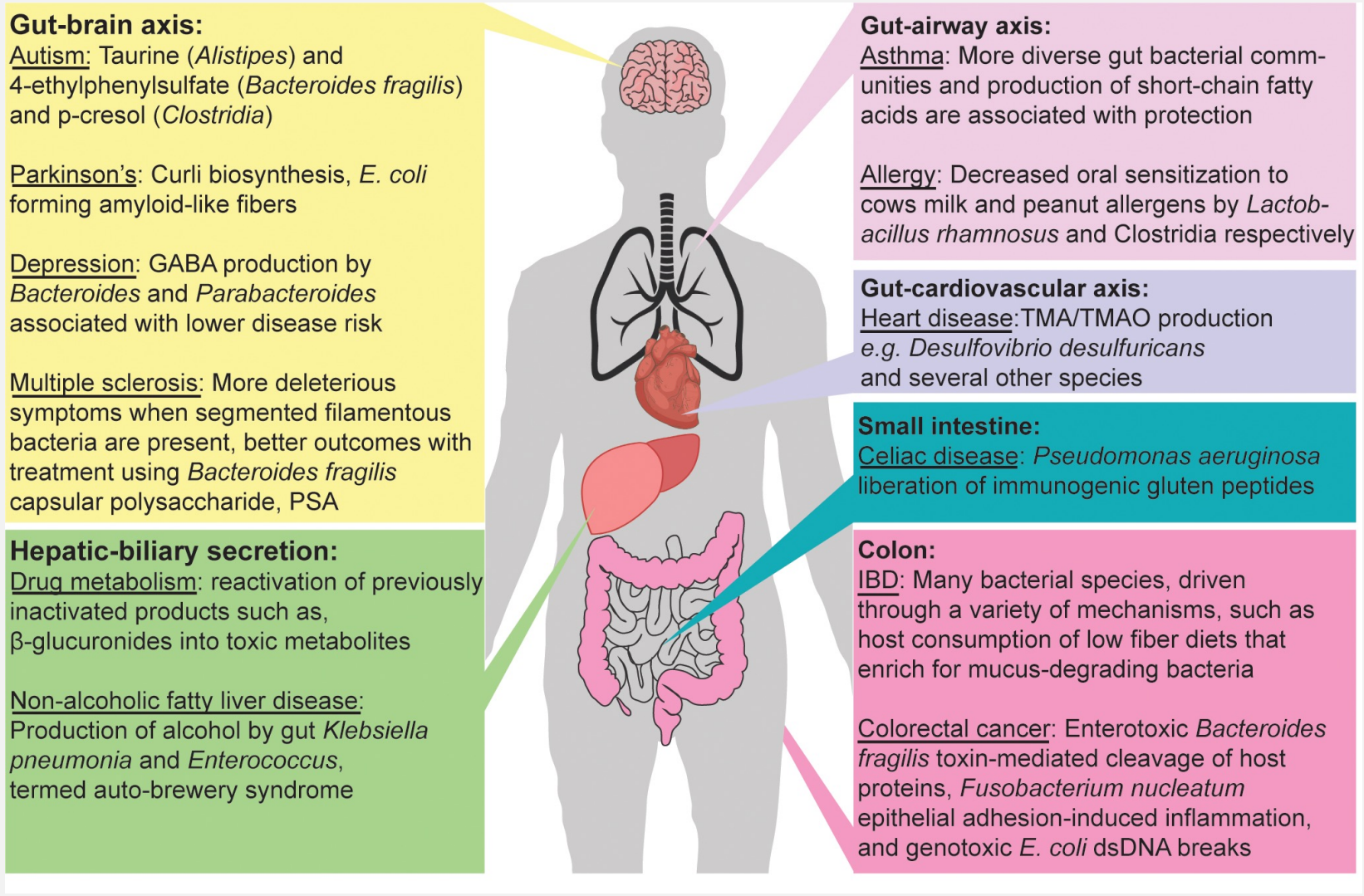
Paone P, Cani PD. *Gut* 2020;**69**:2232–2243. doi:10.1136/gutjnl-2020-322260

MUCIN 1 AND MUCIN 2 LAYERS AND IMMUNE SYSTEM INTERRELATIONSHIP





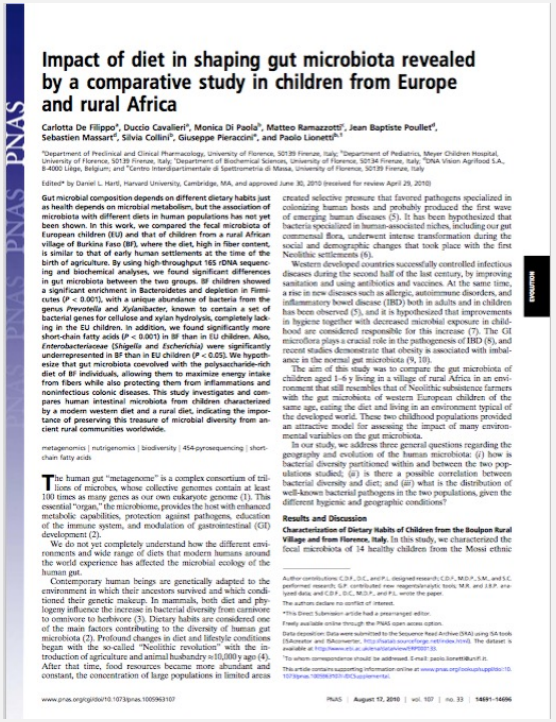
POSSIBLE IMPACT OF MICROBIOME ON HOST HEALTH



Effects of the gut microbiome on host health.
Some of the many known effects of the gut microbiome on diseases of various organ systems are illustrated. References for associations highlighted in the figure that are not mentioned in detail in the main text: autism [21, 33], Parkinson's [64, 65], depression [29], multiple sclerosis [66], drug metabolism [54, 55], nonalcoholic fatty liver disease [67, 68], asthma [69], allergy [70, 71], heart disease [51], Celiac disease [72], IBD [27], and colorectal cancer [73–76]. dsDNA, double stranded DNA; GABA, gamma-aminobutyric acid; *E. coli*, *Escherichia coli*; IBD, inflammatory bowel disease (a collection of several intestinal disorders that includes Crohn's disease and ulcerative colitis); PSA, capsular polysaccharide A from *Bacteroides fragilis*; TMA/TMAO, trimethylamine/ trimethylamine N-oxide.

About 75% of food in the Western diet is of limited or no benefit to the microbiome of the lower gut. Mostly comprised of carbohydrates, which are absorbed in the upper GI tract, what eventually reaches the large intestine is of limited value as this contains only small amounts of the minerals, vitamins, and other nutrients necessary for the maintenance of the microbiota.

Nutrients 2013. 5, 162–207; doi: 10.3390/nu5010162



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[e], and Paolo Lionetti[b,1]

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Edited* by Daniel L. Hartl, Harvard University, Cambridge, MA, and approved June 30, 2010 (received for review April 29, 2010)

Gut microbial composition depends on different dietary habits just as health depends on microbial metabolism, but the association of microbiota with different diets in human populations has not yet been shown. In this work, we compared the fecal microbiota of European children (EU) and that of children from a rural African village of Burkina Faso (BF), where the diet, high in fiber content, is similar to that of early human settlements at the time of the birth of agriculture. By using high-throughput 16S rDNA sequencing and biochemical analyses, we found significant differences in gut microbiota between the two groups. BF children showed a significant enrichment in Bacteroidetes and depletion in Firmicutes ($P < 0.001$), with a unique abundance of bacteria from the genus *Prevotella* and *Xylanibacter*, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, completely lacking in the EU children. In addition, we found significantly more short-chain fatty acids ($P < 0.001$) in BF than in EU children. Also, *Enterobacteriaceae* (*Shigella* and *Escherichia*) were significantly underrepresented in BF than in EU children ($P < 0.05$). We hypothesize that gut microbiota coevolved with the polysaccharide-rich diet of BF individuals, allowing them to maximize energy intake from fibers while also protecting them from inflammations and noninfectious colonic diseases. This study investigates and compares human intestinal microbiota from children characterized by a modern western diet and a rural diet, indicating the importance of preserving this treasure of microbial diversity from ancient rural communities worldwide.

metagenomics | nutrigenomics | biodiversity | 454-pyrosequencing | short-chain fatty acids

The human gut "metagenome" is a complex consortium of trillions of microbes, whose collective genomes contain at least 100 times as many genes as our own eukaryote genome (1). This essential "organ," the microbiome, provides the host with enhanced metabolic capabilities, protection against pathogens, education of the immune system, and modulation of gastrointestinal (GI) development (2).

We do not yet completely understand how the different environments and wide range of diets that modern humans around the world experience has affected the microbial ecology of the human gut.

Contemporary human beings are genetically adapted to the environment in which their ancestors survived and which conditioned their genetic makeup. In mammals, both diet and phylogeny influence the increase in bacterial diversity from carnivore to omnivore to herbivore (3). Dietary habits are considered one of the main factors contributing to the diversity of human gut microbiota (2). Profound changes in diet and lifestyle conditions began with the so-called "Neolithic revolution" with the introduction of agriculture and animal husbandry ≈10,000 y ago (4). After that time, food resources became more abundant and constant, the concentration of huge populations in limited areas created selective pressure that favored pathogens specialized in colonizing human hosts and probably produced the first wave of emerging human diseases (5). It has been hypothesized that bacteria specialized in human-associated niches, including our gut commensal flora, underwent intense transformation during the social and demographic changes that took place with the first Neolithic settlements (6).

Western developed countries successfully controlled infectious diseases during the second half of the last century, by improving sanitation and using antibiotics and vaccines. At the same time, a rise in new diseases such as allergic, autoimmune disorders, and inflammatory bowel disease (IBD) both in adults and in children has been observed (5), and it is hypothesized that improvements in hygiene together with decreased microbial exposure in childhood are considered responsible for this increase (7). The GI microflora plays a crucial role in the pathogenesis of IBD (8), and recent studies demonstrate that obesity is associated with imbalance in the normal gut microbiota (9, 10).

The aim of this study was to compare the gut microbiota of children aged 1–6 y living in a village of rural Africa in an environment that still resembles that of Neolithic subsistence farmers with the gut microbiota of western European children of the same age, eating the diet and living in an environment typical of the developed world. These two childhood populations provided an attractive model for assessing the impact of many environmental variables on the gut microbiota.

In our study, we address three general questions regarding the geography and evolution of the human microbiota: (*i*) how is bacterial diversity partitioned within and between the two populations studied; (*ii*) is there a possible correlation between bacterial diversity and diet; and (*iii*) what is the distribution of well-known bacterial pathogens in the two populations, given the different hygienic and geographic conditions?

Results and Discussion

Characterization of Dietary Habits of Children from the Boulpon Rural Village and from Florence, Italy.

In this study, we characterized the fecal microbiota of 14 healthy children from the Mossi ethnic

Author contributions: C.D.F., D.C., and P.L. designed research; C.D.F., M.D.P., S.M., and S.C. performed research; G.P. contributed new reagents/analytic tools; M.R. and J.B.P. analyzed data; and C.D.F., D.C., M.D.P., and P.L. wrote the paper.

The authors declare no conflict of interest.

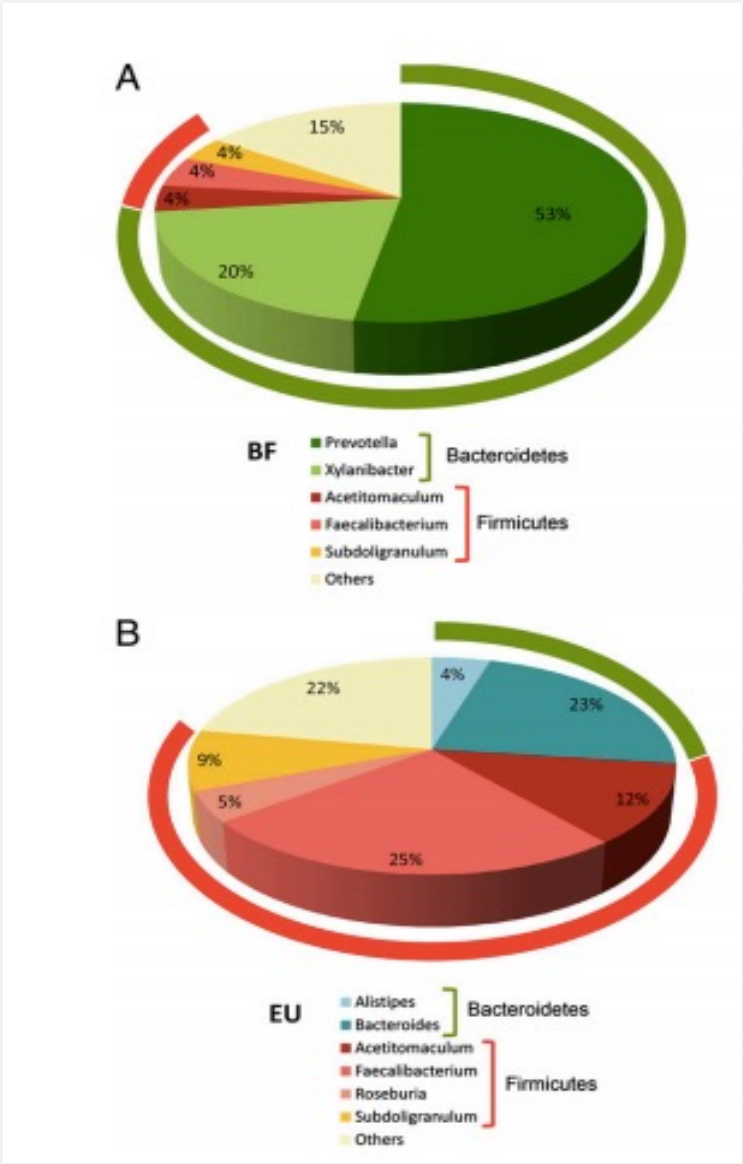
*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

Data deposition: Data were submitted to the Sequence Read Archive (SRA) using SA tools (SAcreator and SAconverter, http://halai.sourceforge.net/index.html). The dataset is available at http://www.ebi.ac.uk/arrayexpress/ERP000135.

[1]To whom correspondence should be addressed. E-mail: paolo.lionetti@unifi.it.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1005963107/-/DCSupplemental.



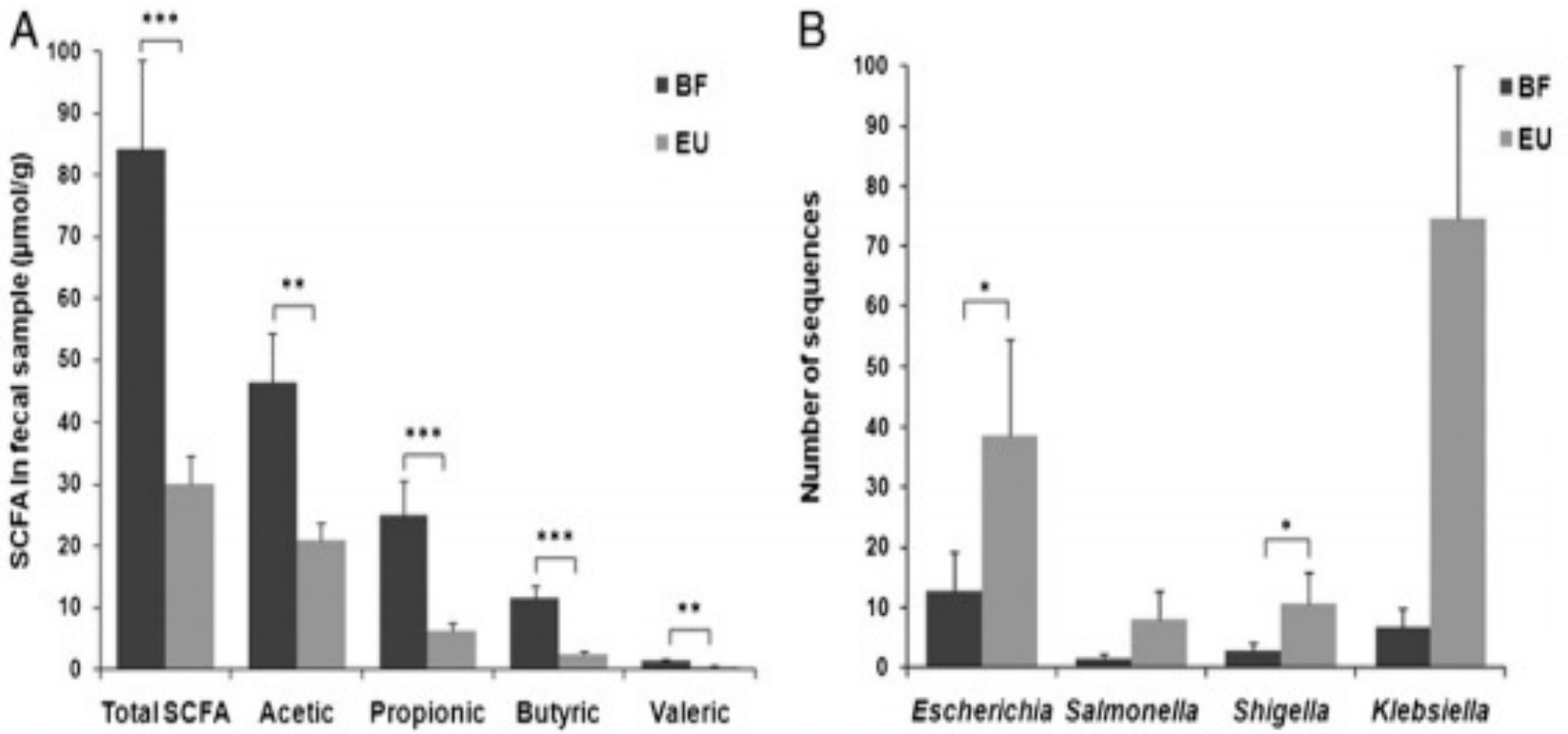
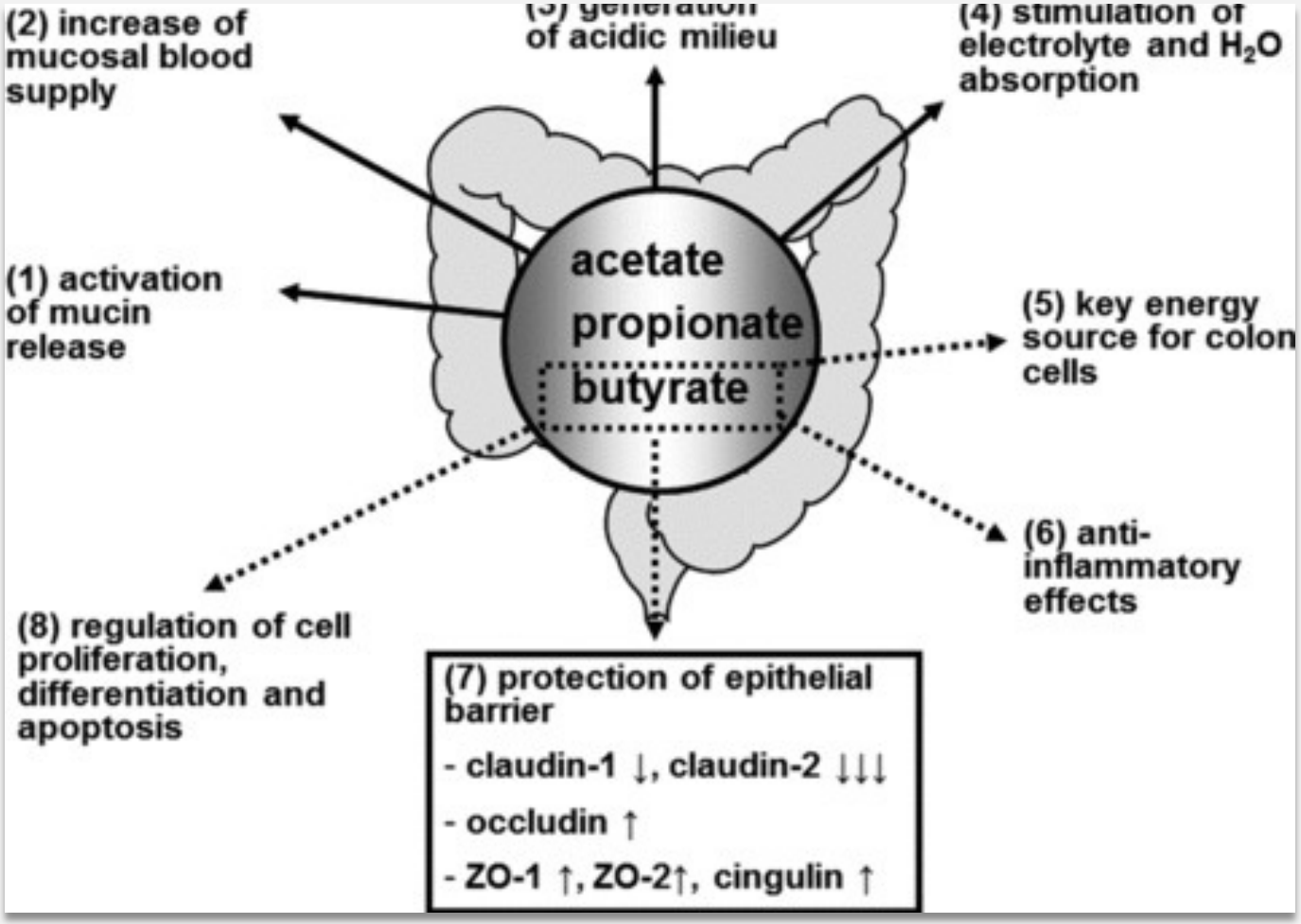


Fig. 3. SCFA-producing bacteria could help to prevent establishment of some potentially pathogenic intestinal bacteria. (*A*) Quantification of SCFAs in fecal samples from BF and EU populations by SPME-GC-MS. (*B*) Number of sequences relative to principal *Enterobacteriaceae* genera, in BF and EU children microbiota. Mean values (±SEM) are plotted. Asterisks indicate significant differences (one-tailed Student *t* test of all data points: $*P < 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).



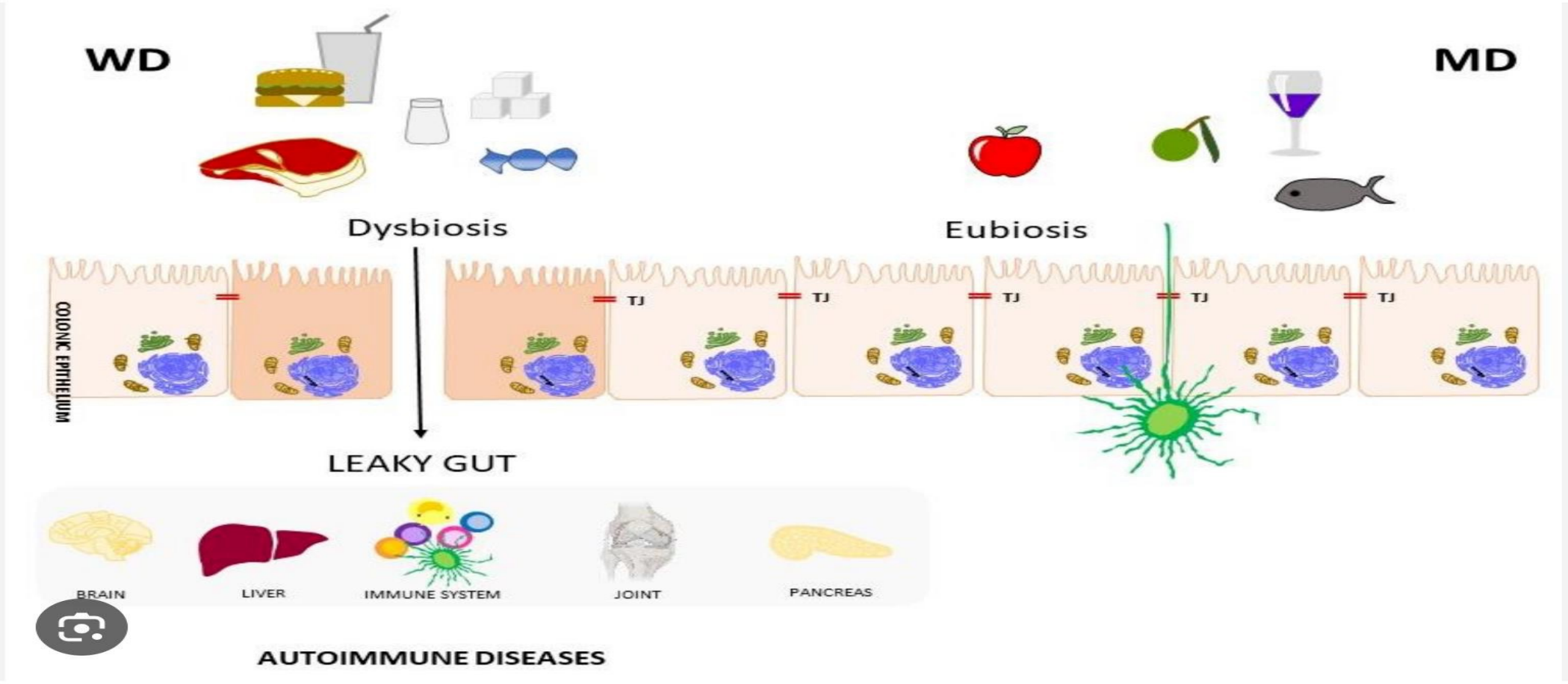
BUTYRATE AND PROPIONATE

Impact of Mediterranean Diet on Disease Activity and Gut Microbiota Composition of Rheumatoid Arthritis Patients

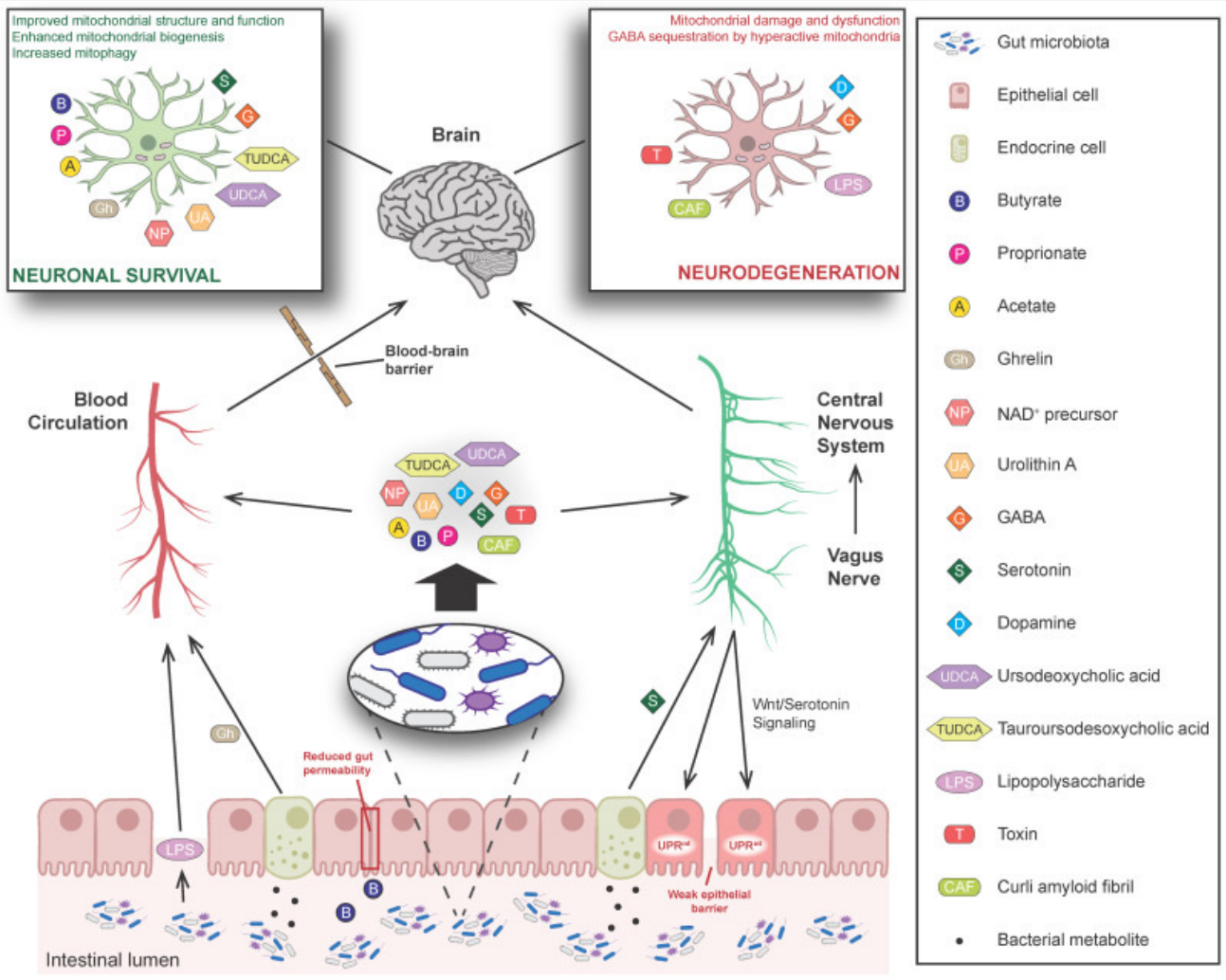
We investigate the protective effect of the Mediterranean diet (MD) on disease activity and the gut microbiota profile in RA patients. Sixty consecutive RA patients were enrolled upon filling a validated 14-item questionnaire for the assessment of adherence to the Mediterranean diet (Prevention with Mediterranean Diet-PREDIMED). Then, 16S analysis was employed to explore the gut microbiota within the two cohorts of patients. Patients with high adherence to MD (20) had a significantly lower C-reactive protein ($p < 0.037$) and disease activity ($p < 0.034$) than the 40 patients with low/moderate adherence to MD. An inverse association between MD and disease activity was confirmed by multivariate analysis after adjustments for all the different demographic, clinical and serologic variables. A healthier gut microbiota composition was observed in the high adherence group, with a significant decrease in Lactobacillaceae and an almost complete absence of *Prevotella copri* with respect to the low/moderate adherence group. In conclusion, our findings support the protective role of MD on disease activity and microbiota composition in RA patients, and suggest the feasibility of shifting the habitual diet to modulate the gut microbiota and promote the benefits associated with MD.

Microorganisms **2020**, *8*, 1989;

PROPOSED IMPACT OF MEDITERRANEAN DIET ON AUTOIMMUNE DISEASES



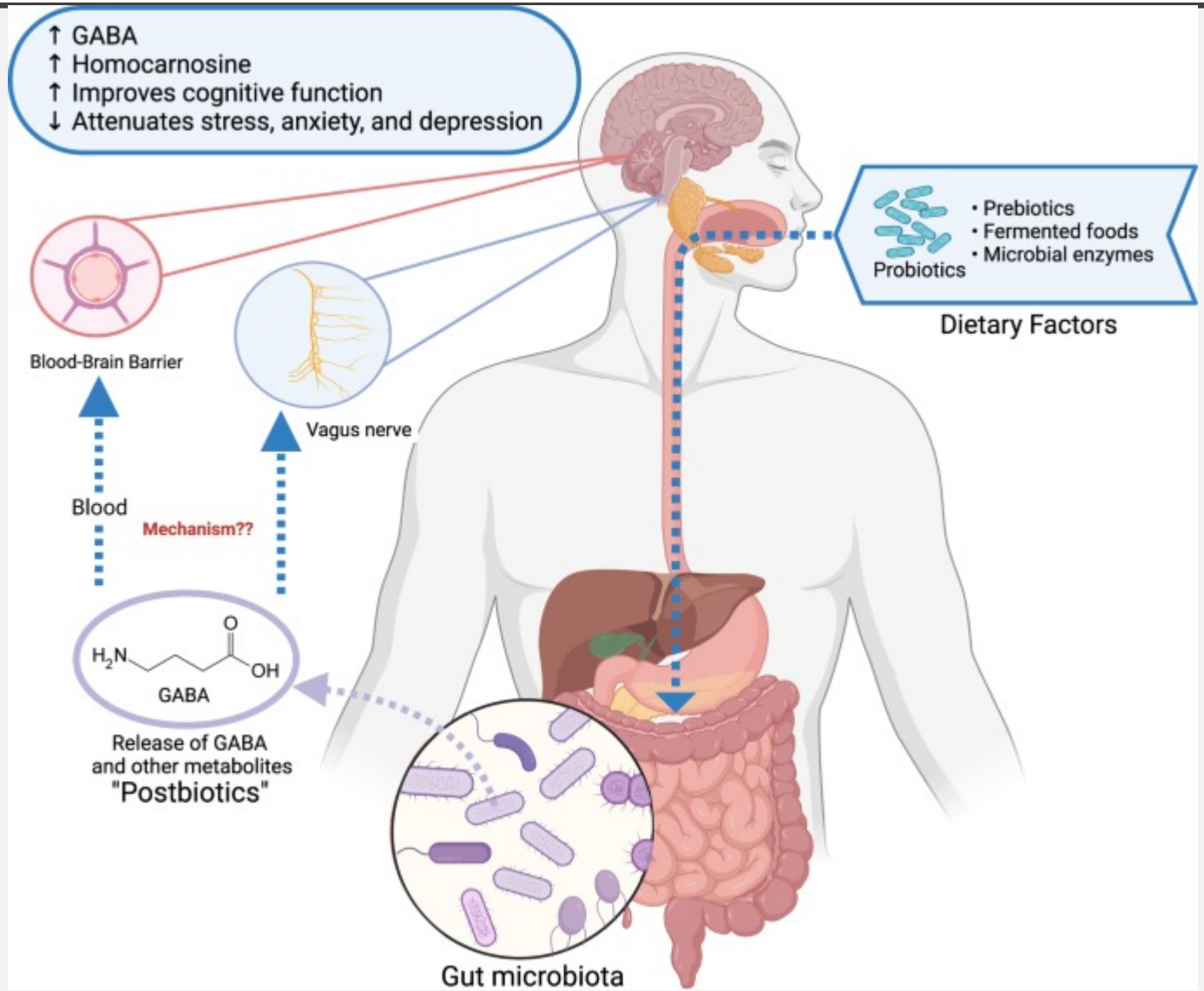
THE CROSSTALK BETWEEN MICROBIOME AND MITOCHONDRIAL HOMEOSTASIS IN NEURODEGENERATION



The implication of mitochondria in the gut–brain axis. Metabolites secreted by commensal microorganisms can affect brain mitochondria by entering the bloodstream and crossing the blood–brain barrier or by acting directly on the central nervous system through the vagus nerve. The impact of such metabolites can promote neuronal survival by improving mitochondrial quality, enhancing mitophagy and promoting organelle biogenesis, or favor neurodegeneration by inducing mitochondrial hyperactivation, damage and dysfunction. Gut microbial metabolites can also act indirectly by affecting the permeability of the epithelium or by modulating the secretion of intestinal endocrine cells. Reversely, the nervous system can affect intestinal microbiota by activating the mitochondrial unfolded protein response (UPR^{mt}) of gut epithelial cells, weakening the epithelial barrier.

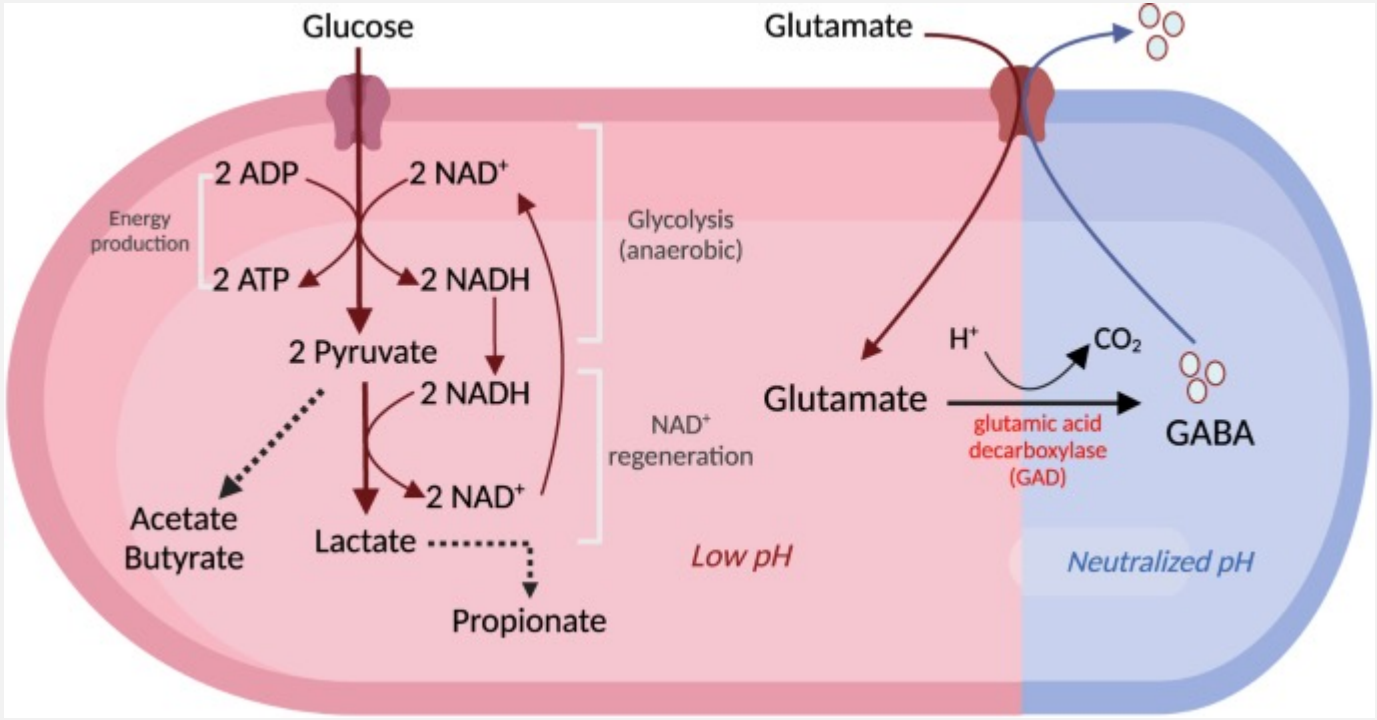
PHARMAGABA

GAMMA-AMINOBUTYRIC ACID AS A POTENTIAL POSTBIOTIC MEDIATOR IN THE GUT–BRAIN AXIS



These dietary factors, including probiotics, prebiotics, fermented foods, and microbial enzymes, positively affect gut microbiota composition that stimulates the release of GABA and other microbial metabolites. As microbial GABA passes through the intestinal barrier, it influences brain compound levels via blood-brain barrier or vagus nerve and improves brain function.

MECHANISM OF GABA PRODUCTION IN MICROORGANISMS.



Under anaerobic and acidic conditions in the human gut and fermentation, it appears that bacteria produce GABA for their own survival purposes under these extreme environments. Under anaerobic conditions, glycolysis takes place in the cytosol, where NAD^+ and ADP are required to convert glucose into pyruvate, in which NADH and ATP are produced from the process[75]. Pyruvate is then converted into lactate or other organic compounds, such as acetate, butyrate, and propionate, where NADH is utilized, and NAD^+ is generated in the process. Then, NAD^+ is fed back and reutilized in the glycolysis process[75]. The acidic fermentation by-products, lactate, and other organic compounds lower the pH, which leads bacteria to utilize the GAD gene system and triggers the production of GABA[76,77]. To produce GABA, exogenous glutamate is transported into the cell by a glutamate/GABA antiporter, then glutamate is converted into GABA by glutamic acid decarboxylase (GAD)[36,76,78]. Then, GABA is exported from the cell via the antiporter, resulting in an increase in the pH of the cytoplasm due to the removal of H^+ ions and a slight increase in the extracellular pH due to the exchange of extracellular glutamate for more alkaline GABA

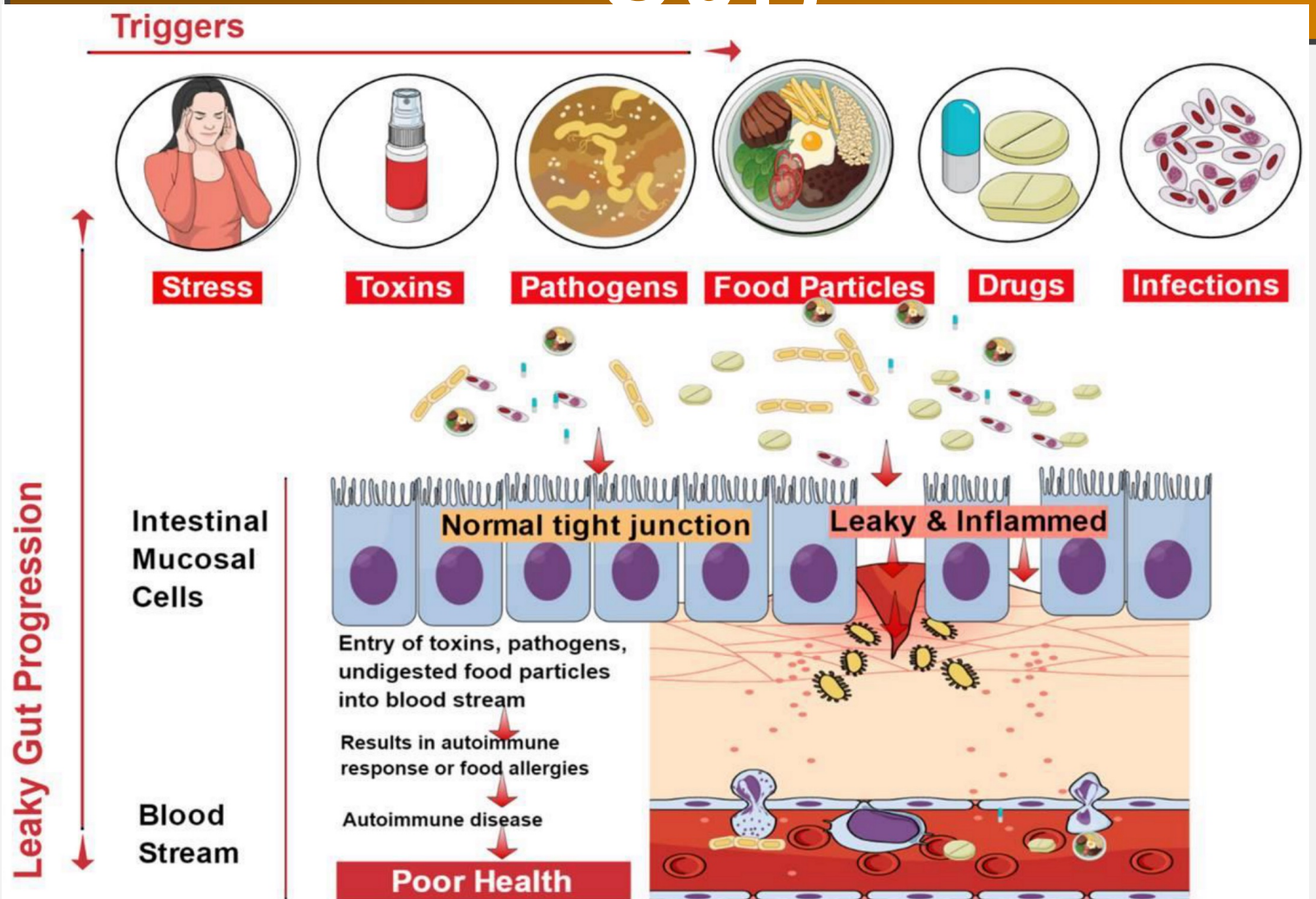
GUT PERMEABILITY (LEAKY GUT)

STRESS
Sympathetic Tone

Increased Gut Permeability

Mitochondrial Dysfunction

CELL ENERGY COMPROMISED

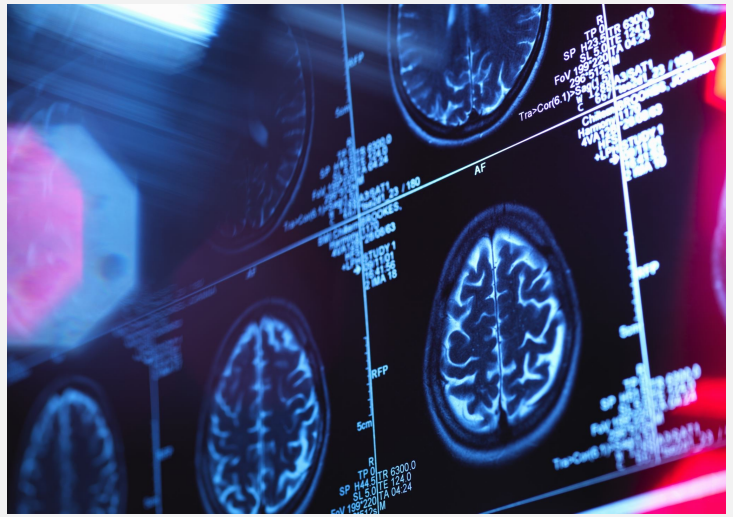


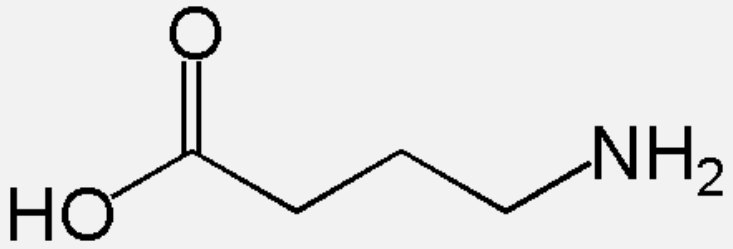
PHARMAGABA

GABA(gamma-amino-butyric acid)

PharmaGABA:

- Natural source of the neurotransmitter found in the human brain
- Clinically researched and proven to promote feelings of relaxation with greater focus, concentration and cognitive function
- One of the most successful natural food ingredients in Japan.
- Very safe with excellent scientific support





CLINICAL EVIDENCE AND SCIENTIFIC VALIDATION SEEN WITH THE USE OF PHARMAGABA

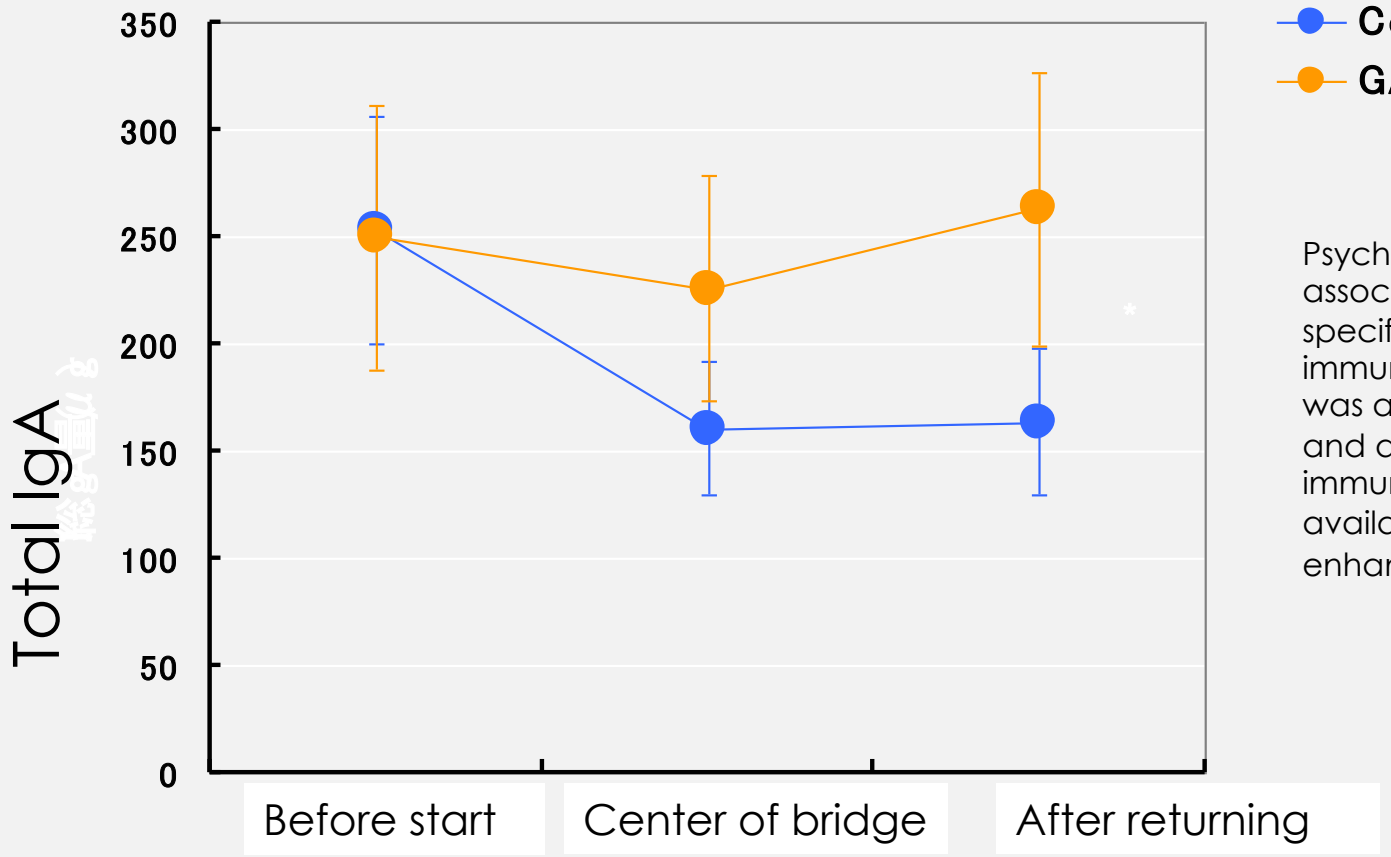
- Maintains blood pressure
- Relieves stress, impacts relaxation, reduces fatigue
- Promotes muscle synthesis
- Improves sleep quality
- Improves cognitive health
- Reduces gut permeability

LET'S LOOK AT THE STUDIES

ANTI-STRESS EFFECT OF PHARMA-GABA

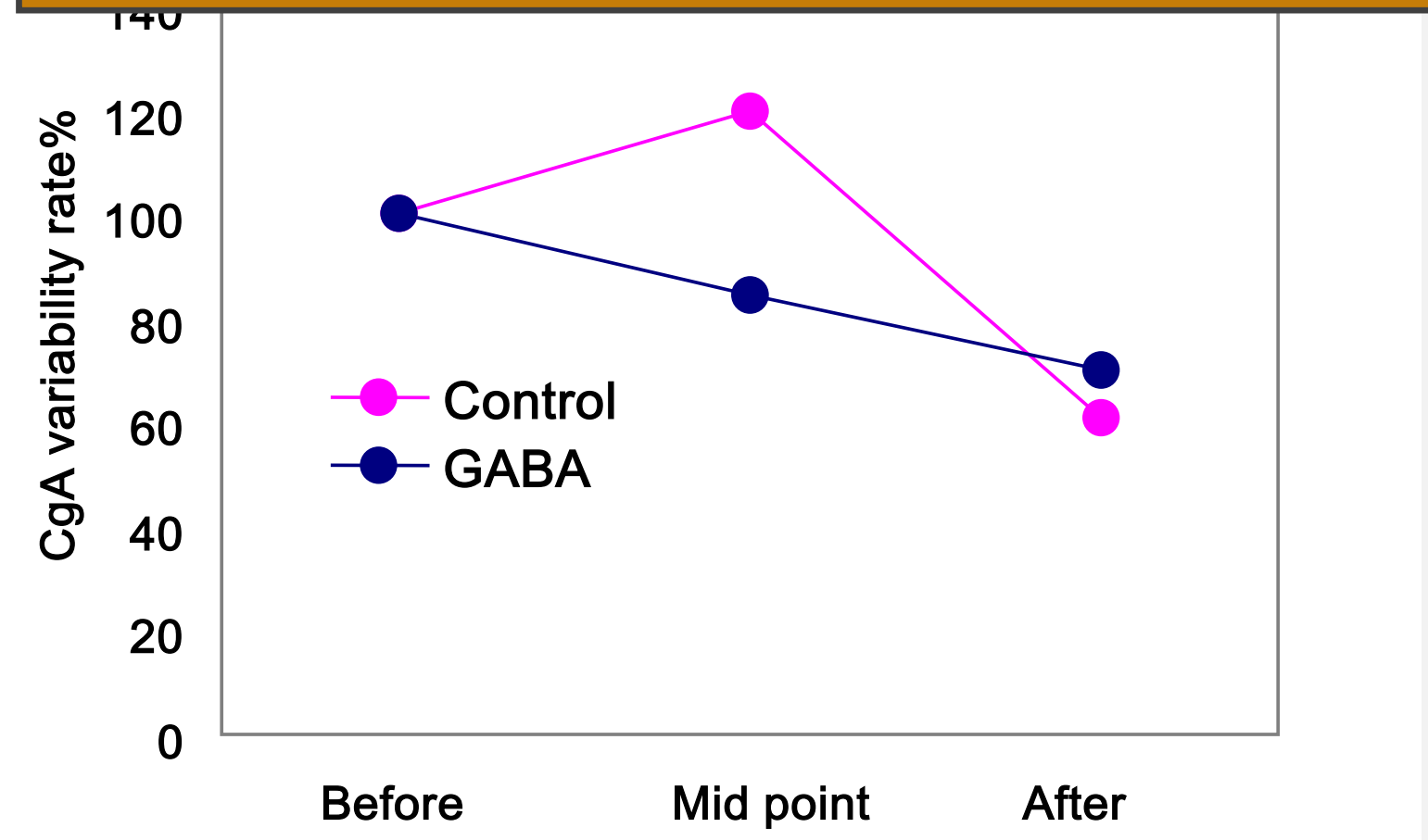
Researchers in Japan studied the calming effects of GABA with 8 volunteers. Study subjects cross a suspension bridge as the stressful stimulus. The placebo subjects in this group showed significant drops in blood level markers indicating high stress levels. While GABA group showed significantly higher blood levels of these same markers.

Change of IgA level in saliva under stress



Psychological stress was negatively associated with secretory immunity, specifically IgA1. The lower immunoglobulin/transporter ratio that was associated with higher loneliness and depression suggested a relative immunoglobulin depletion, whereby availability was not keeping up with enhanced transport demand.

TEST RESULT – CHROMOGRANIN A



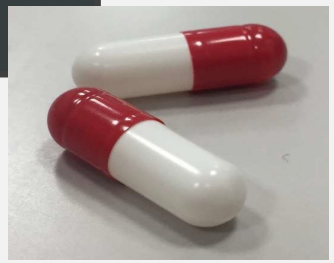
Salivary chromogranin A is often used in stress research as a marker of the sympathetic-adrenal-medullary activity as it is co-released with epinephrine or norepinephrine upon sympathetic stimulation

PHARMAGABA IMPACT ON IMPROVING COGNITIVE FUNCTION

Objective	To investigate the effects of continuous intake of PharmaGABA on cognitive function
Experimental Design	A randomized, double-blind, placebo-controlled, parallel clinical trial
Subjects	Total 120 healthy Japanese people over 40 years old
Selection Criteria	MMSE score of 24 or more (subjects not with dementia) (Mini-mental state examination)
Duration	12 weeks(ingestion of PharmaGABA or placebo)
Assessment	Before the test and after 4th, 8th and 12th week of ingestion
Tests	• Cognitrax • RBANS (Repeatable Battery for the Assessment of Neuropsychological Status） • SF-36 （Short-Form Health Survey）

PHARMAGABA IMPACT ON IMPROVING COGNITIVE FUNCTION

Dosing:
Active: PharmaGABA 100 mg/capsule
PharmaGABA 200 mg/capsule
Placebo: 100 mg Dextrin per capsule
Administration: Oral



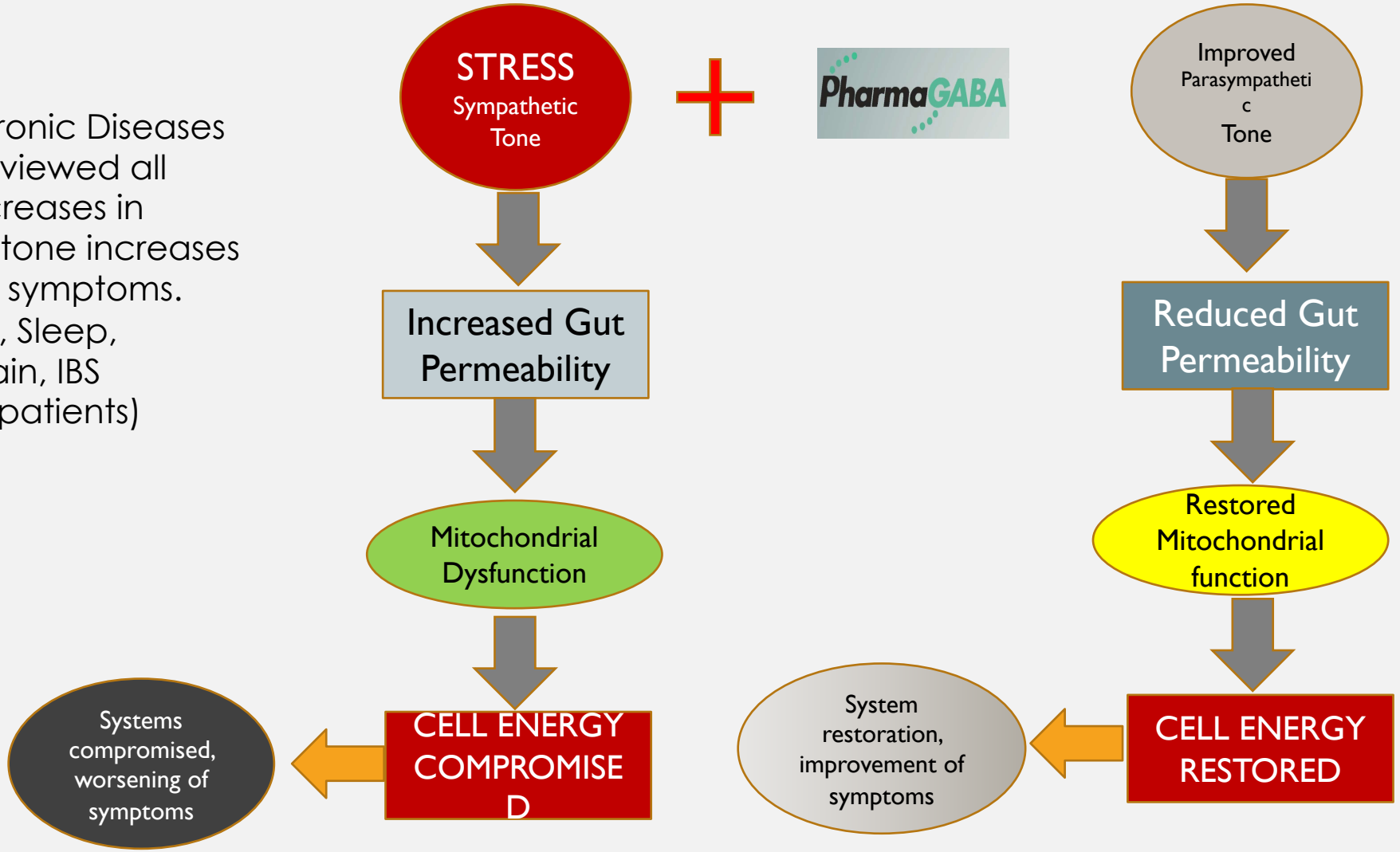
Test	Group	Active capsule	Placebo capsule	Number of subjects analyzed
Test A	Active	2 capsules (PharmaGABA 200 mg)	-	30
	Placebo	-	2 capsules (dextrin 200 mg)	30
Test B	Active	1 capsule (PharmaGABA 100 mg)	-	30
	Placebo	-	1 capsule (dextrin 100 mg)	28 (2 subjects dropout)

PHARMA GABA LEADING TO COGNITIVE IMPROVEMENT

Improved cognitive functions by GABA	200mg	100mg	Brief description
Logical thinking	●		The ability to reason and understand visual or abstract information. The ability to recognize visual and abstract conceptual relationships. ※GABA is a first ingredient to improve Logical thinking
Working memory	●		The ability to store information necessary for work in the short term.
Sustained attention	●		The ability to control and maintain attention.
Visuospatial/ Construction	●	●	The ability to recognize and accurately compose spatial relationships of figures.
Long-term memory	●	●	The ability to recall the words, figures, stories, etc. that you have seen or heard before.
Vitality	●		the state of being strong and active; energetic.
Mental Health	●		Being stable, calm and enjoyable feeling.

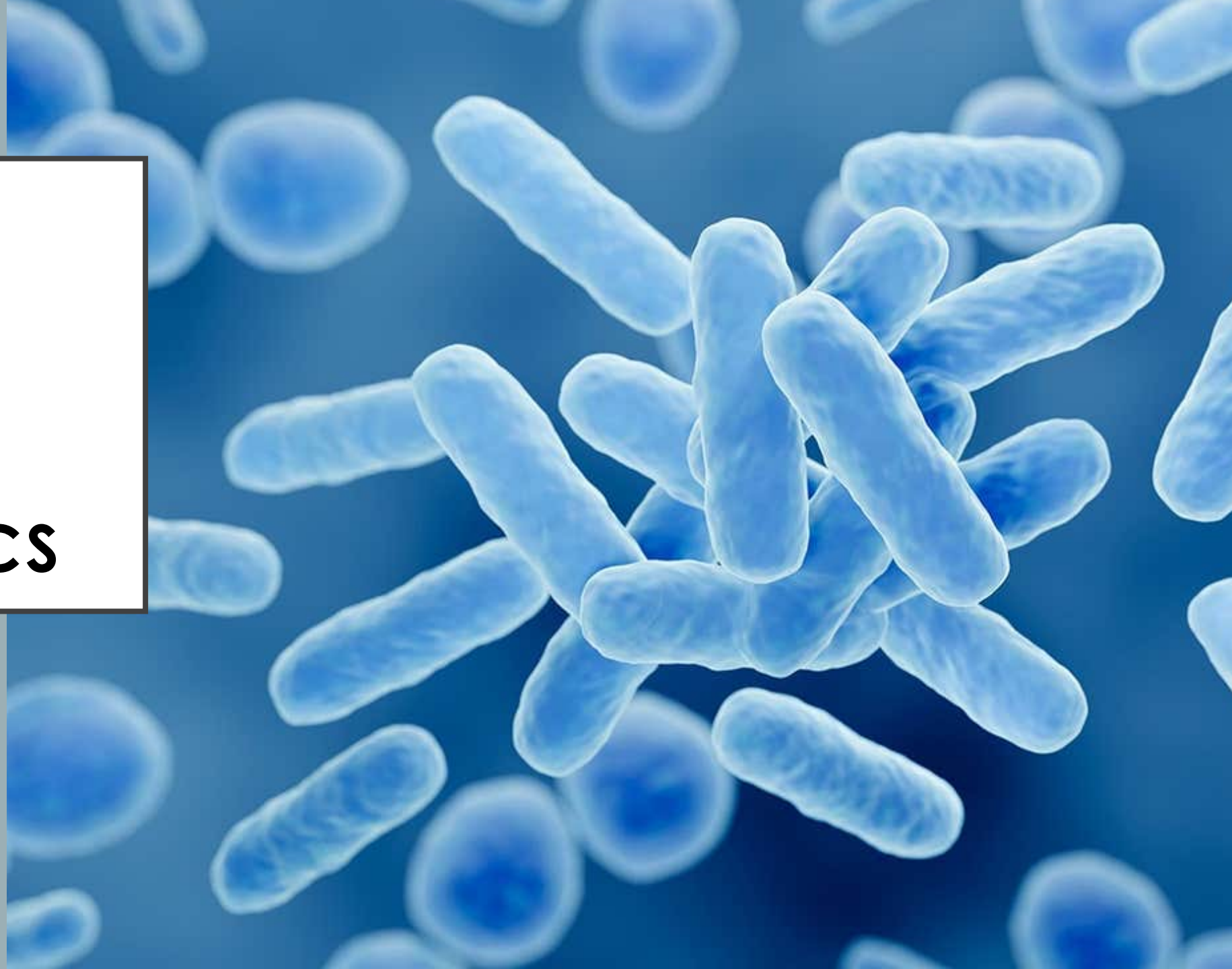
CCD PATIENTS AND INCREASED SYMPATHETIC TONE

Complex Chronic Diseases patients interviewed all state that increases in sympathetic tone increases severity of all symptoms. Fatigue, PEM, Sleep, Cognition, Pain, IBS (over 8,000+ patients)



PROBIOTICS

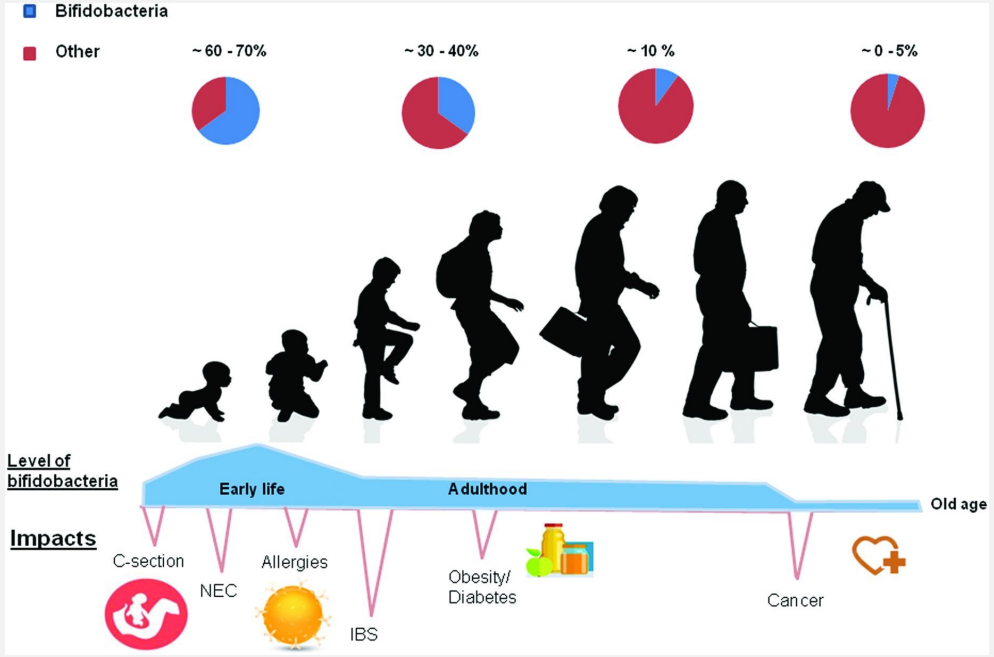
Postbiotics and Prebiotics



DEFINITIONS

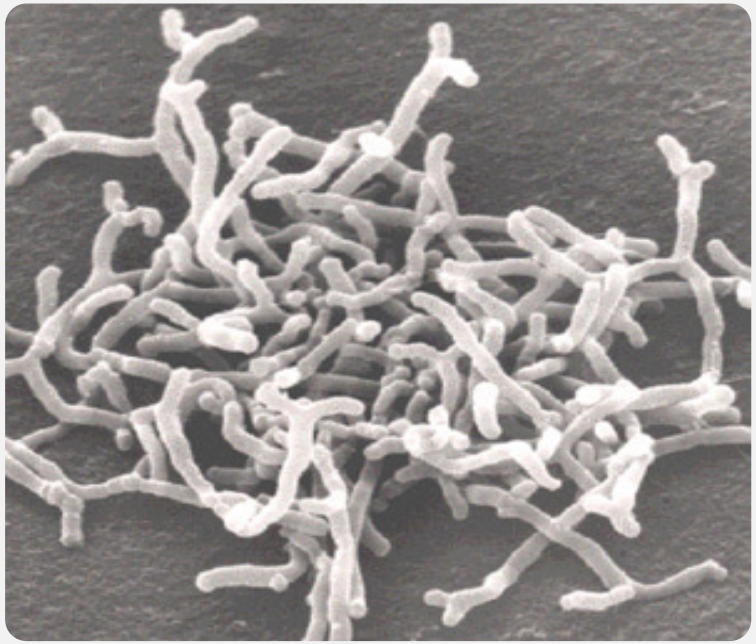
	Probiotics	Postbiotics	Prebiotics
Definition	Live microorganism that when administered in an adequate amount confer a health benefit on the host	Preparation of inanimate microorganisms and/or their components that confer a health benefit on the host	Substrates that are selectively utilized by host microorganisms conferring a health benefit
Key Advantages	Regulation of intestinal functions	Can confer a range of health benefits on the host.	Improves diversity and numbers of beneficial bacterai

BIFIDOBACTERIA



BIFIDOBACTERIUM LONGUM BB536

Used for 25 years



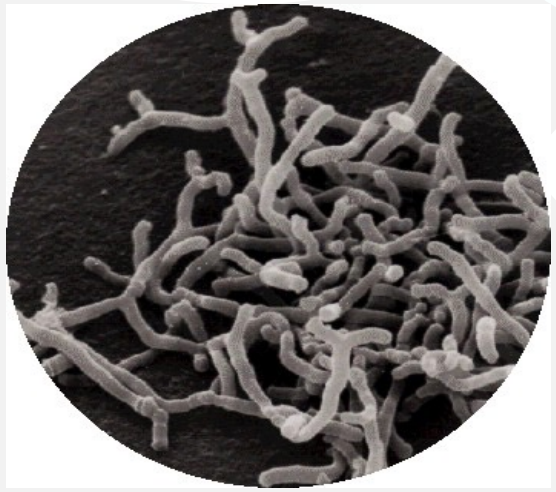
INTRODUCING BB536

Improves Intestinal Environments

Enhances Bone Strength

Stimulates Immunity

Alleviating Constipation



May Improve Epithelial Integrity

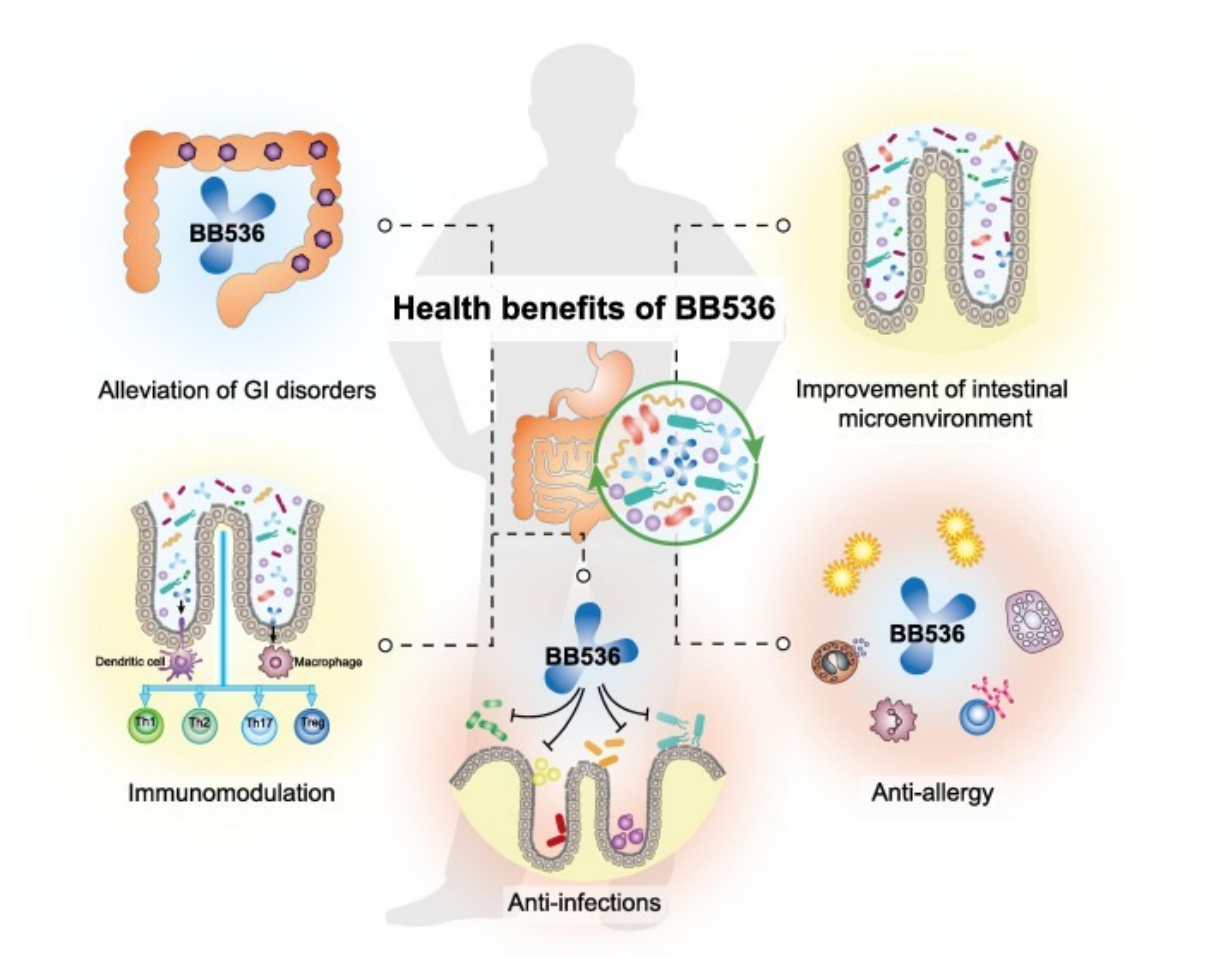
Alleviates the occasional Diarrhea

>supported by over 200 scientific articles

HOW DOES IT DO IT

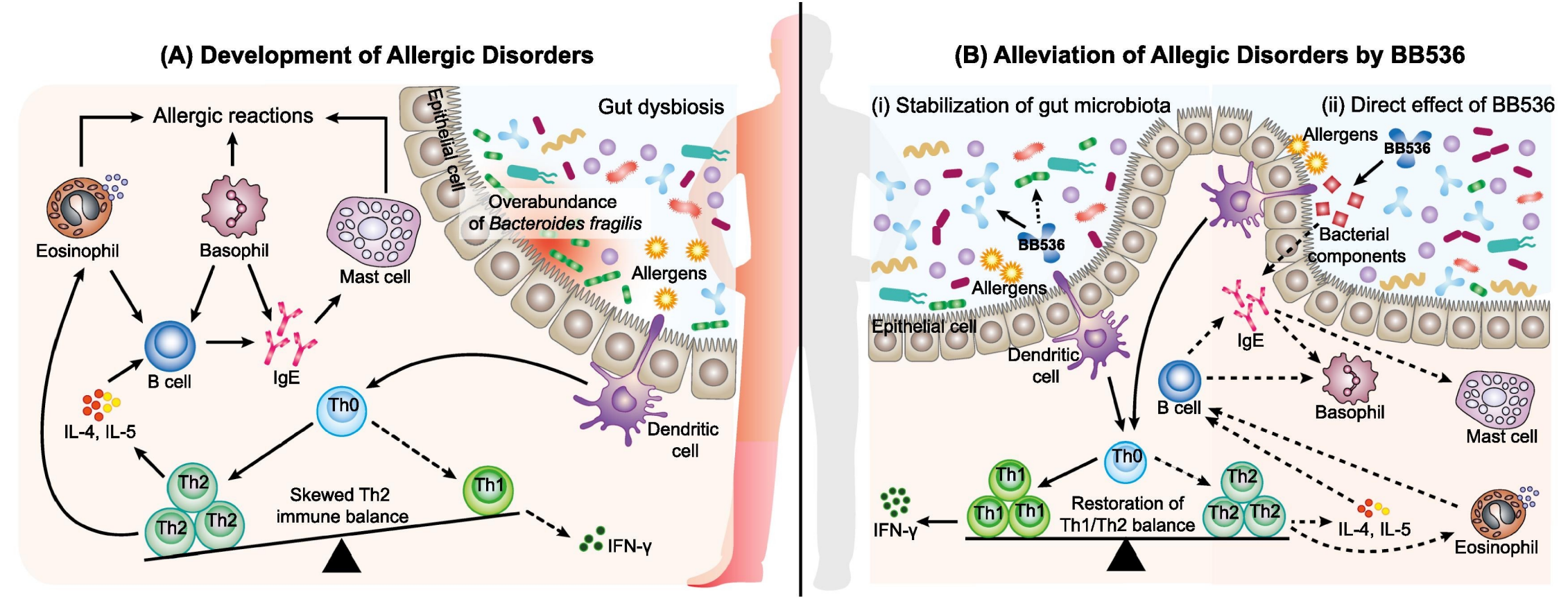
Possible Mechanism of Action of BB536

BB536 HEALTH BENEFITS



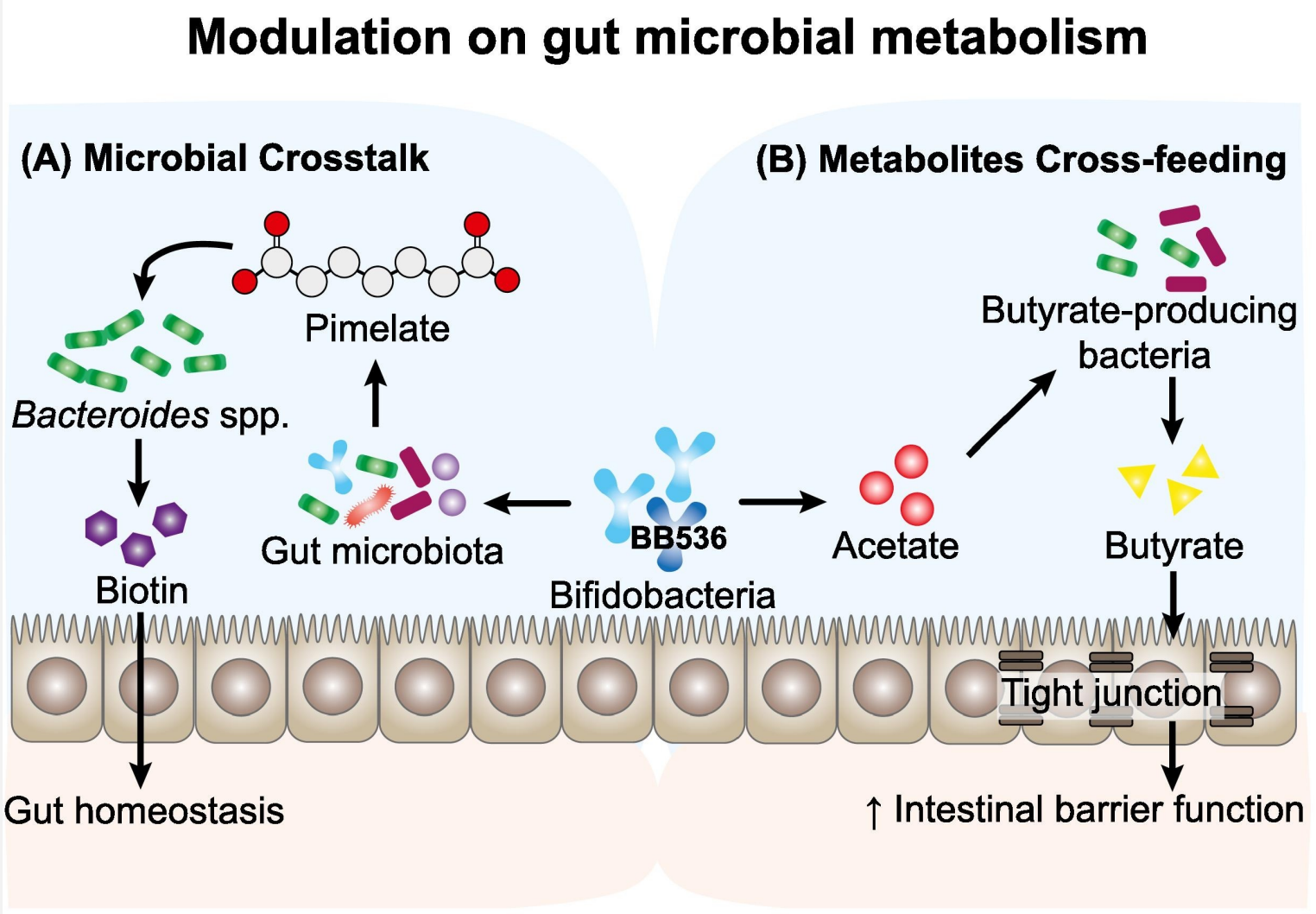
Modulation of gut microbiome is the principal beneficial action of *Bifidobacterium longum* subsp. *longum* BB536 in promoting human health. BB536 acts in concert with the gut microbiota to improve gastrointestinal health, modulate host immune homeostasis, and alleviate allergic disorders and infectious conditions.

BB536 IMMUNE MODULATION



Immunomodulatory effect of *Bifidobacterium longum* subsp. *longum* BB536. (A) Fluctuation of intestinal microbiota, particularly overabundance of Bacteroides fragilis, contributes to perturbation of host immunity and development of allergic disorders. In allergic reactions, an allergen is taken up by dendritic cells, and presented to naïve T cells (Th0) which then transforms into T-helper type 2 (Th2) cells. Th2 cells secrete interleukin (IL)-4 and IL-5 and subsequently stimulated memory B cells to switch to an allergen-specific humoral response that is predominated by the production of immunoglobulin E (IgE) antibodies. These IgE antibodies attach to mast cells and basophils thereby sensitizing them to subsequent exposure and development of allergic symptoms. (B) BB536 modulates immune homeostasis within the host-microbiome interaction and alleviates allergic disorders via both indirect and direct mechanisms. (i) BB536 promotes the stabilization of intestinal microbiota by rectifying the prevalence of *Bacteroides fragilis* and consequently restores Th1/Th2 balance and alleviates allergic symptoms. (ii) BB536 elicits a direct effect on antigen-induced IgE-mediated Th2 skewed immune balance via its bacterial component. Solid arrow line: stimulation; dashed arrow line: inhibition.

BB536 METABOLISM

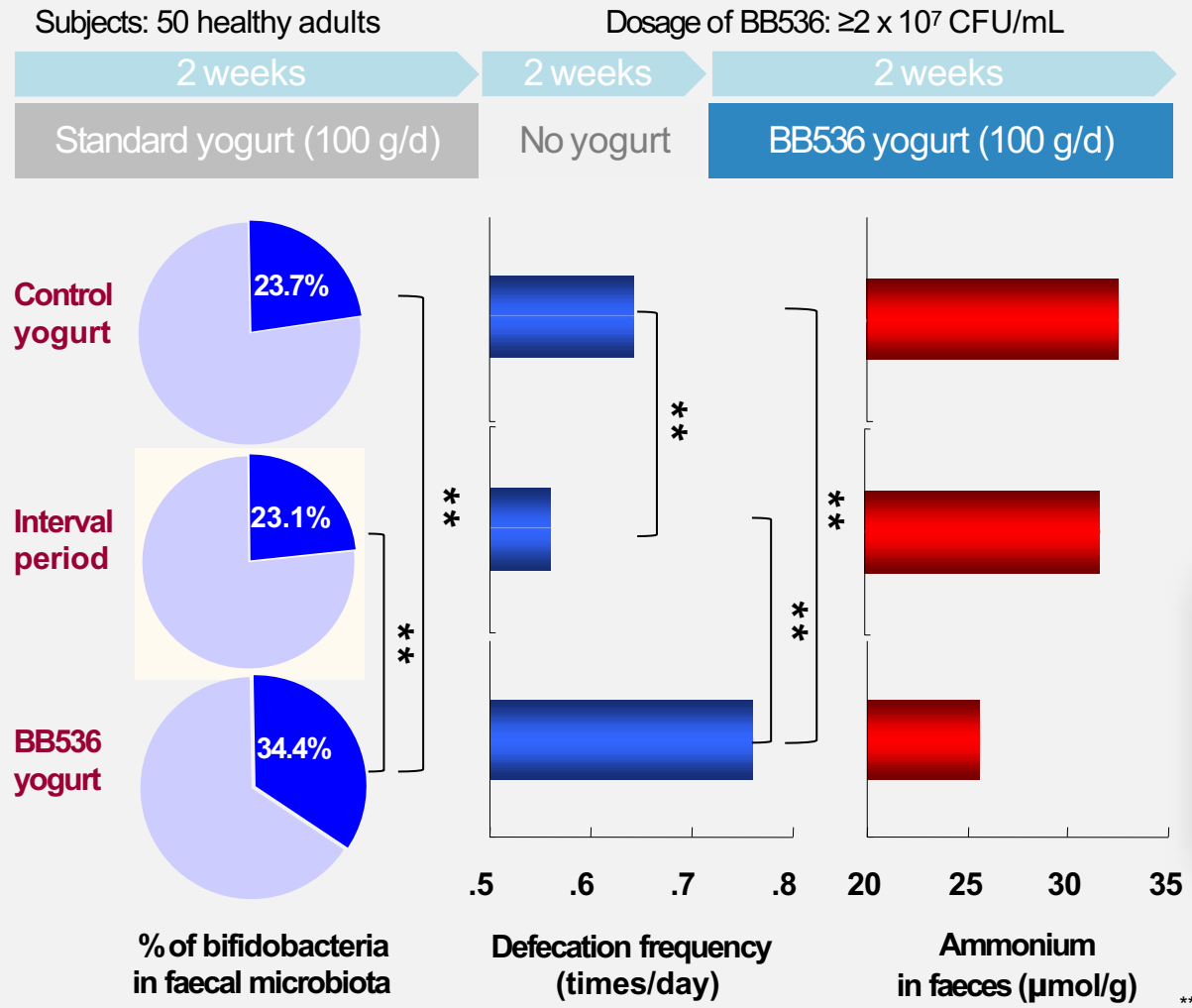


Modulation of gut metabolism by Bifidobacterium *longum* BB536 via microbial crosstalk with human gut microbiota. (A) BB536 modulates biotin biosynthesis by promoting the production of the precursor pimelate and enables Bacteroides caccae to metabolize it further into biotin, thereby contributing to host gut homeostasis. (B) BB536 influences the metabolic activity of the commensal butyrate-producing bacteria (e.g. Eubacterium rectale) through cross-feeding mechanisms. Acetate produced by BB536 in carbohydrate fermentation acts as substrate to sustain the growth of *Eu. rectale* and stimulates the production of butyrate.

Healthy gut regularity

BB536 ON GUT HEALTH

IMPROVE GUT ENVIRONMENT IN ADULTS



**P < 0.01 vs test group

These graphs were reproduced from Yaeshima et al, 1997. *Bioscience Microflora*.

PROTECT AGAINST INFLUENZA INFECTION

Healthy Ageing

BB536 ON ELDERLY'S HEALTH

Seniors aged >65 yo residing in a nursing home (n=27)

Period 1: 6 weeks

BB536 ingestion

W3 (Vaccination) — W5 (Analysis)

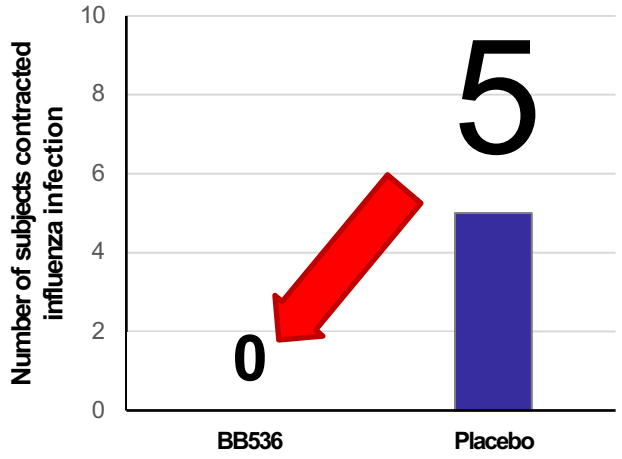
Period 2: 14 weeks

BB536 group

100 B CFU (2g/day)

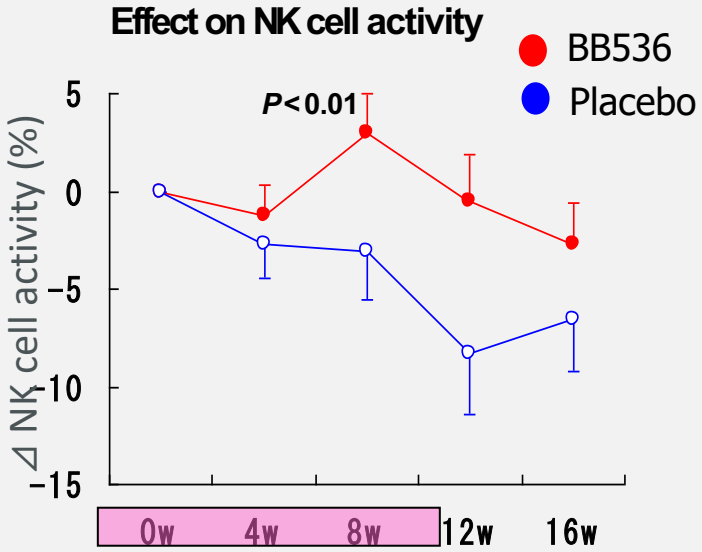
Placebo group

Prevalence of influenza infection



- Subjects: elderly persons age>65 yr residing in a nursing home
- Treatment: administrated with BB536 or placebo for a total of 19 weeks
- Number of subjects: BB536 G=13, placebo G=14

Effect on NK cell activity

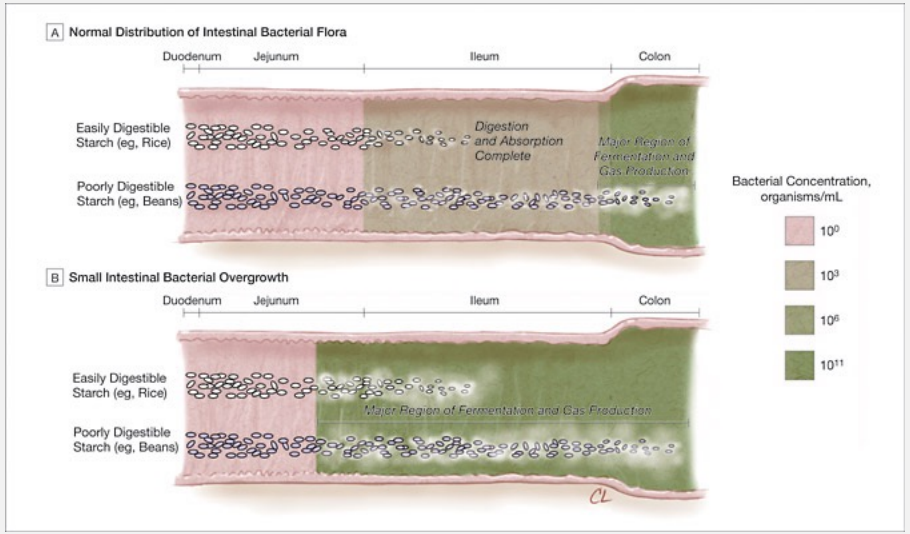


- Subjects: elderly persons age>65 yr residing in a nursing home
- Treatment: administrated with BB536 or placebo for a total of 12 weeks
- Number of subjects: BB536 G=23, placebo G=22

Twenty-seven elderly subjects (mean age 86.7±6.6 years) were pre-administered a test food containing 1×10^{11} cfu of BB536 daily for 5 weeks (P1), during which they also received influenza vaccination at week 3. The subjects were then randomized to a BB536 group and a placebo group for 14 weeks (P2). The proportion of subjects who contracted influenza was significantly lower in BB536 group than in the to placebo group. The proportion of subjects with fever was also significantly lower in the BB536 group than in the placebo group. In the P1 period, the NK cell activity and the bactericidal activity of the neutrophils were significantly higher at week 5 than to before BB536 administration. In the P2 period, although NK cell activity and neutrophilic activities declined at the end of the study in both the placebo and the BB536 group, neutrophil phagocytic activity and NK cell activity tended to maintain slightly higher levels in the BB536 group than in the placebo group. These results suggest that continuous ingestion of BB536 reduces the incidence of influenza and fever, probably by potentiating innate immunity.

These graphs were reproduced from Namba et al., 2010. *Bioscience, Biotechnology, and Biochemistry*

SIBO DEFINED



SIBO: CLINICAL PRESENTATION

Symptoms (gas related)

- Abdominal Pain
- Bloating/Flatulence
- Diarrhea

Other Symptoms

- Malabsorption Syndrome
- Increased GUT Permeability
- IBS

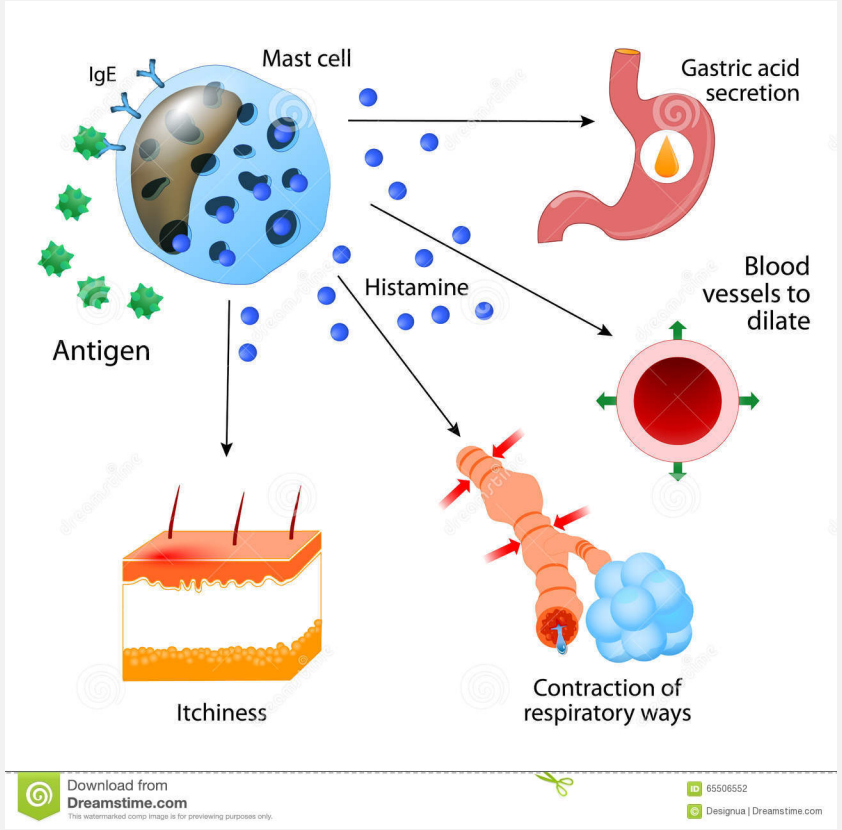
NEW SIBO

- Crohn's dis. *
- Celiac dis. *
- Irritable bowel synd. *
- Chronic liver dis. *
- Restless legs synd.
- Rosacea
- Parkinson's dis. *
- Histamine intolerance
- Renal failure
- Hypothyroidism
- Acromegaly
- Post-chemotherapy
- Fibromyalgia
- Rheumatoid arthritis *
- Interstitial cystitis
- Chronic prostatitis

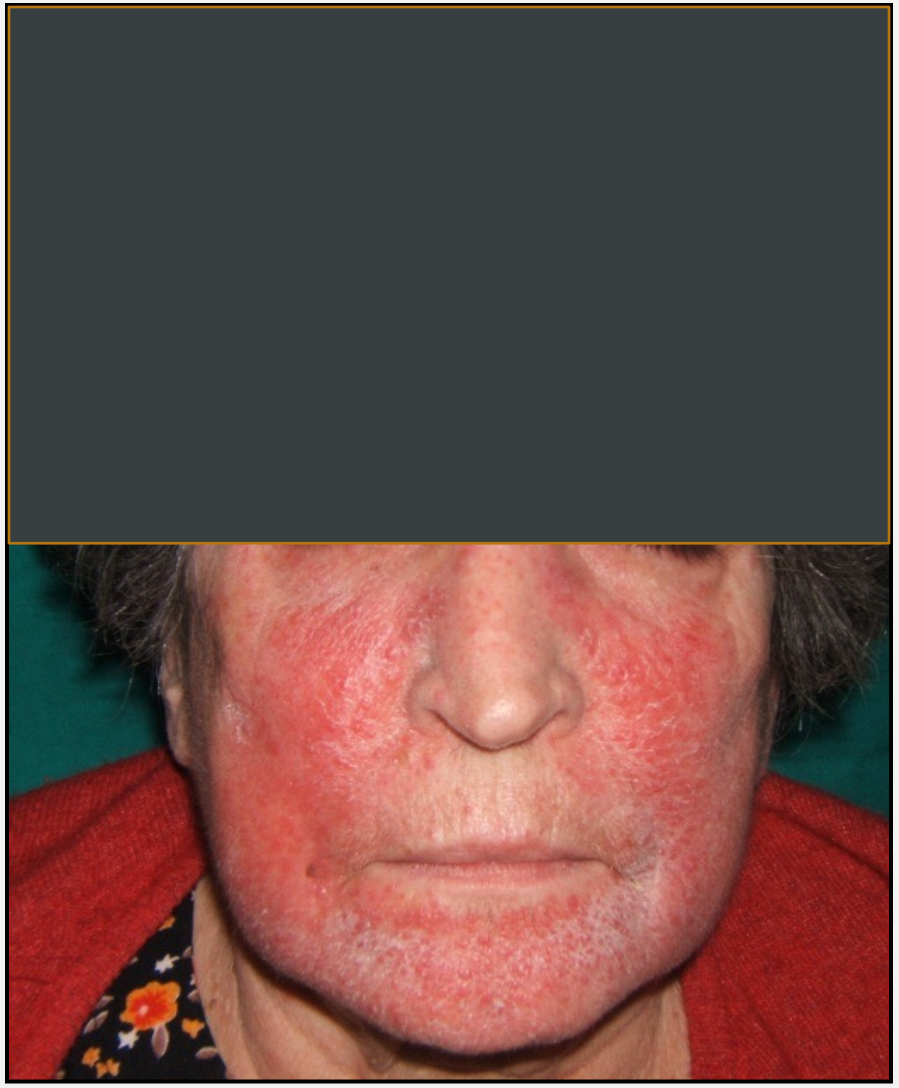
HISTAMINE

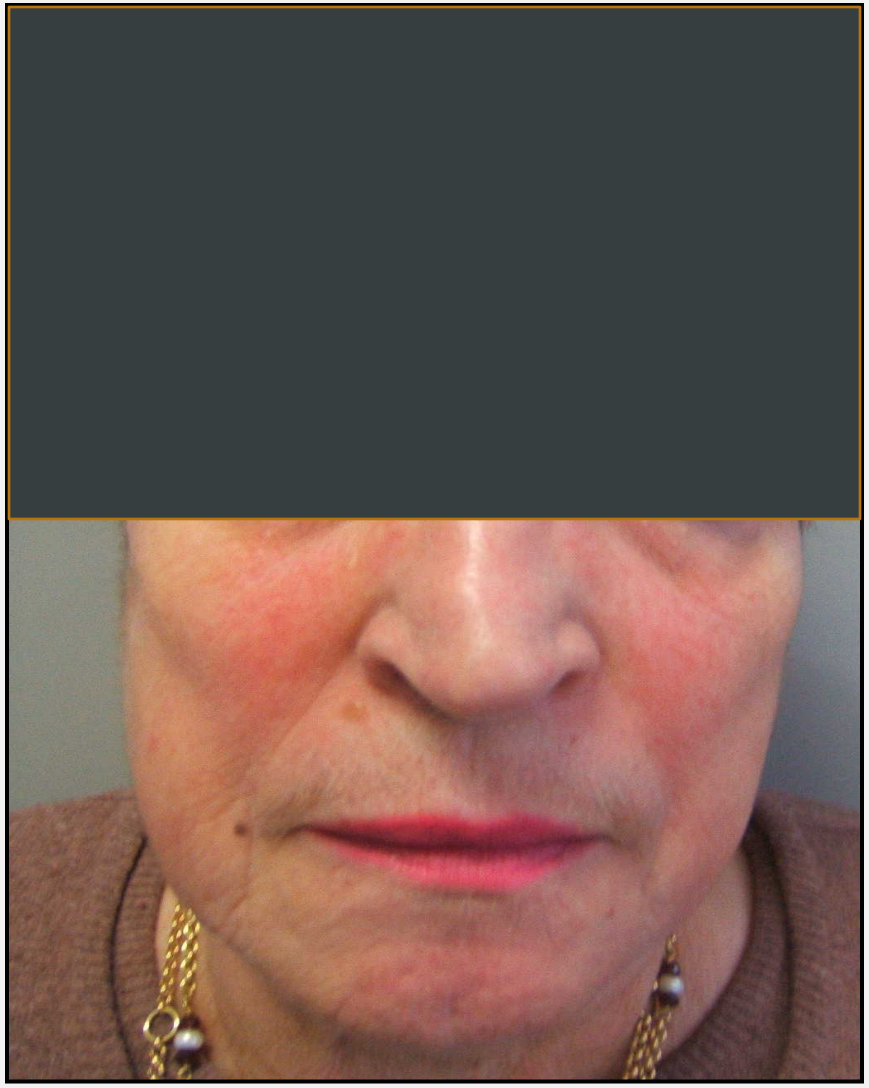
Dysbiosis and SIBO may have an impact on Diamine Oxidase, enzyme that breakdown histamine

Result is increased levels of allergic reaction based on a histamine intolerance issue related to dysbiosis and SIBO



Patient with Rosacea treated for SIBO with Antimicrobial Agents (30 days following tx)





Courtesy of V. Savarino:

What is PEA (Palmitoylethanolamide)

A "pro-resolving lipid signaling molecule"

- Identified nearly 80 years ago as an active biological factor in mammals.
- Later found to be a nutritional factor contained in chicken egg yolk, olive oil, safflower and soy lecithin, peanut meal, and several other foods.
- PEA functions in concert with the endocannabinoid system.
- As a "pro-resolving lipid signaling molecule" PEA acts through impacting central control mechanisms within our cells to resolve factors that lead to cellular stress and inflammation.
- The health benefits of PEA has been demonstrated in over 600 scientific investigations including over 20 double-blind human clinical trials.
- Latest research shows PEA has an impact in reducing gut permeability

A RANDOMISED DOUBLE BLIND CONTROLLED TRIAL EXAMINING THE EFFECT OF PEA AND CBD ON THE PERMEABILITY OF THE HUMAN GUT IN VIVO

DG COUCH, C ORTORI, D BARRETT, JN LUND AND SE O'SULLIVAN
SCHOOL OF MEDICINE, FACULTY OF SCIENCE, UNIVERSITY OF NOTTINGHAM

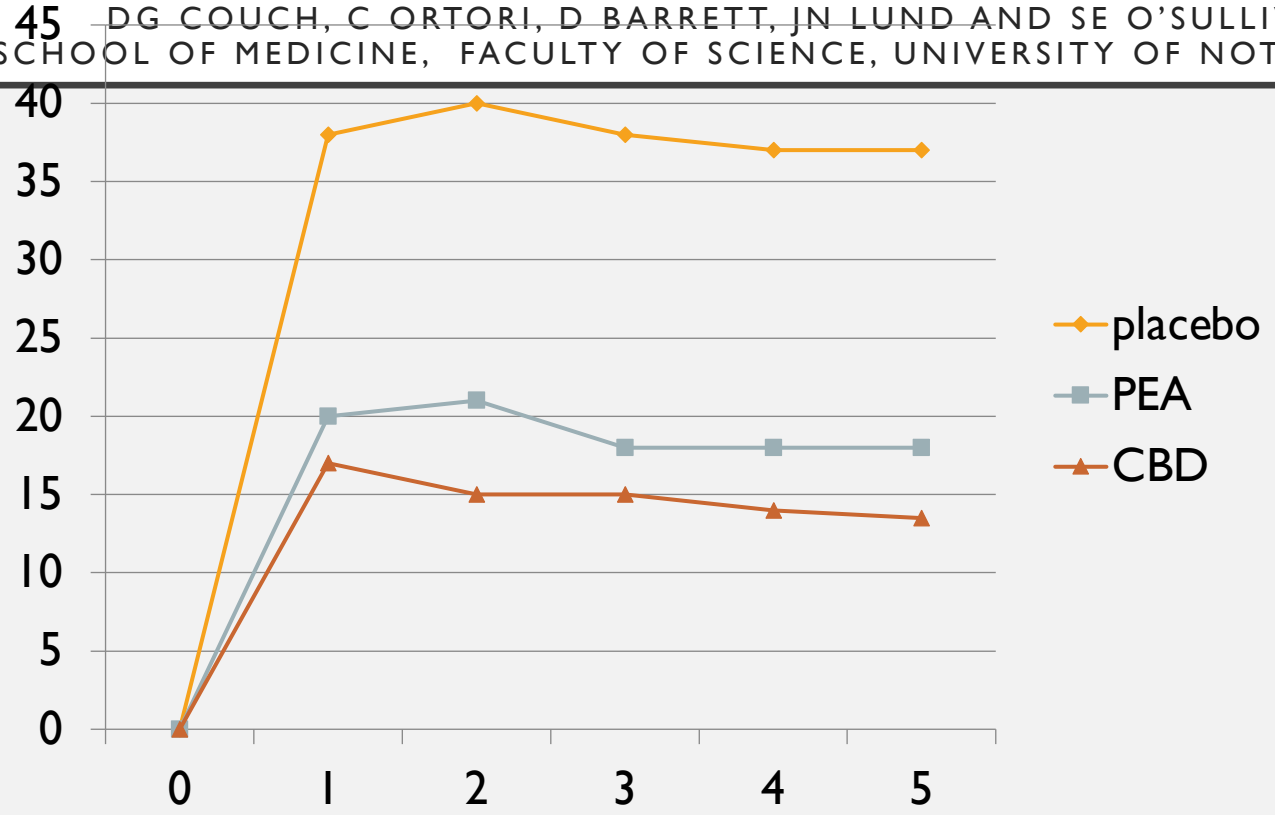
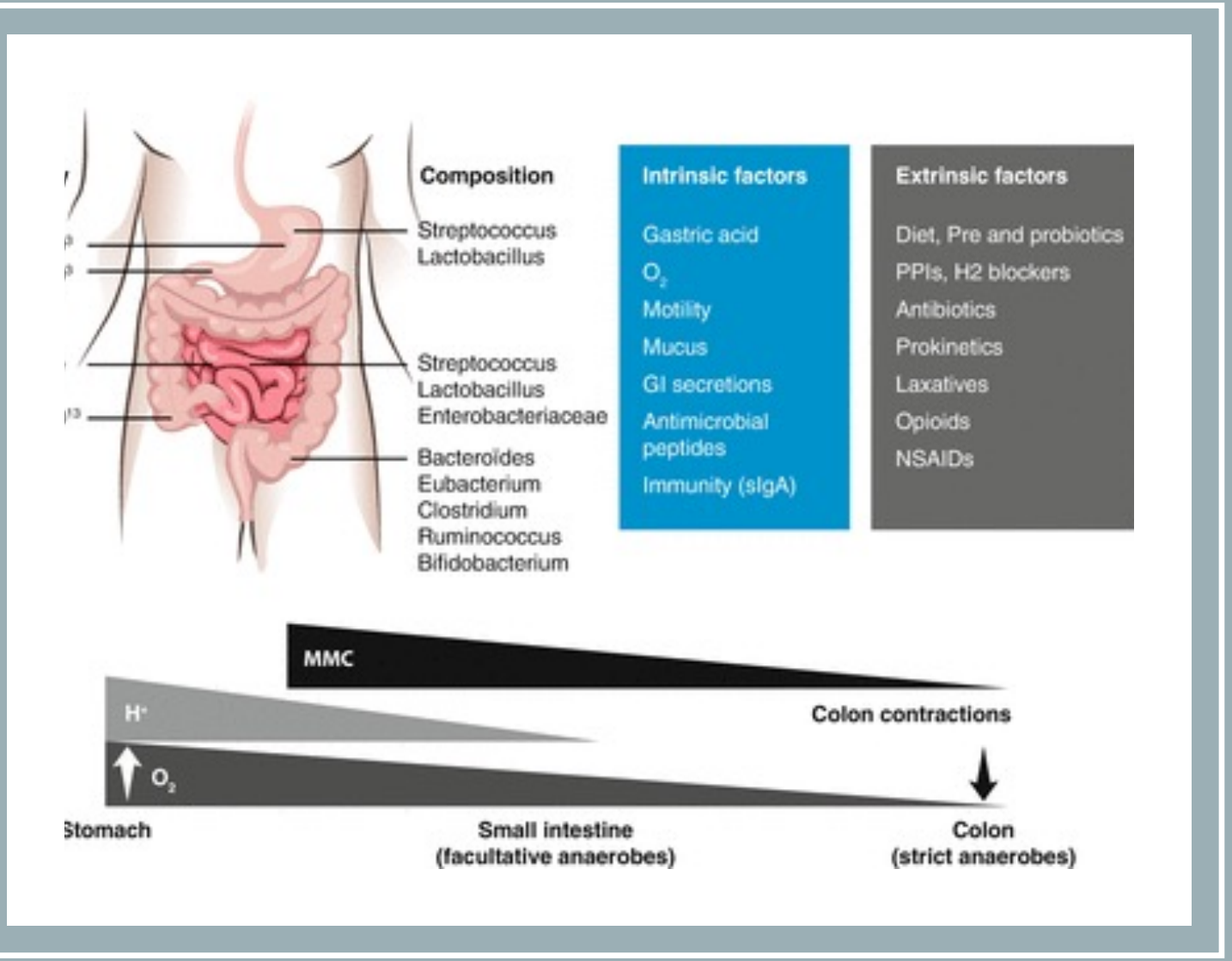
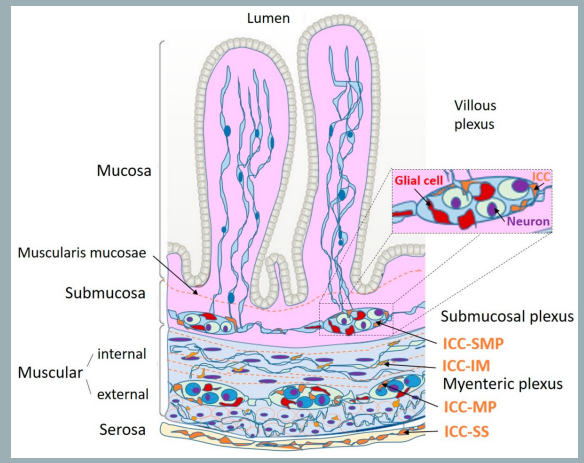


Figure 1 – The concentration ratios of urinary lactulose and mannitol over time in healthy participants treated with aspirin and either placebo, CBD or PEA, measured by LC MS. Results are expressed as mean ratios +/- SEM. Time points between groups were compared using two-way ANOVA using Dunnett's multiple comparisons test comparing to placebo at the same time point (*p



EXTRINSIC AND INTRINSIC FACTORS AFFECTING THE DISTRIBUTION AND COMPOSITION OF THE MICROBIOME

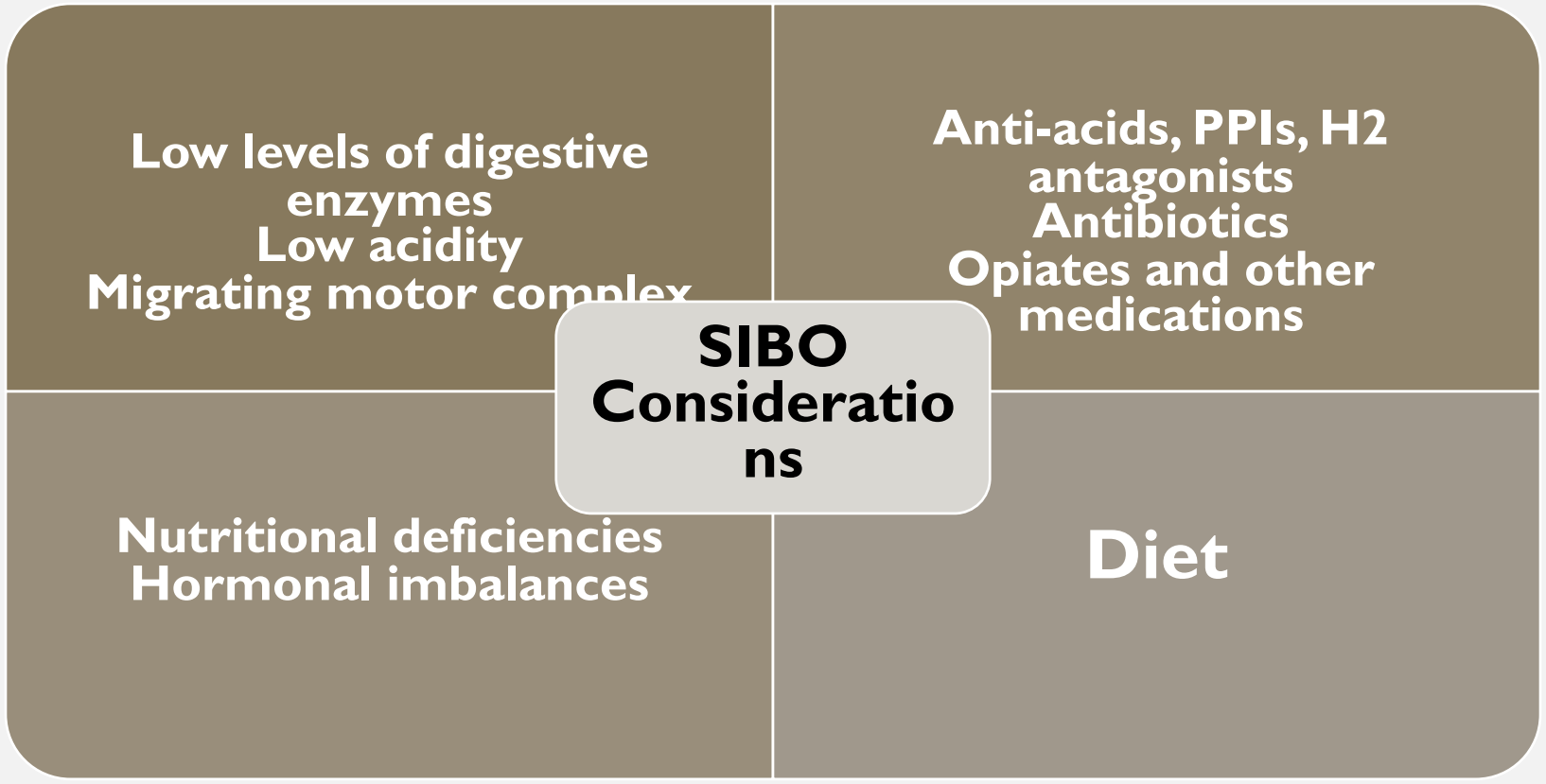


Organization of interstitial cells of Cajal (ICC) at submucosal and myenteric plexuses in the digestive tube.

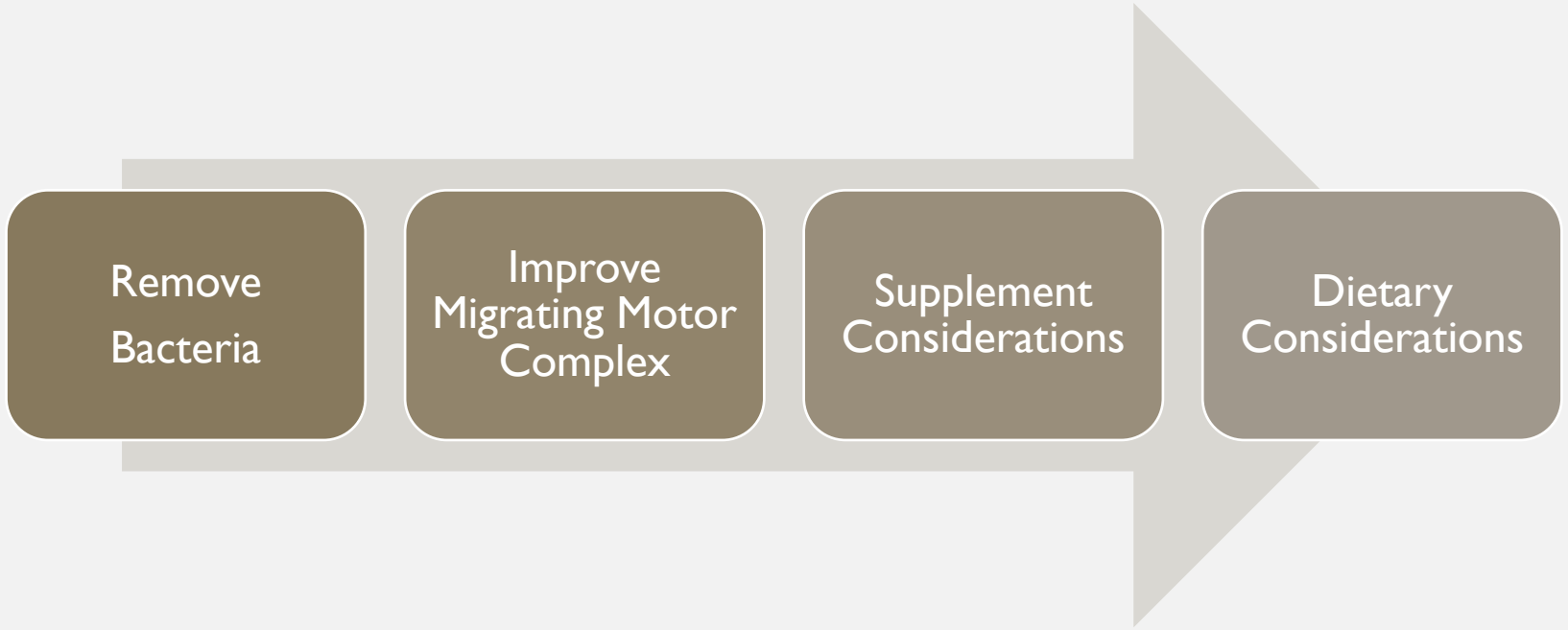
GABA-PRODUCING MICROORGANISMS ISOLATED FROM THE HUMAN GUT

Microorganism	Characteristic	References
Lactobacillus brevis DPC6108	Converts 10 and 20 mg/ml of MSG to GABA at 100% conversion rate	41
Lactobacillus brevis 15 f *Bifidobacterium angulatum* GT102	Efficient GABA producer	42
Bifidobacterium adolescentis 150	Possess antibiotic-resistant and antioxidant activity	43
Lactobacillus plantarum 90sk	GABA production is affected PLP addition; possesses antibiotic-resistant and antioxidant activity	42,43
Bacteroides fragilis KLE1758 *Bacteroides caccae KLE1911* *Bacteroides vulgatus KLE1910* *Bacteroides ovatus KLE1170* *Bacteroides dorei KLE1912*	Abundant levels negatively correlated with brain signatures associated with depression	37
Bacteroides spp.	Regulation of the GABAergic system in the human gut	36
Bifidobacterium adolescentis PRL2019 *Bifidobacterium adolescentis* HD17T2H	In vivo production of GABA with potential implication in gut–brain axis interactions	44

CONTRIBUTING FACTORS IN SIBO



THERAPEUTIC ACTION PLAN



TREATMENT PATHS

Antibiotic Treatment

1. Rifaximin 550 mg 3 times per day
   - Taken for 10-14 days
2. Followed by prokinetic
   - Erythromycin 50 mg at night for 3 months

Botanical Extracts

- **Garlic** (*Allium sativum*) – *allicin, alliin, gamma-Glutamylcysteine:*
  - 1 softgel 3 times per day for 30 days, then 1 daily
- **Berberine:**
  - 3 capsules 2 times per day
- **Organic Oil of Oregano** (*Origanum vulgare*) – minimum 80% carvacrol:
  - 2 capsules 2 times per day for 14 days, then 2 capsules daily for 15 days

Chedid, V., Dhalla, S., Clarke, J.O., et al. (2014). Herbal Therapy is Equivalent to Rifaximin for the Treatment of Small Intestinal Bacterial Overgrowth. *Global Advances in Health and Medicine, 3*(3), 16–24.

PROKINETICS

Prokinetics are substances that promote motility; they are promoters of the MMC.

- **Chewable Ginger:** 1,000 mg per day
- **5-HTP:** 50 mg at night

ENTERIC COATED PEPPERMINT OIL (ECPO)

ECPO Double Blind Study

Summary:

110 pts. with IBS were give ECPO or placebo 3–4 times daily 15 minutes before meals for 1 month. Percentage of patients improving:

Parameter	Placebo	ECPO
Abdominal pain	**79%**	**43%**
Abdominal distension	**83%**	**29%**
Stool frequency	**83%**	**33%**
Flatulence	**79%**	**22.5%**

Gastroenterology 32:765-8, 1997

ECPO is one of the most underutilized nutraceutical compounds

Has impressive clinical outcomes for gas/bloating associated with IBS and SIBO

Enteric coated allows bypassing of low pH environment (stomach).

Note: If stomach alkaline ECPO will NOT be effective. Need to bypass Stomach.

Supportive Nutrients

- **Pharma GABA® 100 mg:**
  - Start at 100 mg twice per day, move to 200 mg twice per day.
  - Helps up regulate parasympathetic response.

ELEMENTAL DIET

What is it?

1. Predigested nutrient drink that replaces all meals for up to 2 to 3 weeks.
2. Macronutrients are broken down into elemental forms; proteins into amino acids, fats in the from of medium chain triglycerides, carbohydrates simple sugars and supplemented with essential vitamins/minerals
3. This is used as an alternative to Antibiotic treatment; evidence shows its just as effective. As such there is a potential for this diet to limit the nutrients for more distally located bacteria of the small intestine.
4. ED starves bacteria but feeds patient. End result is improved breath test.

ELEMENTAL DIET

Background:

- Most studied strategy in dietary management
- Has been shown to be effective in reducing relapses in Crohn Disease patients

Surg Today. 2017 Dec;47(12):1519-1525. doi: 10.1007/s00595-017-1545-5. Epub 2017 May 22.

Adherence to an elemental diet for preventing postoperative recurrence of Crohn's disease.

Ohara N[1], Mizushima T[2,3], Iijima H[4], Takahashi H[1], Hiyama S[5,4], Haraguchi N[1], Inoue T[4], Nishimura J[1], Shinzaki S[4], Hata T[1], Matsuda C[1], Yamamoto H[1,6], Doki Y[1], Mori M[1].

Author information

Abstract

PURPOSE: An elemental diet (ED) can suppress inflammation in patients with Crohn's disease (CD); however, adherence to this diet is difficult. We examined the correlation between ED adherence and the postoperative recurrence of CD.

METHODS: The subjects of this study were 38 patients who underwent intestinal resection with anastomosis. We defined ED adherence as consuming the average daily ED dose (≥900 kcal/day) for 2 years after surgery. Patients who did not adhere to the ED were allocated to the non-ED group. We diagnosed symptomatic recurrence using the CD activity index and endoscopic recurrence using the Rutgeerts' score.

RESULTS: The ED and non-ED groups comprised 21 and 17 patients, respectively, with ED adherence of 55.3% (21/38). At the initial endoscopy, symptomatic and endoscopic recurrence rates were 4.8 and 14.3%, respectively, in the ED group, and 23.5 and 41.2%, respectively, in the non-ED group (P = 0.152 and P = 0.078, respectively). The overall symptomatic recurrence-free duration was significantly longer than the endoscopic recurrence-free duration (P = 0.022). Symptomatic and endoscopic recurrence-free durations were longer in the ED group than in the non-ED group (P = 0.003 and P = 0.021, respectively), and ED adherence was a prognostic factor for endoscopic recurrence (HR = 2.777, 95% CI = 1.036-8.767, P = 0.042).

CONCLUSION: Maintaining ED adherence for 2 years after surgery improved the symptomatic and endoscopic recurrence-free durations.

KEYWORDS: Adherence; Crohn's disease; Elemental diet; Postoperative recurrence

Aliment Pharmacol Ther. 2006 Nov 1;24(9):1333-40.

Effectiveness of an 'half elemental diet' as maintenance therapy for Crohn's disease: A randomized-controlled trial.

Takagi S[1], Utsunomiya K, Kuriyama S, Yokoyama H, Takahashi S, Iwabuchi M, Takahashi H, Takahashi S, Kinouchi Y, Hiwatashi N, Funayama Y, Sasaki I, Tsuji I, Shimosegawa T.

Author information

Abstract

BACKGROUND: Although thiopurines have a proven role in maintenance therapy for Crohn's disease, an alternative therapy is needed for patients intolerant or resistant to thiopurines.

AIM: To evaluate the effectiveness of home enteral nutrition as a maintenance therapy regimen in which half of the daily calorie requiremer is provided by an elemental diet and the remaining half by a free diet. We refer to this home enteral nutrition therapy as 'half elemental diet

METHODS: Between 2002 and 2005, 51 patients in remission from two hospitals were randomly assigned to a half elemental diet group (n 26) or a free diet group (n = 25). The primary outcome measure of this study was the occurrence of relapse over the 2-year period.

RESULTS: The relapse rate in the half elemental diet group was significantly lower [34.6% vs. 64.0%; multivariate hazard ratio 0.40 (95% 0.16-0.98)] than that in the free diet group after a mean follow-up of 11.9 months. Compliance was similar in the two groups. No adverse event occurred in any of the patients throughout the study.

CONCLUSION: This randomized-controlled trial shows the effectiveness of an half elemental diet, which is a promising maintenance therap for Crohn's disease patients.

ELEMENTAL DIET (HALF) PROTOCOL

1. Half Elemental Diet:

Patient consumes 50% of daily caloric needs from ED and the other 50% from whole foods. Half ED's or sometimes called partial ED's are found in the scientific literature to help with maintaining remission of Crohn's Disease. They can also be used when compliance becomes difficult for patients on Full ED's for SIBO and IBS. Half ED's can also be used as starting and exiting conduits to Full ED's easing the patient experience and possibly improving compliance.

Application: Maintaining remission of Crohn's after completion of Full ED, used as conduits to Full ED's and in place of ED's for difficult compliant patients. Again this will be at the discretion of physician.

Dosage: The dosage supplied by the Half ED is 50% of the daily total calories divided into 200-300 calorie servings, consumed every 2 hours (use either first or second half of day). Whatever part of the day where the Half ED is NOT used the patient consumes a whole food diet as per usual. Calories consumed by Half ED would be approximately 900 calories, however, to accurately calculate the patient total needs the physician should determine BMR and then use the Harris-Benedict equation (see below) to calculate total caloric requirement (divide this by half to give you the calories needed from the Half ED).

Duration: There are no published reports regarding the duration of a Half ED, however, 4-6 weeks can be a good starting point. The duration would be calculated at the discretion of the physician taking into account various patient symptomology and other markers deemed important.

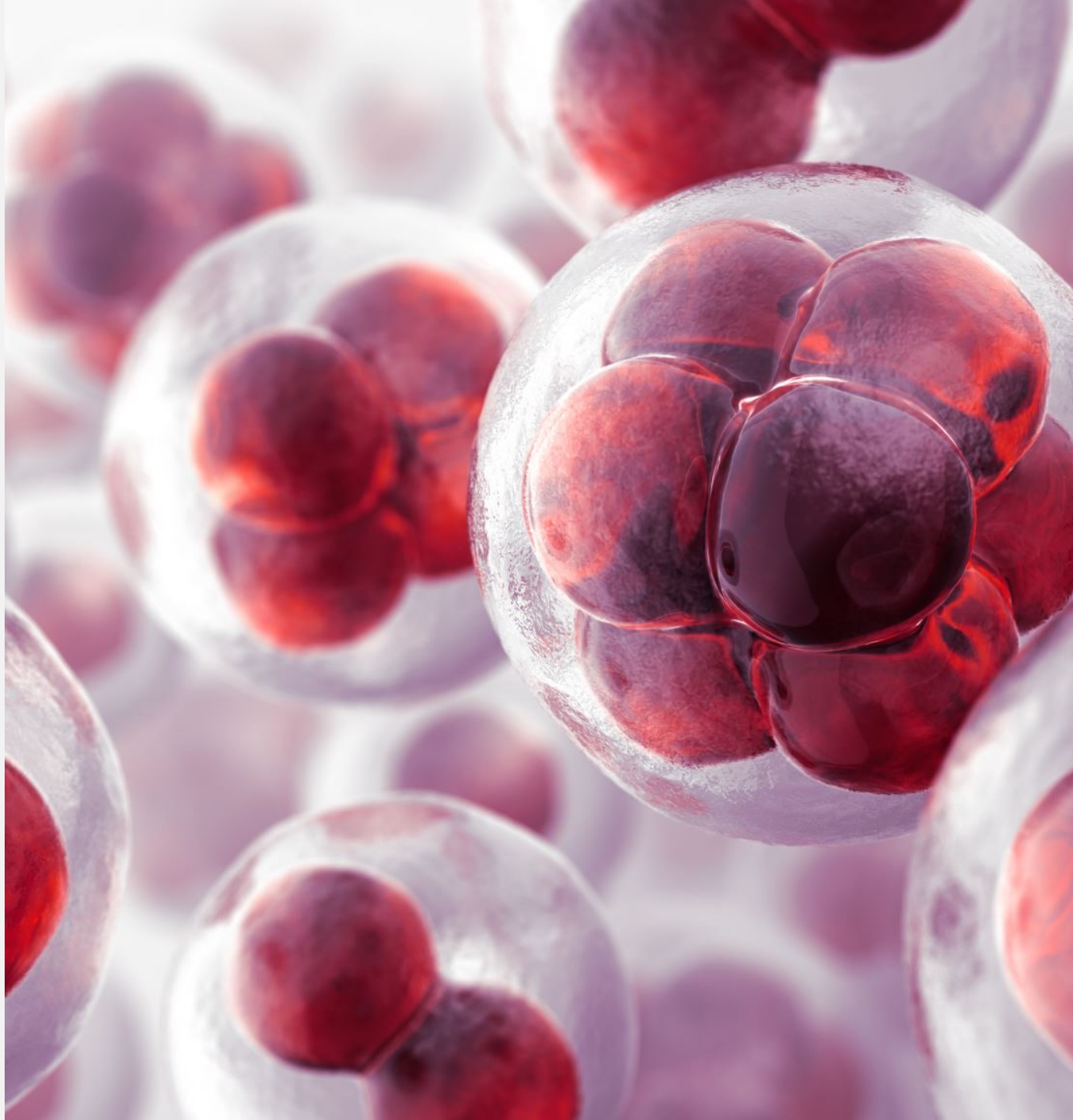
ELEMENTAL DIET

- Do for 14 days – starves and kills bacteria while feeding the patient
- Patient lives off drink – cannot eat anything for 2–3 weeks
- Absorbed in first two feet of small gut
- Kcal: 1500–1800 kcal/day – 5–6 packs a day (packet = 300 kcal)
- Can do a ½ elemental diet when one meal is the drink
- DO NOT TAKE antibiotics at same time

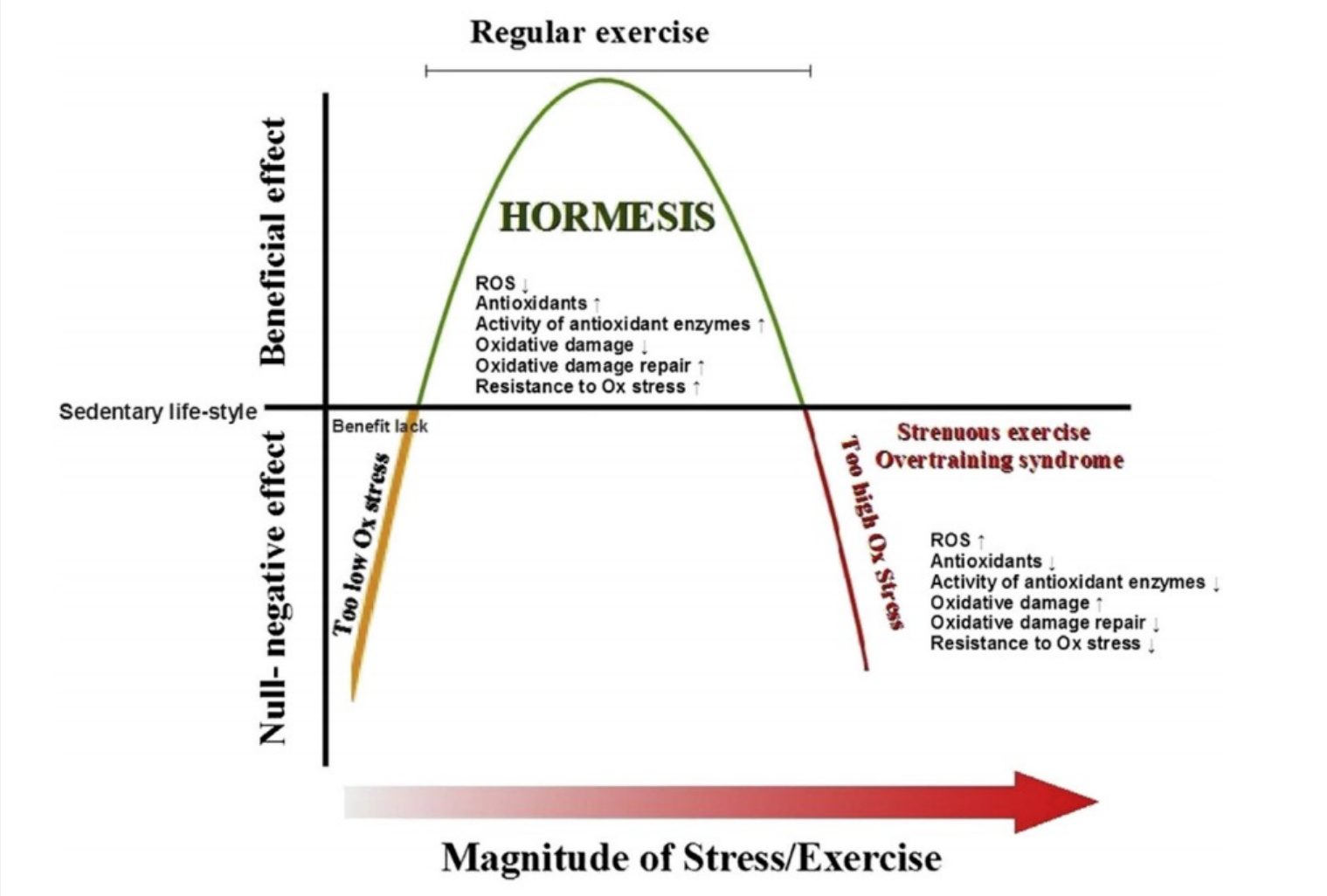
HORMESIS

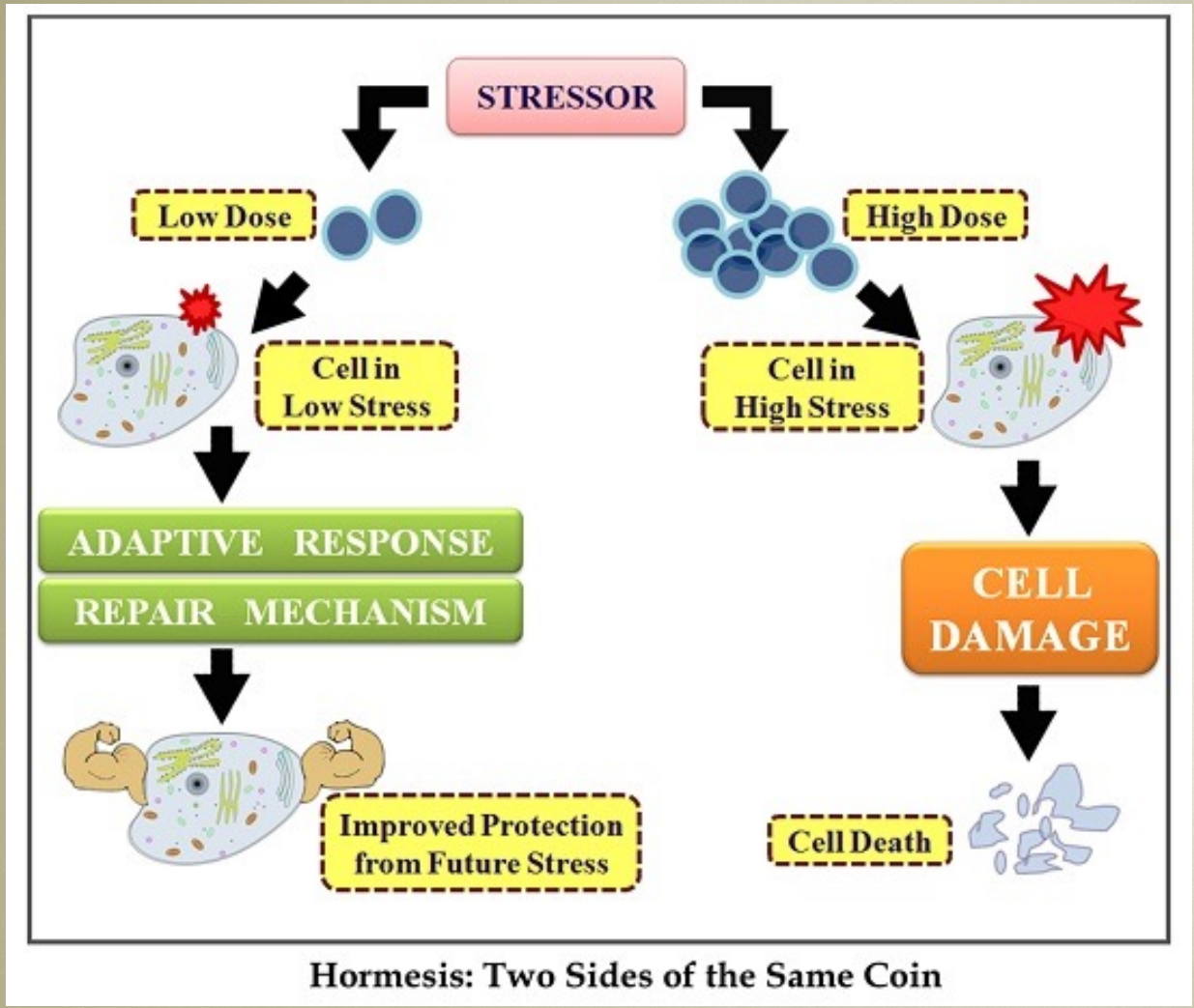
Definition:

- The biology of beneficial adaptions to stress
- A process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at a higher doses induces and adaptive beneficial effect on the cell or organism



HORMESIS


Regular exercise
HORMESIS
ROS ↓
Antioxidants ↑
Activity of antioxidant enzymes ↑
Oxidative damage ↓
Oxidative damage repair ↑
Resistance to Ox stress ↑
Beneficial effect
Sedentary life-style
Benefit lack
Too low Ox stress
Too high Ox Stress
Strenuous exercise
Overtraining syndrome
ROS ↑
Antioxidants ↓
Activity of antioxidant enzymes ↓
Oxidative damage ↑
Oxidative damage repair ↓
Resistance to Ox stress ↓
Null- negative effect
Magnitude of Stress/Exercise



Hormesis: Two Sides of the Same Coin

HORMESIS SPECTRUM

Hormesis defines the limits of lifespan

This commentary provides a novel synthesis of how biological systems adapt to a broad spectrum of environmental and age-related stresses that are underlying causes of numerous degenerative diseases and debilitating effects of aging. It proposes that the most fundamental, evolutionary-based integrative strategy to sustain and protect health is based on the concept of hormesis. This concept integrates anti-oxidant, anti-inflammatory and cellular repair responses at all levels of biological organization (i.e., cell, organ and organism) within the framework of biphasic dose responses that describe the quantitative limits of biological plasticity in all cells and organisms from bacteria and plants to humans. A major feature of the hormetic concept is that low levels of biological, chemical, physical and psychological stress upregulate adaptive responses that not only precondition, repair and restore normal functions to damaged tissues/organs but modestly overcompensate, reducing ongoing background damage, thereby enhancing health beyond that in control groups, lacking the low level "beneficial" stress. Higher doses of such stress often become counterproductive and eventually harmful.

Ageing Research Reviews 91 (2023) 102074

Some Considerations: Why We Age…			
Concept	Genetic Program	Damage Accumulation	Hyperfunction
Advocates	Jay Olshanisky	Dentram Harman	Valter Longo
Drivers	Fixed Program Maximum Lifespan Telomeres	Free Radicals DNA damage Mito damage	Overactive cellular processes: mTOR, inflammation, cell division, lipogenesis
How To Live Longer	Hack system, Program with hormones Supplements and telomerases	Take anti-oxidants Avoid Toxins, UV Cell Phones	Hormesis, especially after middle age, calorie restriction, exercise, cold, hypoxia, bitter herbs
Problems with theory	Hormones and telomerase actually increase cancers	Antioxidants impair oxidative cellular signaling and repair Naked mole rat	Limited evidence in humans

THEORIES ON WHY WE AGE