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THE MICROBIOME AND WELL BEING: FROM GUT-BRAIN AXIS TO INFLAMMATION

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Dr. Mahmoud Ghannoum

"Mahmoud Ghannoum, the scientist who is now known as the leading microbiome researcher in the world"
- The Washington Post

- Received MSc in Medicinal Chemistry and PhD in Microbial Physiology from University of Technology in England
- MBA from the Weatherhead School of Management at Case
- Tenured Professor and Director of the Integrated Microbiome Core and Center for Medical Mycology, Case Western Reserve University and University Hospitals Cleveland Medical Center
- Past President of the Medical Mycological Society of the Americas
- Fellow of the American Academy of Microbiology, and the European Confederation of Medical Mycology
- Awarded the Freedom to Discover Award from Bristol-Myers Squibb for his pioneering work on microbial biofilms
- **Top 1%** of most cited scientists in the world, and **Top 0.1 percent** of cited microbiologists (143 out of 134,369 microbiologists)
- Coined the term "Mycobiome"
- Fellow of the Infectious Disease Society of America

450+
Peer-Reviewed Publications

23,000+
Citations in Scientific Literature

$25M+
NIH Funded Research

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MICROBIOME OVERVIEW

- Recent advances in Next Generation Sequencing ushered an explosion of research into the communities of microorganisms living in our bodies referred to as the microbiome
- Identity of the microbes residing in our body
- Describe the associations/correlation between the microbiome and digestive and non-digestives issues and diseases
- Rebalancing the microbiome to promote human health and combating disease

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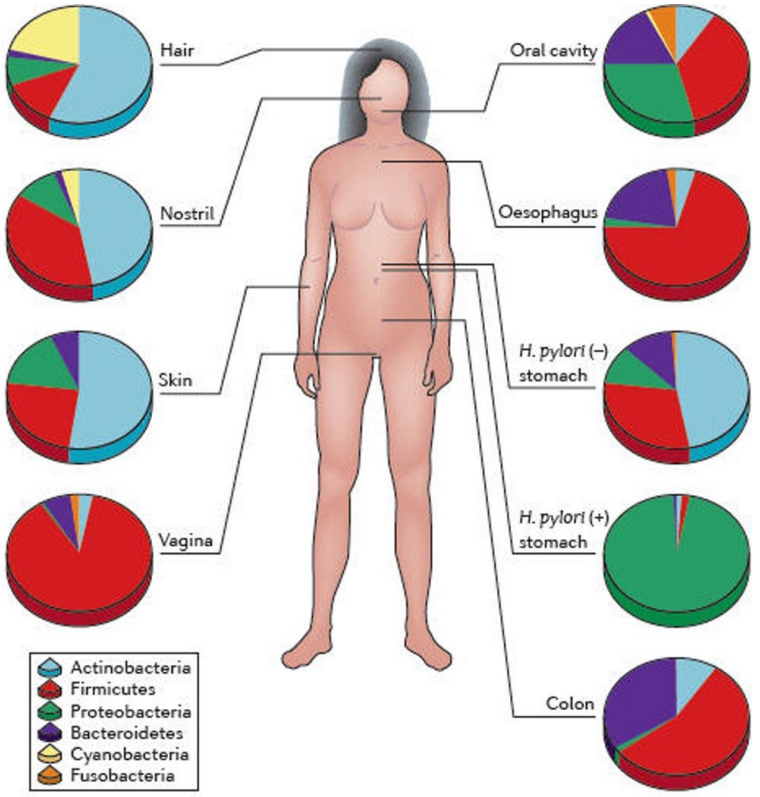
Microbiome Definition: Meet Your MICROBIOME

- The microbiome is a collection of microbes that live in and on the human body.
- One hundred trillion bacteria make up the microbial ecosystem of the human body far exceeding human cells.
- Each individual has 23,000 human genes and about 600,000 microbial genes. Thus, the human component contributes only to less than 4% of the total hologenome (Host + all its symbiotic microbes).
- Interactions with these microbes take place daily at the level of the skin, in the urogenital tract, mouth, pharynx, and respiratory system, and the digestive tract which contains the largest density and diversity of microorganisms.



Compositional differences in the microbiome by anatomic site
Cho, L and **Blaser, M. The Human Microbiome: at the interface of health and** Disease, 2012. Nat Rev Genet. ; 13(4): 260–270. doi:10.1038/nrg3182.

Najla El-Jurdi1 ... Mahmoud A. Ghannoum. Curr Clin Micro Rpt. DOI 10.1007/s40588-017-0063-9

Gregory et al. Cell Host & Microbe *28*, 724–740, November 11, 2020 a 2020 Elsevier Inc.

The Microbiota is More Than Bacteria!

- Fungi also inhabit our body alongside bacteria, viruses, archaea and parasites
- When one microbial community is knocked out another can cause overgrow and take over
- The three main microbial communities are known as: bacteriome, mycobiome, and virome
- Imbalance of the microbiota can lead to **dysbiosis** or an **overgrowth of pathogenic bacteria and/or fungi**

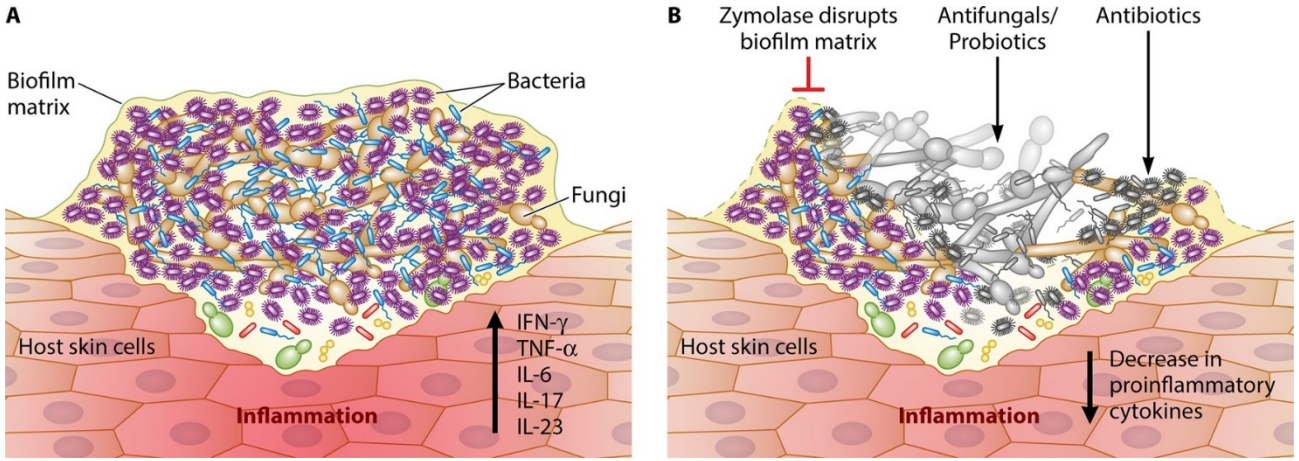
As an example:

- Eliminating *Lactobacillus* will lead to overgrowth of *Candida*

Lactobacillus (a beneficial bacteria) 

Candida (Pathogenic fungi) 

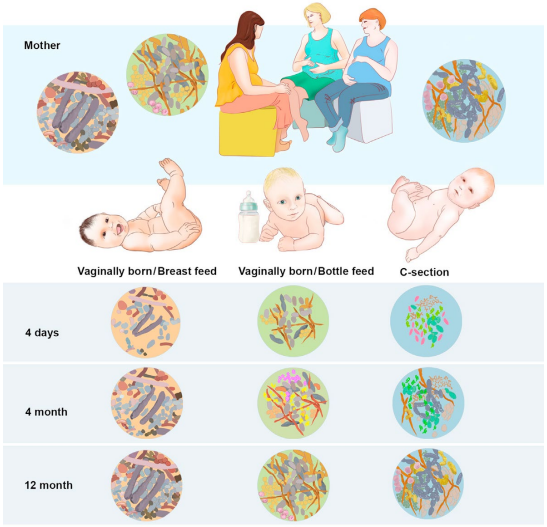
Cooperation Between Fungi and Bacteria



- Chronic wounds are complex systems of multispecies fungal and bacterial biofilms.
- Biofilms provide a protected milieu for these microbes
- The fungal hyphae and microbial-secreted enzymes/metabolites facilitate invasion of the skin leading to host tissue damage and inflammatory response (increase in proinflammatory cytokine production, panel A)
- Disruption of the biofilm matrix by zymolase unmasking the microbes (Panel B)
- Consequently, treatment with antimicrobials leads to microbial cell death and a decrease in the production of proinflammatory cytokines

Ghannoum M. 2016. mBio 7(6):mBio.01951-16. doi:10.1128/mBio.01951-16

When Do We Acquire Our Microbiome: It Starts Before Birth!



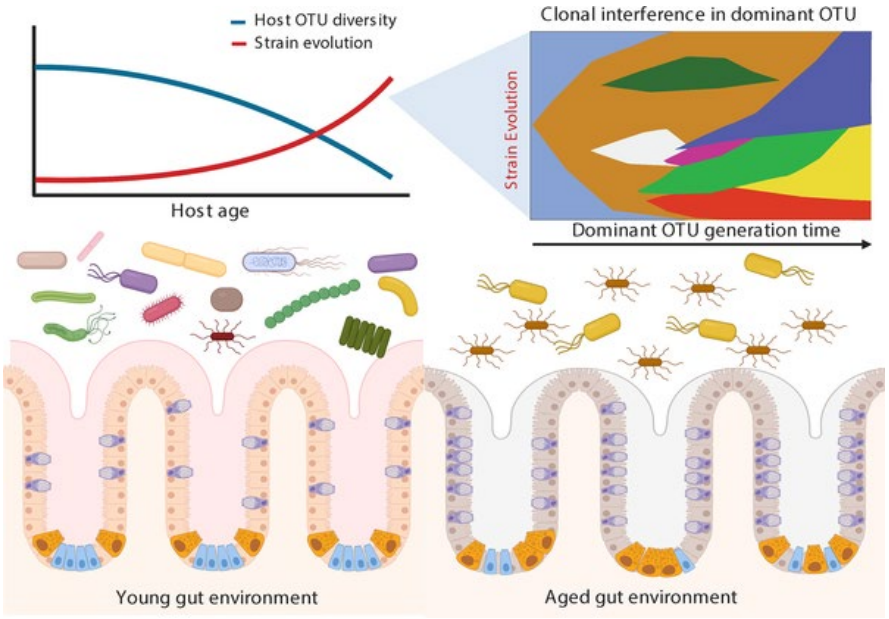
- Immediately after birth, humans become colonized by a range of microorganisms (on the skin, oral cavity, gastrointestinal tract and urogenital tract).
- Large majority are acquired from the mother during delivery influenced by whether the delivery is through the vagina or C-section.
- The microbiota in the gut of newborns is dramatically shaped by diet and varies depending on whether the infant is fed with maternal milk or formula.

Bäckhed et al., 2015, Cell Host & Microbe17, 690–703May 13, 2015ª2015 Elsevier Inc.http://dx.doi.org/10.1016/j.chom.2015.04.004

Fredrik BackhedJosefine RoswallJovanna DahlgrenJun Wang

Gut Microbiota Undergoes Dynamic Changes During Host Aging

- Changes in host intestinal cell composition and architecture occurring during aging are matched by a decrease in the microbiota diversity
- Age-related decrease in microbial diversity leads to larger population size for a few age-associated microbial species, increasing the chances for the evolution of novel potentially pathogenic microbial strains
- Although individual gut microbiota are largely unstable in the first years of life, they become more stable during adulthood
- The microbiome stability is affected by onset of disease and frailty



Aleman FDD, Valenzano DR (2019) Microbiome evolution during host aging. PLOS Pathogens 15(7): e1007727. https://doi.org/10.1371/journal.ppat.1007727

OTU, Operational Taxonomic Unit

Why is There Heightened Attention on the Microbiome?

Science has shown that the gut microbiome is linked to good health and diseases conditions

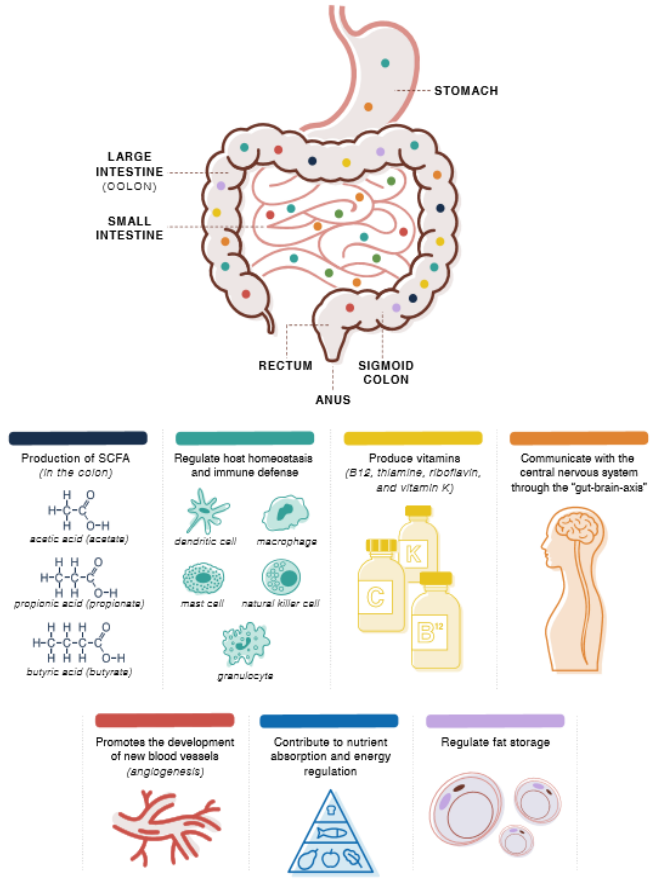
Microbiome directly/indirectly influences:

- Weight
- Sleep
- Digestion
- Skin health
- Immune support
- Chances for developing disease
- Mood
- How you respond to certain drugs (e.g. checkpoint inhibitors)

Microbes play a number of functions that support our well-being

The microbiome supports different bodily functions by:

- Regulating homeostasis (balance)
- Producing a number of beneficial compounds (e.g. vitamins)
- Communicating with the brain (e.g. Gut-Brain Axis)
- Promoting development of blood vessels
- Contributing to nutrient absorption and energy regulation
- Regulating fat storage



The Microbiome Contributes to the Digestive System in Many ways

- **Supports the health of your gastrointestinal lining (cells that cover your gut)**
  - Promote tight junction integrity (sealing the gut)
  - Maintain gut impermeability
- **Supports digestion**
  - Water re-absorbed in colon
  - Increase nutrient absorption/biosynthesis
  - Vitamin K (esp. K2) + B12
- **Produce essential metabolites (e.g. SCFA) can help our body in different ways:**
  - Main energy source for colonocytes
  - Improving immune function
  - Anti-inflammatory (Butyrate)
  - Helps regulate pH balance
- **Helps in excretion**

Beyond the Gut: When Your Microbiome is Diverse, It Benefits You in Many Ways

Immune System
- Cytokines (pro vs anti-inflammatory)
- Systemic inflammation

Hormone Balance
- Estrobolome (betaglucuronidase)
- Ghrelin & leptin
- Hormone-like metabolites & precursors (ex: Tryptophan)
- HPA hormones

Neurotransmitter Production
- ~90% of Serotonin is produced in the gut

Gene Expression
- Alteration of the epigenome

Control pathogens via distinct mechanisms:

- Compete for nutrients and produce antimicrobial molecules/metabolites that affect the survival/virulence of pathogens
- Promote the production of antimicrobial peptides by epithelial cells and reinforce tight junctions
- Modulate the function of dendritic/innate cells that promotes the induction of effector T and B cells responses against pathogens
- **When uncontrolled, this adjuvant property of the microbiota can promote inflammatory and autoimmune disorders**

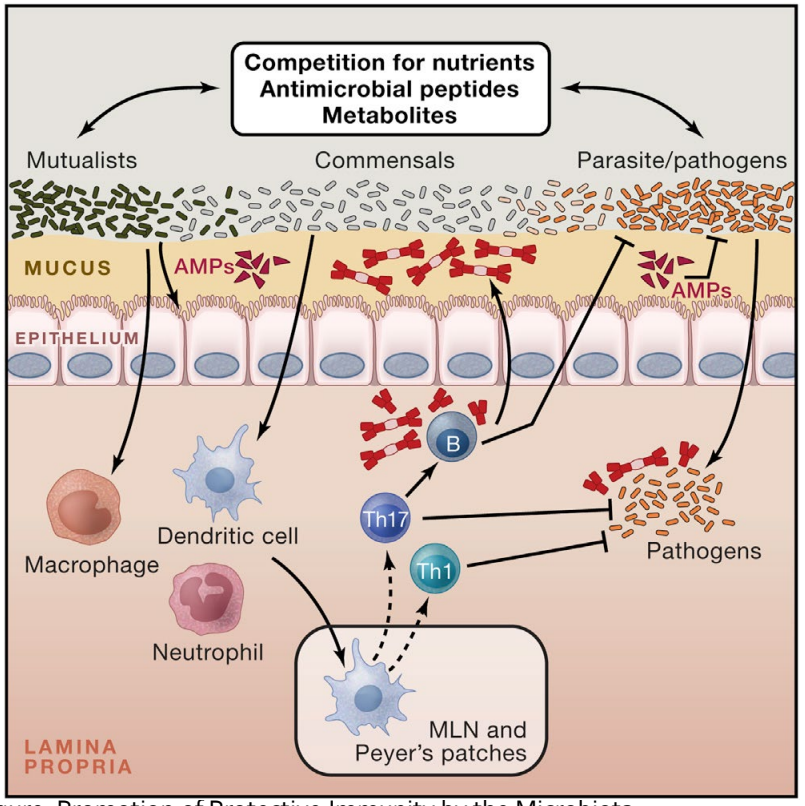


Figure: Promotion of Protective Immunity by the Microbiota

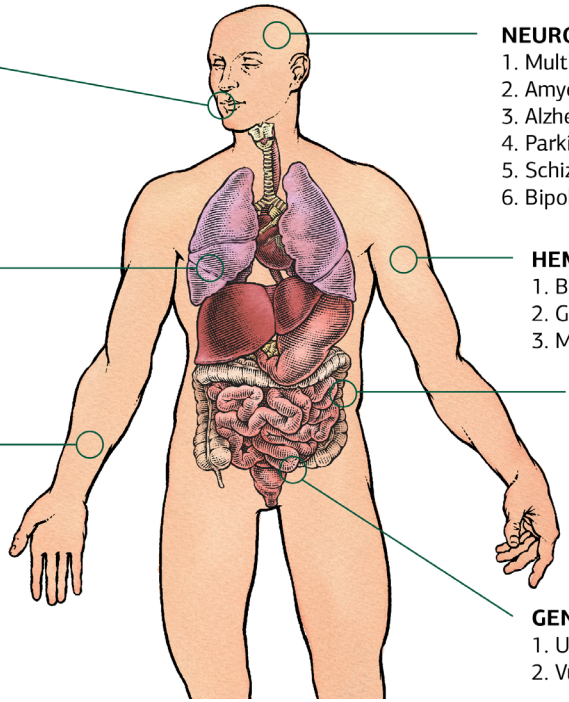
Belkaid, Y and Hand, T. Role of the Microbiota in Immunity and Inflammation. http://dx.doi.org/10.1016/j.cell.2014.03.011

Health Implications of The Microbiome Negatively Impact a Number of Conditions

ORAL CAVITY
1. Tongue cancer
2. Oral candidiasis
3. Dental caries
4. Halitosis
5. Periodontal & Endodontia
6. Oral squamous cells carcinoma

RESPIRATORY TRACT
1. Cystic Fibrosis
2. Asthma
3. Lung transplant
4. Pulmonary disease in HIV

SKIN
1. Atopic dermatitis
2. Psoriasis
3. Seborrheic dermatitis
4. Wound healing
5. Tinea versicolor



NEUROPSYCHIATRIC
1. Multiple sclerosis
2. Amyotrophic lateral sclerosis
3. Alzheimer
4. Parkinson's disease
5. Schizophrenia
6. Bipolar disorder

HEMATOLOGY
1. Bone marrow transplant in multiple myeloma
2. Graft versus host disease
3. Mycobiome in HIV

GASTROINTESTINAL TRACT
1. Inflammatory bowel disease
2. Pancreatic cancer
3. Alcoholic liver disease
4. Colorectal cancer
5. *C. difficle infections*
6. Irritable bowel syndrome

GENITOURINARY
1. Urinary tract candidiasis
2. Vulvovaginal candidiasis

Ghannoum, Mukherjee. The Human Mycobiome and its Impact on Health and Disease. *Current Fungal Infection Reports* 2013; 7: 345-350

Health Implications of the Microbiome

Specifically, studies have shown that dysbiosis is associated with the development and/or progression of a broad spectrum of health conditions or diseases, including the following:

- Inflammatory Bowel disease
- Irritable Bowel Syndrome
- Celiac disease
- Systemic Lupus Erythematosus
- Type-1 Diabetes
- Type-2 Diabetes
- Rheumatoid Arthritis
- Atopic disease such as food allergies and childhood allergic asthma
- Obesity
- Hypertension

The recognition that the microbiome can impact our health positively and negatively led to a growing interest in personalized microbiome analysis among health care practitioners and their patients.

Your Microbiome is Special: It is Like Your Fingerprint

- Each person has his/her microbiome that is different from those of others
- Diet and lifestyle can predict many aspects of your microbiome profile (i.e. what bacteria and fungi live in and on you)
- Studies have shown that "normal" subjects can fall into one of three different bacterial profiles:
  - Group 1: Healthy human profile
  - Group 2: Inflammatory microbiome profile
  - Group 3: Obesity-prone microbiome Profile
- Each one of these profiles may/may not contain the fungus *Candida*
- Therefore, "normal" individuals could fall into one of six profiles

Microbiome in Balance: Homeostasis

- **Homeostasis:** a state of physiological stability within a system, and it is a marker of good health.
- The balanced state of the internal workings of the human body.
- When *homeostasis* is disrupted, problems can take root and ripple throughout the whole system. If uncorrected, will result to disease, dysfunction, even death.
- The microbiome follows similar path. Microbiome in balance leads to better digestive as well as overall health.

When the Microbiome is in Balance You have Good Health

What is microbiome balance?

- Microbial communities (bacterial and fungal) in your body are in harmony with each other, as well as in symbiosis with you, the host
- This leads to a better gut health with less inflammation and associated gastrointestinal symptoms
- Therefore, having a balanced microbiome is our target to maintain optimal health and wellness
- What is critical to know is that balancing and maintaining the microbiome is in your hand to a large extent and it is essential to having good gut health and overall health

When the Microbiome is Out of Balance (in Dysbiosis) Your Body Suffers

- Microbiome imbalance, called Dysbiosis, is the opposite of homeostasis
- Dysbiosis refers to microbial imbalance or maladaptation on or in the body
- Having imbalance:
  - Puts your beneficial microbes at risk and
  - Gives the pathogenic microbes a foothold, leading to uncomfortable symptoms and serious disease or infection
- This imbalance is the result of complex interactions between fungi and bacteria brought about by diet, lifestyle, and/or disease
- These communities work together for their benefits and ours or their benefits and not us

Ghannoum, M. 2016. Cooperative Evolutionary Strategy between the Bacteriome and Mycobiome Commentary mBio 7(6):mBio.01951-16. doi:10.1128/mBio.01951-16.

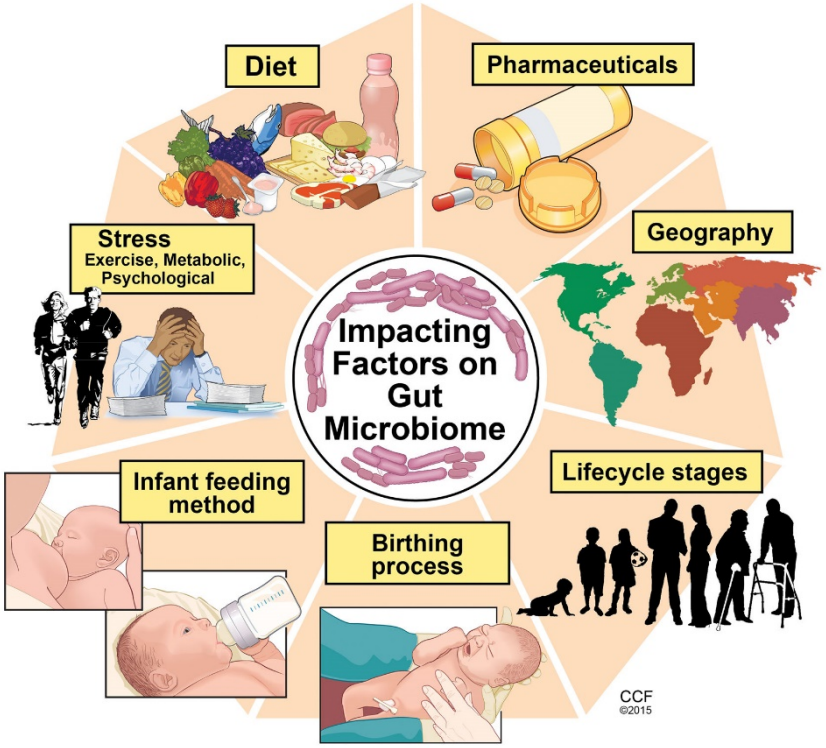
Dysbiosis has been linked to countless diseases

- Intestinal disorders [e.g. inflammatory bowel disease, and irritable bowel syndrome (IBS)]
- Allergies
- Asthma
- Metabolic syndrome
- Cardiovascular disease
- Obesity

Therefore, there is an urgent need to identify approaches that can rebalance and maintain a healthy microbiota.

Factors That Influence the Balance of Your Microbiome

A pre-request to rebalancing and maintaining Microbiome balance is knowing what factors can affect it positively and negatively.



Cresci G. and Bawden 2015. The Gut Microbiome: What we do and don't know Nutr Clin Pract. 2015 December ; 30(6): 734–746. doi:10.1177/0884533615609899.

Medications Can Contribute to Dysbiosis

Broad spectrum antibiotics
Antibiotic means ***antibacterial***, and these medications wipe out bacteria— not just the pathogenic bacteria causing your illness, but many of the good guys as well

Antifungals
Antifungals can be just as disruptive as antibiotics because they remove fungi that could be contributing to healthy microbiome balance

Certain other medications
Talk to your doctor before stopping any prescribed medication, but you should know that the following kinds of medications could be contributing to microbiome imbalance:

- Acid-reducing drugs
- Non-steroidal anti-inflammatory drugs (NSAIDs)
- Hormone-manipulating drugs
- Corticosteroids
- Diabetes drugs
- Antipsychotics and anticancer/chemotherapy drugs

Personal care products can disrupt microbiome balance, including:

- Mouthwash, shampoo, skin cream, soap
- Anything toxic that goes into or onto your body is a potential microbiome disruptor

Impact of antibiotics on long-term physiology through microbiota changes

- The gut microbiota has been shown to influence the development of the host's immune system in addition to being implicated in adipose, muscle, and bone tissue growth.
- New evidence indicate that the gut microbiota may impact stem-like cell populations suggesting a new way in which the gut microbiota may be regulating tissue development.
- Antibiotics alter the gut microbiota, which may change the course of these developmental pathways, leading to variations in long-term physiology.

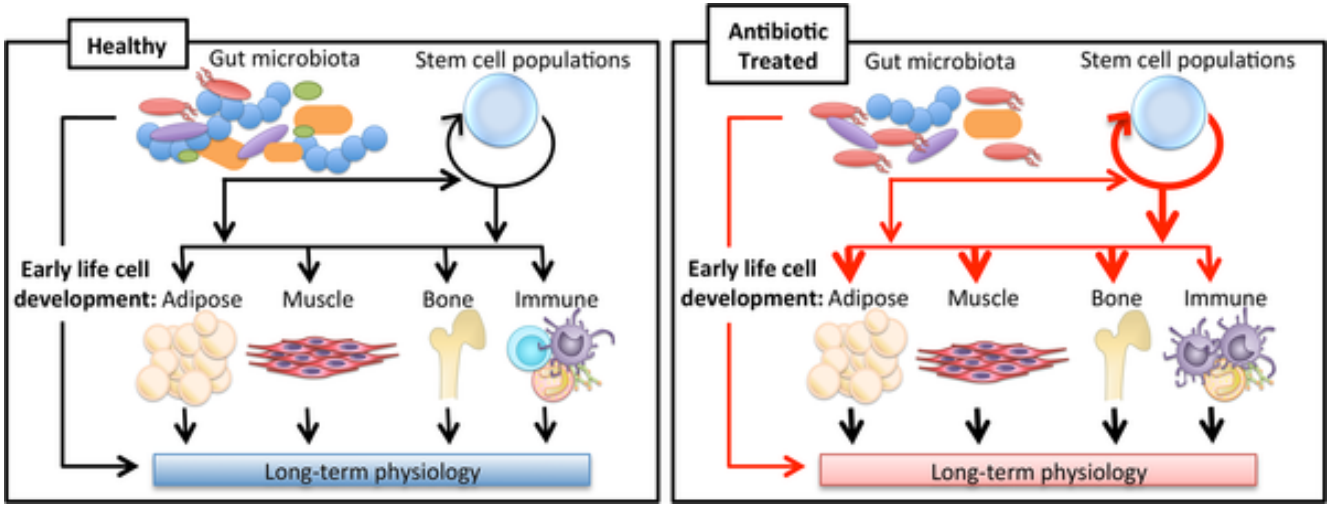
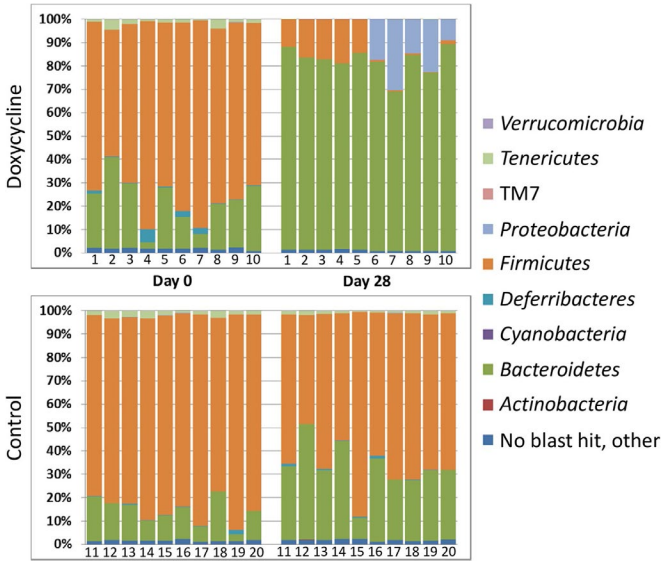


Figure. Impact of antibiotics on long-term physiology through microbiota changes

Schulfer A, Blaser MJ (2015) Risks of Antibiotic Exposures Early in Life on the Developing Microbiome. PLOS Pathogens 11(7): e1004903. https://doi.org/10.1371/journal.ppat.1004903
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004903

Effect of Broad Spectrum Antibiotics on Gut and Skin Microbiome



Doxycycline, a broad-spectrum antibiotic, induces dysbiosis in female C57BL/6NCrl mice relative abundance at the phylum level.

- Bar charts showing the bacterial composition of the same control and doxycycline-treated mice on days 0 and 28
- Each bar represents an individual animal (DOX animals numbered 1–10, control animals numbered 11–20)

Boynton et al. 2017. Doxycycline induces dysbiosis in female C57BL/6NCrl mice. BMC Res Notes (2017) 10:644 DOI 10.1186/s13104-017-2960-7

Minocycline, A Broad Spectrum Antibiotic

Causes microbial dysbiosis in the skin and gastrointestinal tract of acne patients

- Just as with the intestinal microbiome, skin microbiota is also impacted by the use of broad-spectrum oral antibiotics
- A study by Thompson *et. al.* investigating the effect of minocycline showed that this drug taken orally not only affected the gut microbiome but also the skin microbial community
- Post treatment, the **gut microbiota** of acne patients was significantly depleted in probiotic species including: *Lactobacillus salivarius*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudolongum*, and *Bifidobacterium breve* (*P* values <0,042)
- While the **skin bacterial community** of these patients was:
    - Enriched with *Leuconostoc mesenteroides*, a gram-positive, non-motile, vancomycin-resistant pathogen *(P*=0.028), and
    - Depleted in *Staphylococcus epidermidis* (*P*=0.009), a skin probiotic bacterium, that inhibits *C. acnes* growth, and affect the inflammatory process

Thompson KG, *et al. 2020.* Minocycline and its impact on microbial dysbiosis in the skin and gastrointestinal tract of acne patients. Annals of Dermatology. 2020;32(1):21-30.

A number of lifestyle choices can impact your microbiome:

- Level of stress
- Amount of sleep
- Eating junk food
- Eating refined sugar
- Eating saturated and trans fats
- Eating fatty meat
- Fiber intake
- How much exercise you get

Take Home Message

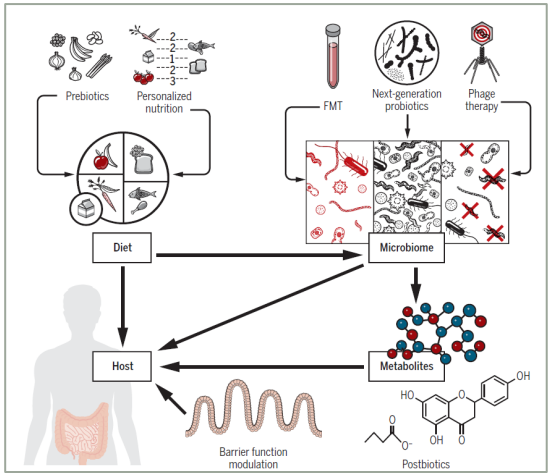
Bacteria and fungi live together in a way like a garden. In the garden you have roses and weeds. If you have weeds, then you will not enjoy the summer days as much.

When it comes to the microbiome, we want to have the right community of bacteria and fungi **(the roses)** that are working together to produce the right chemicals for your body that will support your health and wellness.

The key thing is that it is in **your hand** to grow the beneficial microbes and kill those that cause disease **(the weeds)**.

In these modules we will teach you how to grow roses and kill weeds!

Ways to Rebalance Your Microbiome:
Interventions for Promoting Human Health and Combating Disease



Will microbiome research help us become healthier?

- Advances in the treatment of *C difficile* provided proof of concept that manipulating the microbiome could treat a disease: Patients who received Fecal Microbiome Transplant (FMT) from healthy donors were rapidly cured
- Based on this, it is believed that microbiome research could lead to the development of new therapies (a targeted intervention of the microbiome for health)

Different Approaches to Balance the Gut Microbiome:

- **Microbiome modulation**: Nutritional intervention (prebiotics or individualized diets)
- **Directly impacting the host:** Probiotic supplementation or FMT
- **Bacteria-derived metabolites (post-biotics):** Product of probiotic strains, also known as metabolites (e.g. short chain fatty acids)
- **Manipulation of host gut barrier function**

These approaches, alone or in combinations, will affect the host-microbiome interface.

Rebalancing Microbiome is Critical for Optimal Health

2 Ways to Rebalance the Microbiome:

1. Probiotics

2. Diet

Bacteriome-Mycobiome Interactions told as "A Tale of How to Create a Probiotic Guided by Crohn's Disease"

Crohn's disease (CD) is mediated by multitude of factors:

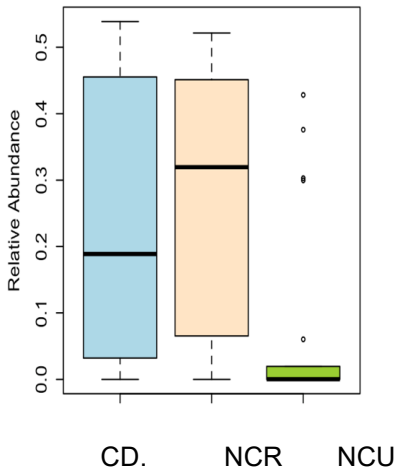
- Host genotype (genetic factor)
- Dysregulated immune responses
- Intestinal microbiota: Dysbiosis of the intestinal flora plays a key role in modulating CD-associated inflammatory processes

Characterization of the Mycobiome and Bacteriome in Crohn's Patients and Healthy Relatives in Multiplex Families

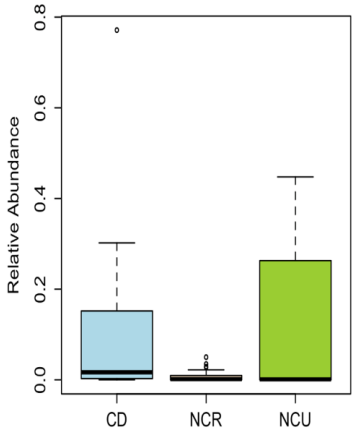
Variable	CD	NCR	NCU	Total
Families	9	9	4	13
Individuals	20	28	21	69
Female	12	13	13	38
Male	8	15	8	31
Age (mean, yrs)	44.5	48.4	41.3	45.1

CD – Crohn's disease patients
NCR – Non-Crohn's, Related individuals
NCU – Non-Crohn's, Unrelated individuals

The Level of Beneficial Fungi and Bacteria Decrease in Crohn's Disease, While Pathogenic Ones Increase



***S. cerevisiae:* Beneficial Fungi**



C. tropicalis: Pathogenic fungus

The same was seen with bacteria:

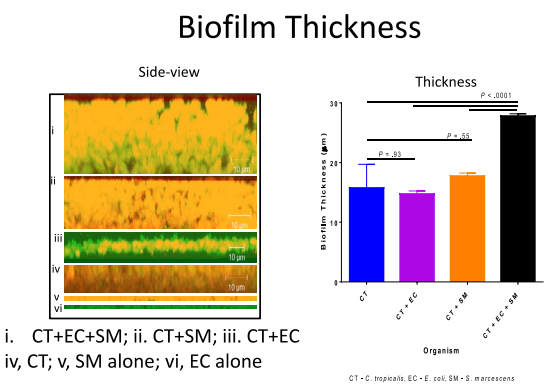
Faecalibacterium prausnitzii: An anti-Inflammatory bacteria decreased

E. coli: a pathogen increased

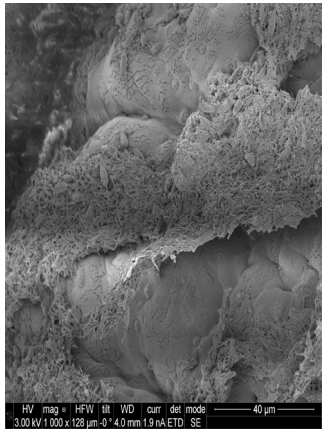
S. marcescens and *E. coli* significantly correlated with *C. tropicalis* in CD patients

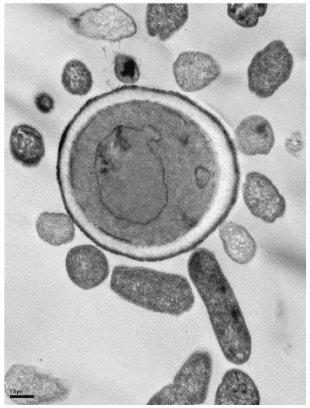
In the gut, these bacteria and fungi exist in biofilms, we wanted to know whether they work together in Crohn's patients

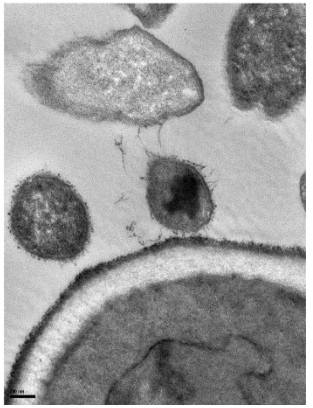
Biofilm Thickness



i. CT+EC+SM; ii. CT+SM; iii. CT+EC
iv, CT; v, SM alone; vi, EC alone

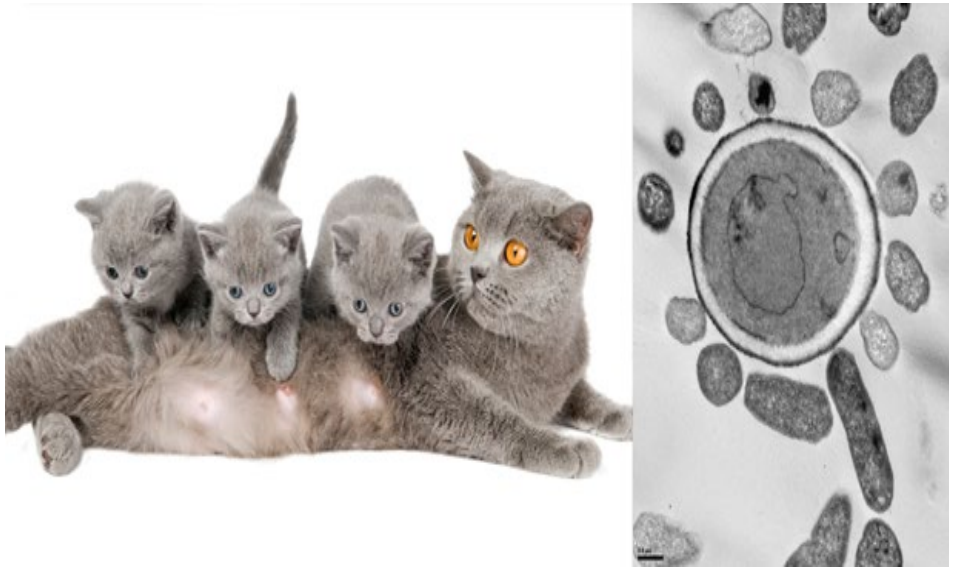






Hoarau, G., … , and Ghannoum, 2016. M. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease mBio 7(5):e01250-16. doi:10.1128/mBio.01250-16.

The Way of the Future: Polymicrobial Interactions



We need to investigate not only the babies (bacteria) but also the mother (fungi)!

Identification of Probiotic Strains That Can Impact the Host

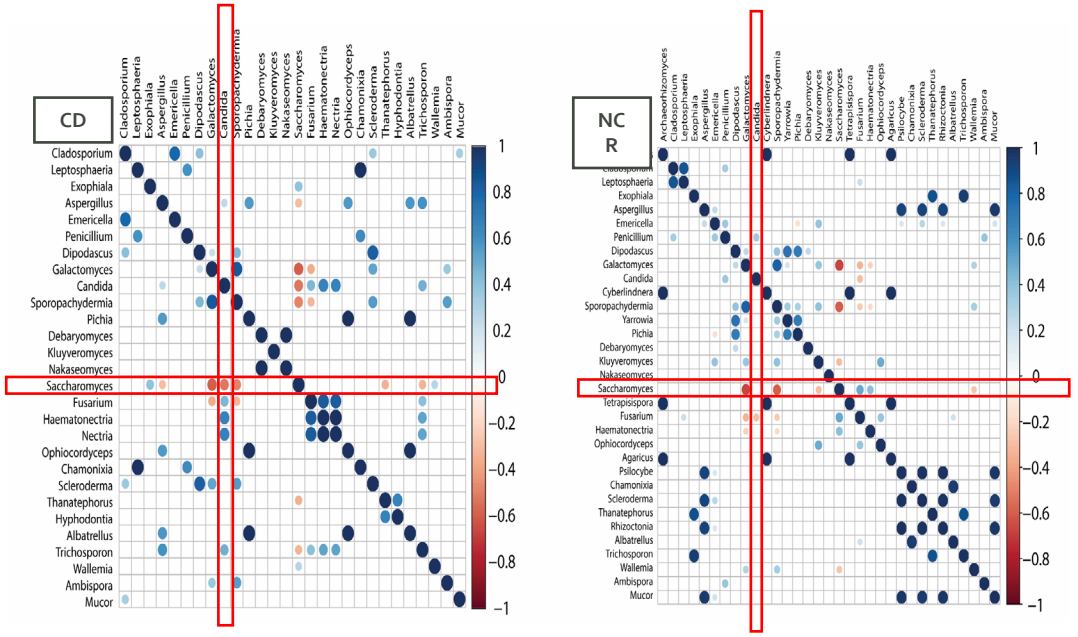
Armed with the study findings, the objective was to identify probiotic strains that can inhibit pathogenic bacteria and fungi (*Candida*) while supporting beneficial ones.

Step 1: Correlation analysis of bacterial-bacterial and bacterial-fungal interactions was conducted. Identified strains that are able to antagonize the bad microbes while simultaneously being beneficial to our gut.

Step 2: Incorporation of an anti-biofilm enzyme. Since fungus and bacteria cooperate to form biofilms, an enzyme with anti-biofilm activity and is safe was included.

Correlation Analysis:

A Way to Detect How Microbes Interact Together

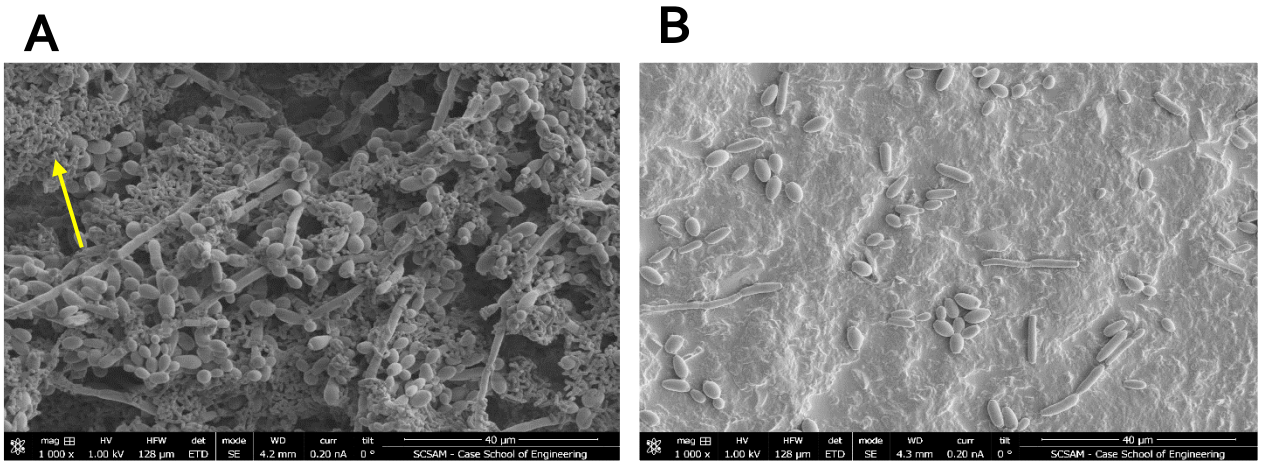


Number of genera = 28

Number of genera = 33

Red circles: negative associations (work against each other)
Blue circles positive associations (help each other).

Effect of probiotic on prevention of *C. tropicalis*, *E. coli*, and *S. marcescens* poly-microbial biofilms (PMB)

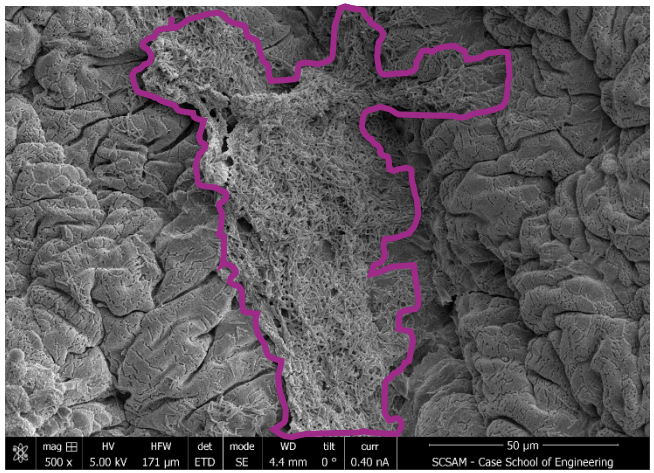


A) Untreated PMB showing dense biofilm with yeast and hyphal structures, as well as bacterial aggregates (arrow)

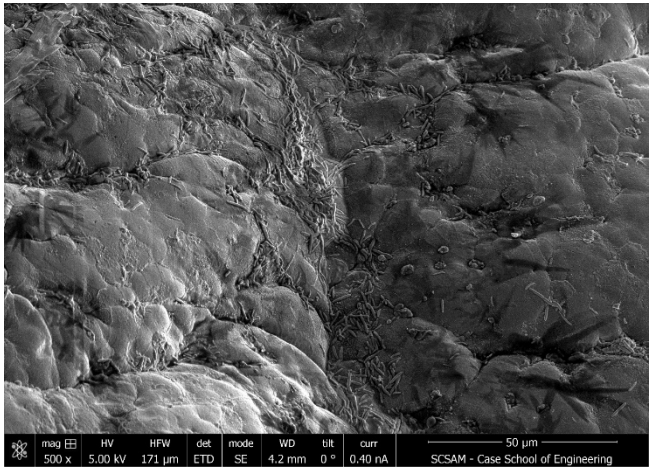
B) Probiotic-treated PMB showing absence of matrix, very few yeast cells, and no visible bacteria

Hager et al. mBio. 2019; 10(2): e00338-19. doi: 10.1128/mBio.00338-19

Prevention of gut polymicrobial biofilm formation by probiotic



Gut micrograph of untreated control



Gut micrograph of probiotic treated

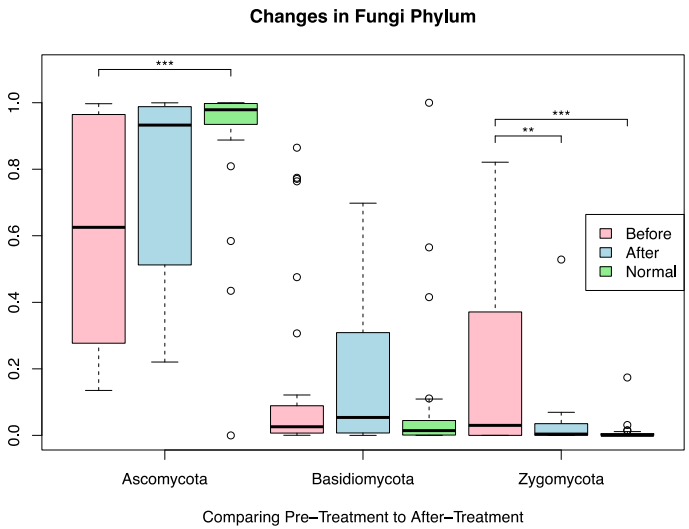
Effect of the Designed Probiotic on the Microbiome Structure of Healthy Volunteers

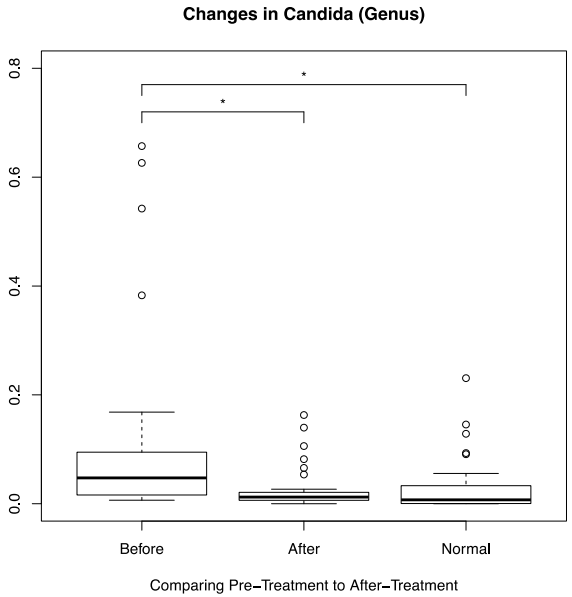
Schrom K *et al.* 2018. Consumption of a Novel Probiotic Leads to Beneficial Structural Functional Changes in the Gut Bacteriome and Mycobiome of Human Volunteers. Presented at ASM Microbes, Atlanta, Georgia.

Clinical Testing:
Determine the Effect of the Designed Novel Probiotic on the Bacteriome and Mycobiome Profiles of Healthy Individuals

- Fecal samples were collected from healthy volunteers (n = 49) at baseline and following 4 weeks of once a day probiotic capsule
- 57% females and 43% males
- Collected samples analyzed for bacterial and fungal communities using Ion-Torrent sequencing platform
- Pre- and post data were analyzed for each sample
- Statistical significance levels were calculated comparing the changes across groups by t-test for a given species or phylum.
- A *P* value < 0.05 was considered significant

Schrom K *et al.* 2018. Presented at ASM Microbes, Atlanta, Georgia.





Profile of the Mycobiome Community after Novel Probiotic :

1. Reduction in Zygomycota levels were observed (*P* value < 0.01), with abundance level becoming normal-like
2. Increase in Ascomycota was noted with abundance levels becoming normal-like levels
3. Abundance of *Candida* was significantly decreased

Summary of Mycobiome Results

Profile of the Mycobiome Community Before and After Probiotic Consumption:

1. Reduction in Zygomycota levels were observed (*P* value < 0.01), with abundance level and becoming normal-like
2. Increase in Ascomycota was noted (albeit not statistically significant) noted with abundance levels becoming normal-like levels
3. Significant reduction in the abundance of *Candida* spp. (*P* value < 0.006)

Profile of the Bacterial Community Following Probiotic Use:

- Significant increase in Bacteroidetes (*P value < 0.017)*
- Reduction in the abundance of Firmicutes phylum (albeit not significant) was noted to become similar to the NHMPS
- **Conclusion:** Use of the designed novel probiotic modifies the gut fungal and bacterial microbiota indicating that consumption of this probiotic may lead to improvement in indicators of digestive health
- More studies are warranted

Rebalancing Microbiome is Critical for Optimal Health

2 Ways to Rebalance the Microbiome:

1. Probiotics

2. Diet

Microbiome Modulation:
By Nutritional Intervention (Individualized Diets)

Western Diet has contributed to global health crisis

Diets high in processed foods and sugar have caused severe microbiome dysbiosis

Why the Mycobiome Diet?

Dysbiosis has been linked to countless diseases, such as: Intestinal disorders include inflammatory bowel disease, irritable bowel syndrome (IBS), allergies, asthma, metabolic syndrome, cardiovascular disease, and obesity to name a few.

By restoring and maintaining a healthy balance in the microbiome, we can eliminate dysbiosis and reverse adverse health conditions.

The Mycobiome Diet is:

- Nutritionally balanced
- Whole-food-based
- Low-glycemic
- Rich in fiber and resistant starches
- Low in sugar
- Full of good mono- and polyunsaturated plant fats
- Low in saturated fat and animal fat
- Rich in lean plant protein and animal protein primarily from seafood, with a bit of poultry thrown in
- Ultimately flexible, diverse, and customizable

Mahmoud Ghannoum, Eve Adamson, 2019. Total Gut Balance, The Countryman Press, New York

Mycobiome Diet is designed to:

Work on all parts of the microbiome (bacteria and fungi) and lifestyle practices:

- Limit the growth of pathogenic fungi (e.g. *Candida*) with a diet designed to stifle its growth:
    1. Vitamins A, C, and Bs,
    2. Low-carb,
    3. A good balance of plant-based protein and fatty acids

- Enhance the growth of beneficial bacterial (*Bifidobacteria* and *Lactobacillus*): by providing prebiotic food thereby keeping fungal growth under control and at the same time crowding out pathogenic bacteria that cooperate with *Candida* in biofilm-making efforts
- Break down existing biofilms using particular kinds of vegetables, fruits, garlic
- Reduce overall inflammation with potent anti-inflammatory and antioxidant foods rich in targeted vitamins, minerals, and other beneficial constituents

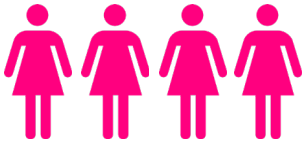
Mycobiome Diet Preliminary Trial

10 healthy volunteers followed the Mycobiome Diet for 28 days

Ages 30 to 70

6 Males

4 Females



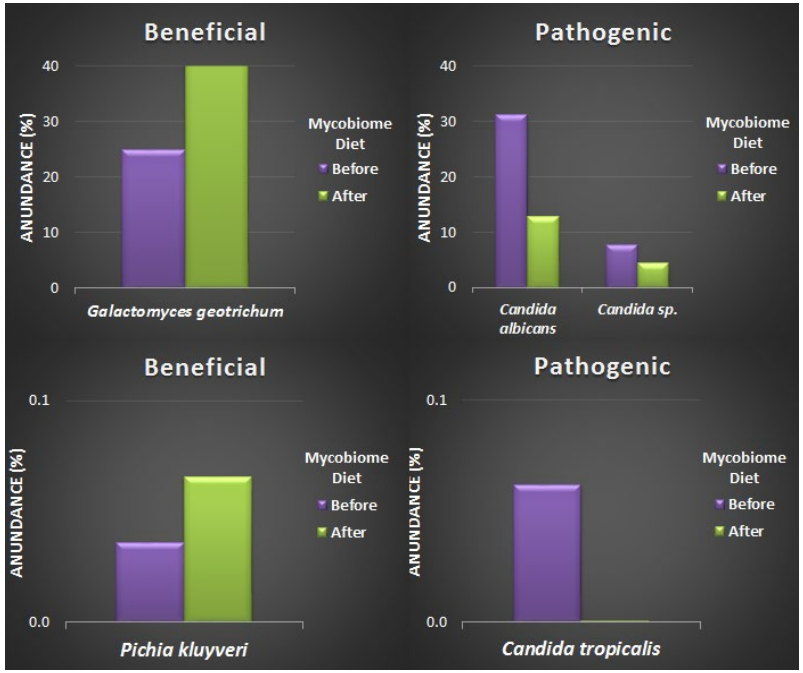
Mycobiome Diet Clinical Trial

Study Design

- Fecal samples were collected and mycobiome and bacteriome analysis was conducted on samples collected at baseline (pre-diet), during (14 days), and 28 days post the study
- Participants completed daily food logs, checking off the foods required for daily and weekly consumption, and noting various health measures such as weight and aspects of digestion and symptoms
- They also filled a questionnaire and exit feedback

Profiles of the Beneficial and Pathogenic Fungi Before and After the Mycobiome Diet Study

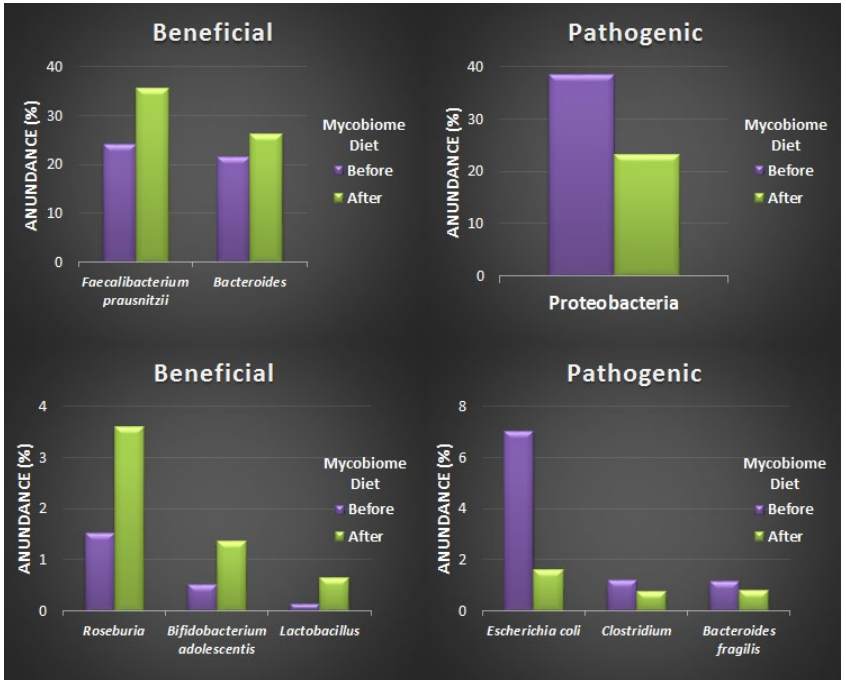
- **Decrease in pathogenic** *Candida* species (72.4%), as well as *C. albicans* (142%)
- ***C. tropicalis* was undetected** post the Mycobiome Diet
- **Increase in Beneficial fungi:** *Pichia kluyveri* and *Galactomyces geotrichum* after initiation of the Mycobiome Diet



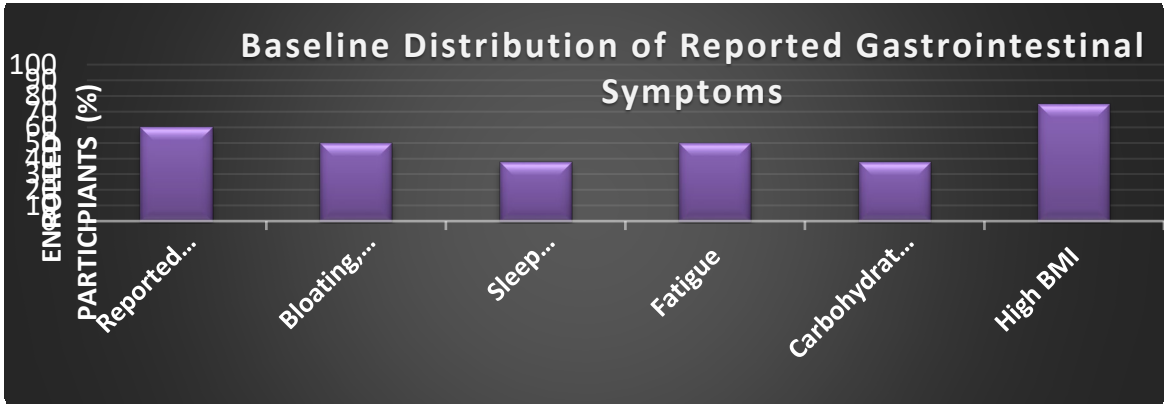
Profiles of the Beneficial and Pathogenic Bacteria Before and After the Mycobiome Diet Study

Following the Mycobiome Diet led to:

- Increase in beneficial bacteria (*Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis*, *Roseburia*, and *Lactobacillus*), and
- Decrease in the pro-inflammatory Proteobacteria, *E. coli*, *Clostridium*, and *Bacteroides fragilis*



Adhering to the Mycobiome Diet Led to Improvement in Gastrointestinal Symptoms



Mycobiome Diet Study Clinical Trial

Results

Before the Study

- 6 participants reported health issues
- 3 participants had **SIBO** (small intestinal bacterial overgrowth)
- 1 participant had **celiac disease**
- Many reported problems with gas, **bloating**, constipation, heartburn, and diarrhea
- Other reported issues included fatigue, low energy, **cravings** for sugar, bread, or salty carbs, and **sleep** disturbances, especially waking in the middle of the night

After the Study

- All participants with GI symptoms reported moderate or **dramatic improvements**
- 4 out of 6 participants who chose to track their **weight lost significant weight** in our weeks:
  - 2 people lost 10 pounds
  - 1 person lost 6 pounds
  - 1 lost 2 pounds
- 3 participants reported **better sleep**, less waking up at night, and even reduced hot flashes
- 3 participants reported moderate or dramatically **improved fatigue** and **higher energy** levels

Mycobiome Diet Study Summary

Adhering to the Mycobiome Diet Study leads to a positive shift in fungal and bacterial communities, as well as systemic improvements in GI health

Further studies are warranted

Ghannoum M, Smith C, Adamson E, Isham N, Salem I, Retuerto M (2019) Effect of Mycobiome diet on Gut Fungal and Bacterial Communities of Healthy Adults. J Prob Health. 7:215. DOI: 10.35248/2329-8901.19.7.215

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- 10.1097/MNH.0000000000000293 + doi: 10.2459/JCM.0000000000000900 + 10.3389/fped.2017.00138 + https://doi.org/10.3389/fphar.2020.00258
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WholisticMatters™

MICROBIOME, GUT-BRAIN AXIS AND YOUR HEALTH

- Gut-brain axis, a bidirectional relationship
- How does the gut talk to our brain and vice versa
- Gut-brain axis influence on neurodegenerative diseases
- Relationship between the microbiome and stress
- Relationship between the microbiome and depression
- Dietary and lifestyle approaches to address stress and depression

The Gut-Brain Axis (GBA)

The first evidence of the gut–brain axis **(GBA)** came from an army surgeon who examined gastric juices secreted by intragastric fistula and found that **intestinal function was related with mood**.

Recent studies show that our brain talks to our gut and vice versa.

We used to think top-down **(brain to gut axis**), however, we should also start thinking bottom-up **(gut to brain axis).**

This is a two-way communication called **bidirectional** communication.

In reality, the two opposite directions mutually affect and depend on each other.

The purpose of this communication is to maintain homeostasis and protect the body against detrimental factors.

Communication Between the Microbiome and the Brain

- The two-way communication between the gut microbiome and the brain occurs through neural, inflammatory, and hormonal signaling pathways.
- The gut microbiome is actively involved in processes linked to brain development, physiology, psychology, and behavior.
- Specifically, the gut microbiome plays a critical role in the regulation of mood, anxiety, and pain.
- The GBA modulates stress responsiveness, prefrontal myelination, brain biochemistry, immune function, neurotransmission, and neurogenesis.

Cryan JF, Dinan TG. Nat Rev Neurosci 2012;13:701-12.

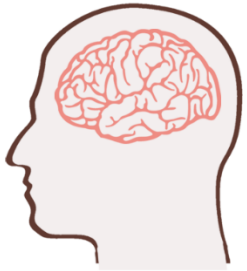
Osadchiy V et al. Compr Physiol 2019;10:57-72.

Amaral FA et al. Proc Natl Acad Sci U S A 2008;105:2193-7.

Bravo JA Proc Natl Acad Sci U S A 2011;108:16050-5.

Dinan TG, Cryan JF. Psychoneuroendocrinology 2012;37:1369-78.

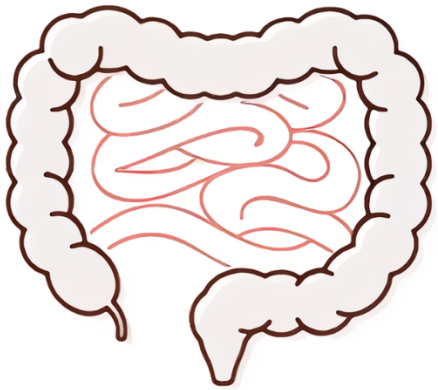
Marx W et al. Proc Nutr Soc 2017;76:427-36.



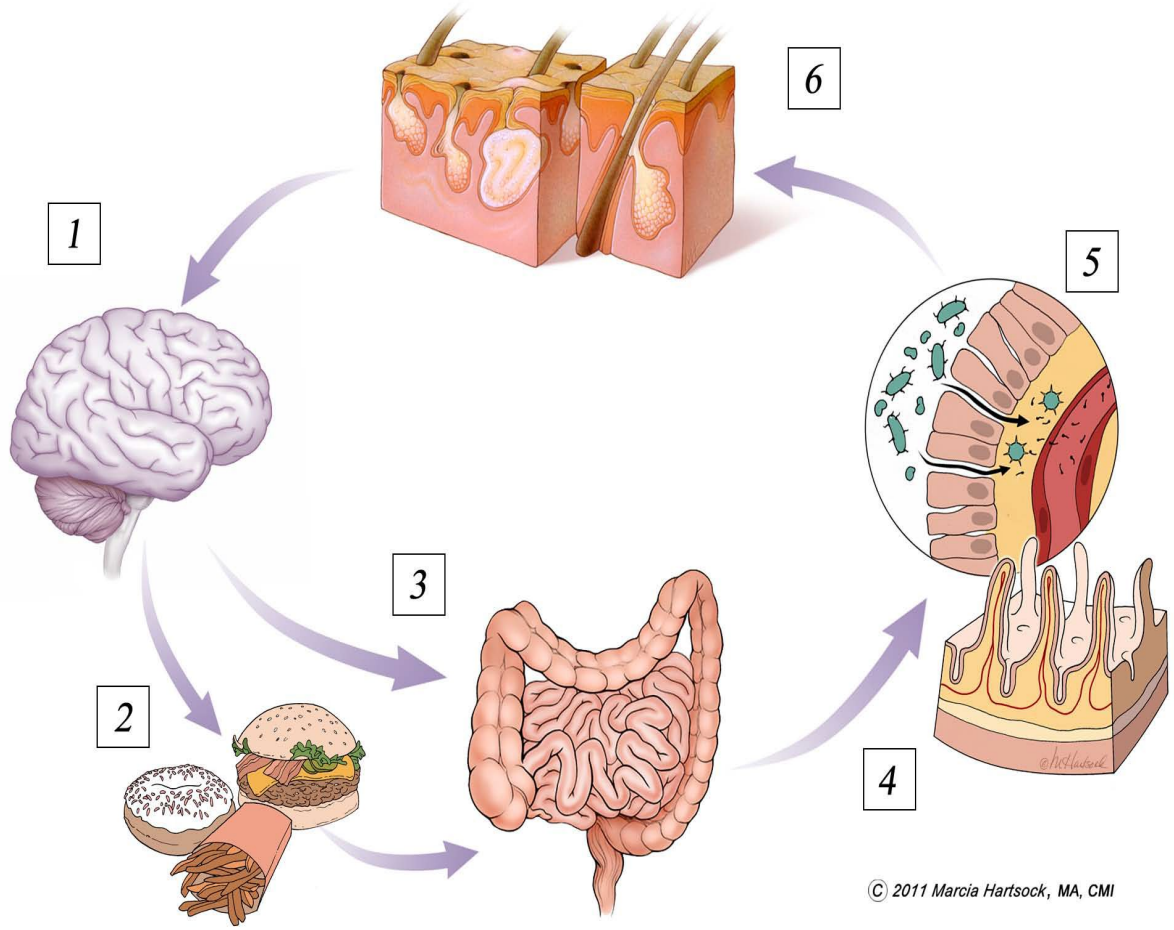
Dysbiosis in the gut alters certain chemicals in the brain (e.g. serotonin and dopamine) that directly impact the mood.
These changes can lead to increase in stress and depression, leading to change in eating habits that cause further imbalance of the gut microbiota

THE "GUT - BRAIN - AXIS"

Changes in cortisol levels affect the body respone to stress which can cause gastrointestinal disturbance leading to imbalance in gut microbiota



The Gut-Brain-Skin Axis and Skin Health & Wellness



Both probiotics and antimicrobials may play a role in interrupting this cycle at the gut level.

Bowe and Logan Gut Pathogens 2011, 3:1

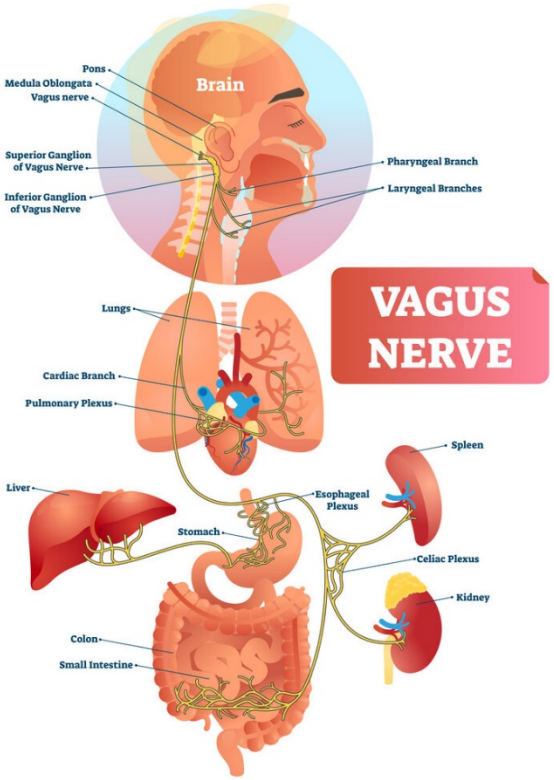
How does the gut talk to our brain?

The vagus nerve (a cranial nerve connecting the brain to visceral organs) is the primary connection between brain and gut.

The vagus nerve also **mediates CNS effects on behavior**.

Modulation of this connection by the microbiota may affect:

- Fat storage and energy balance
- GI barrier function
- General low-grade inflammation (in the GI and systemically)
- Increased stress reactivity
- Increased anxiety and depressive-like behaviors



Exploiting The Vagus Nerve: Future Perspective

Imbalance can increase intestinal vagus nerve activity, inducing low grade inflammation leading to anxiety-like behavior in animals.

Interestingly, animals whose vagus nerve is removed did not experience behavioral disturbance indicating that the vagus nerve plays an important role in anxiety behavior.

Therefore, the vagus nerve could be seen as the pathway for behavioral change, through the use of probiotics.

In fact, studies showed that probiotics (e.g. *Lactobacillus rhamnosus*) regulate behavioral and physical reactions through the vagus nerve, by regulating anxiety behavior related receptors (e.g. $GABA_A$).

We are well positioned to tackle outstanding questions and develop innovative approaches to prevent and treat stress-related disorders, including anxiety and depression.

Does the fungal community play an important role in neurological diseases?

- Increasing interest of the mycobiome in neurological disease is largely driven by findings that fungi can modulate the host immunological response and hence may be a risk factor for immune diseases
- Most research exploring a fungal association to date has focused on Multiple Sclerosis (MS)
- MS is an immune-mediated inflammatory disease that shares overlapping epidemiological/ etiopathophysiology characteristics with IBD
- Based on these observations, it is conceivable that the mycobiome, which has been shown to be perturbed in IBD (Hoarau et al., 2016), is similarly altered in neurological disease.
- Perturbed gut mycobiome community has been reported in neurodevelopmental conditions such as autism spectrum disorder and Rett syndrome
- Fungal toxin produced by pathogenic fungi in the gut may cross blood-brain barrier and play a role in myelin degradation
- Increased prevalence of fungal infections in blood and CSF of MS patients
- Potential gut health benefits or probiotic effects of some fungal species (e.g. *Saccharomyces boulardi*) are also being explored. Thus, there is emerging supportive evidence to suggest a potential association.

Lin et al., 2014.

Kosmidou et al., 2017.

Dworecka-Kaszak et al., 2016.

Strati et al., 2017.

Strati et al., 2016.

Ward et al., 2017.

Brain-affecting disorders

- Gastrointestinal manifestation are seen in brain-affecting disorders
- Where the intestinal microbiome and enteric nervous networks are actively involved

Diseases	Conditions
Parkinson's disease	Depression
Autism spectrum disorder	Anxiety
Amyotrophic lateral sclerosis	Behavior
Alzheimer diseases	Cognition
Prion diseases	Mood
Creutzfeldt-Jakob disease	Stress
Transmissible spongiform encephalopathies	Fatigue
	Aging

Chandra S,*et. al. 2020.* Curr Top Med Chem. 20(13):1142-1153. doi: 10.2174/1568026620666200413091101
Sarkar S, and Banerjee S. 2019. J Neuroimmunol. 2019 Mar 15;328:98-104.doi: 10.1016/j.jneuroim.2019.01.004.Epub 2019 Jan 9.
Zhu S *et al. 2020. Journal of Neuroinflammation* volume 17, Article number: 25
Fang P. *et al*. DOI:https://doi.org/10.1016/j.chom.2020.06.008
Wilms E. *Gut.* 2018; 67: 2213-2222

Gut-Brain Axis and the Microbiome

Examples:

- Stress
- Depression
- Autism

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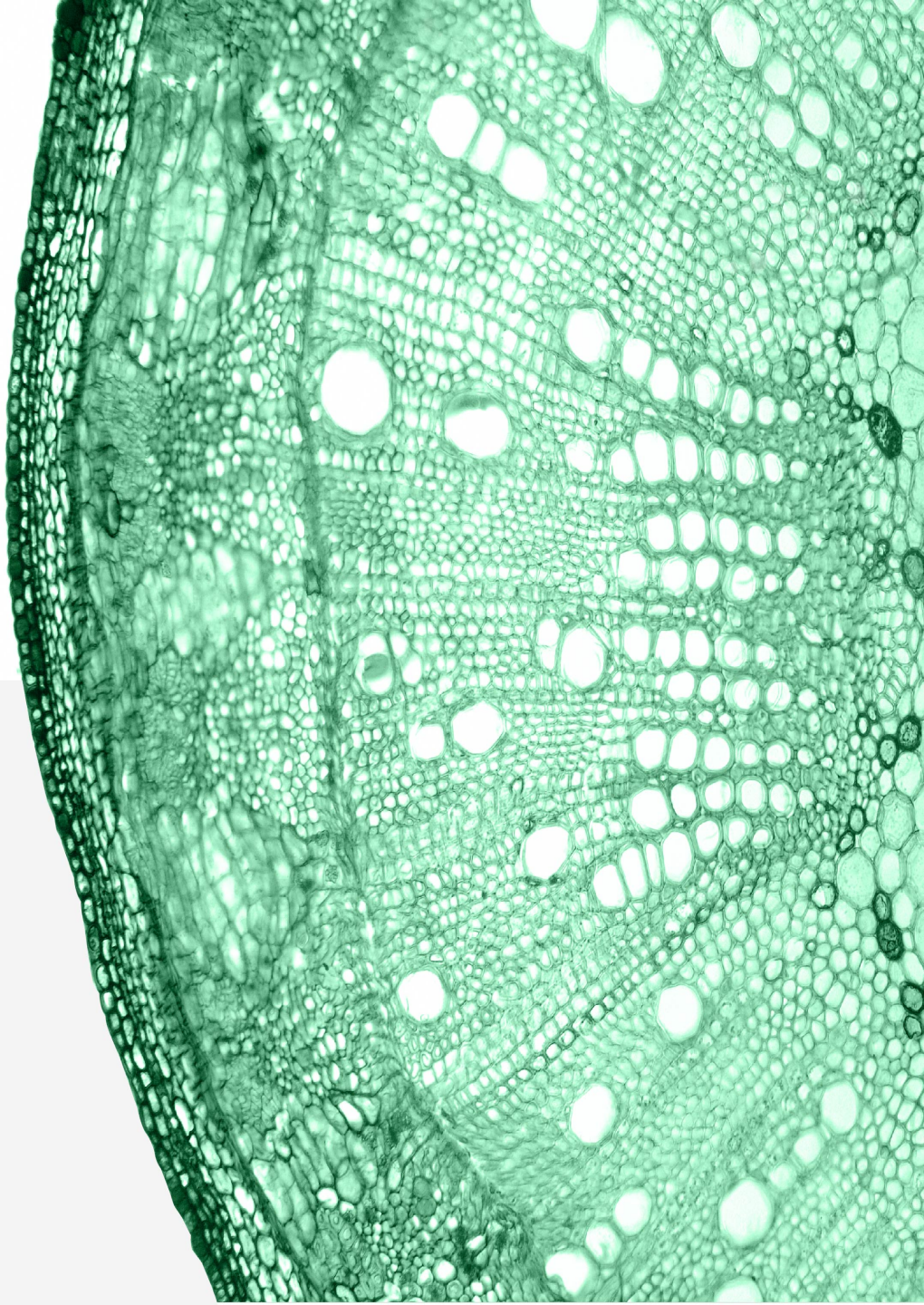
Gut-Brain Axis and The Microbiome

Stress and The Microbiome



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Stress Definition

Stress is defined as a person's total response to environmental demands or pressures.

Several different types of stressors can be distinguished:

- Acute or chronic
- Some may occur only once, while others are repetitive
- Stress can be anticipated, unpredictable and uncontrollable, mild or severe, and occur in or out of context
- The perception of stress is variable between individuals, and so is the persistence of its consequences
- Exposure to stressors has long been known to increase susceptibility to disease, including GI disorders
- Stress contributes to many disabilities worldwide and represents a severe economic burden

Lucassen et al. 2014. Neuropathology of stress. Acta Neuropathol 127, 109–135.

Relationship Between the Microbiome and Stress

- 40 years ago, it was shown that environmental and dietary stress alters the gut microbiota in mice, favoring the establishment of pathogenic bacteria
- Stressor-induced changes in the microbiota may enhance the ability of enteric pathogens (e.g. *Citrobacter rodentium*) to colonize the intestine
- Social stress increases the risk of inflammation-related diseases, promoting pro-inflammatory gene expression and monocyte differentiation
- Exposure to a social stressor affects the gut microbiota and circulating levels of cytokines (e.g. interleukin-6 and monocyte chemotactic protein-1)
- Acute and repeated stress affects levels of intestinal secretory IgA, impacting intestinal homeostasis and probably resulting in inflammation
- Altered levels of intestinal secretory IgA might cause shifts in commensals and possibly result in dysbiosis

Konturek et al. 2011. J Physiol Pharmacol 62, 591–599. Tannock & Savage. 1974. Infect Immun 9, 591–598.

Tannock & Smith. 1972. J Med Microbiol 5, 283–289. Bailey et al. 2011. Brain Behav Immun 25, 397–407.

Powell et al. 2013. Proc Natl Acad Sci U S A 110, 16574–16579.

Gut-Brain Communication During Stress

- During stress, alterations at the level of the central nervous system can influence:
  a. gut neuromotor and secretory function
  b. immunity
  c. microbiota composition
- In turn, dysbiosis may contribute to perpetuate inflammation, further disrupting gut–brain communication
- Some of these effects may be mediated by direct host–microbial interactions at the level of the intestinal epithelium
- The sequence of events can occur in a top-to-bottom or bottom-to-top fashion, but once initiated can perpetuate and exacerbate maladaptive responses that promote a state of disease

De Palma *et al.* J Physiol 592.14 (2014) pp 2989–2997

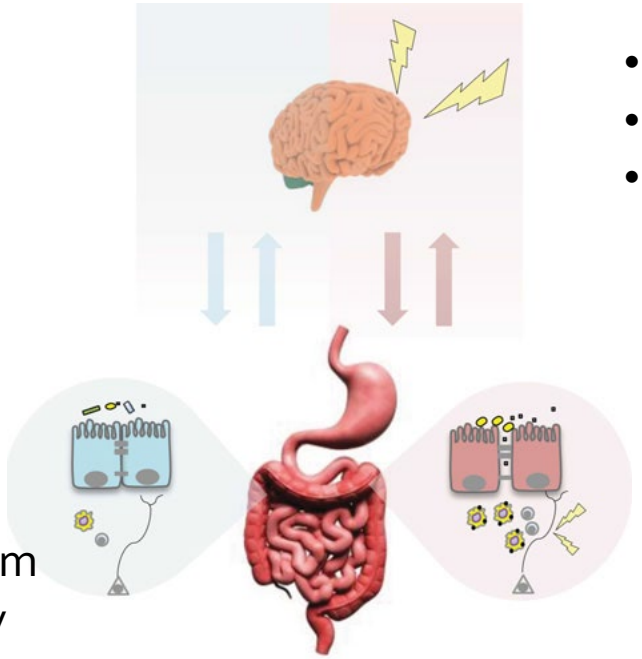
Communication between the gut and the brain during stress, through multiple pathways

Well-Being

- Functional HPA axis
- Normal Behavior
- Normal nociception

Stress

- Dysfunctional HPA axis
- Altered Behavior
- Increased pain perception



- Balanced Microbiota
- Normal GI function
- Balanced Immune system
- Normal gut morphology

- Dysbiosis: neurotransmitters Catecholamines, GABA
- Gut Dysfunction
- Low grade or overt inflammation
- Tissue damage

De Palma et al. J Physiol 592.14 (2014) pp 2989–2997

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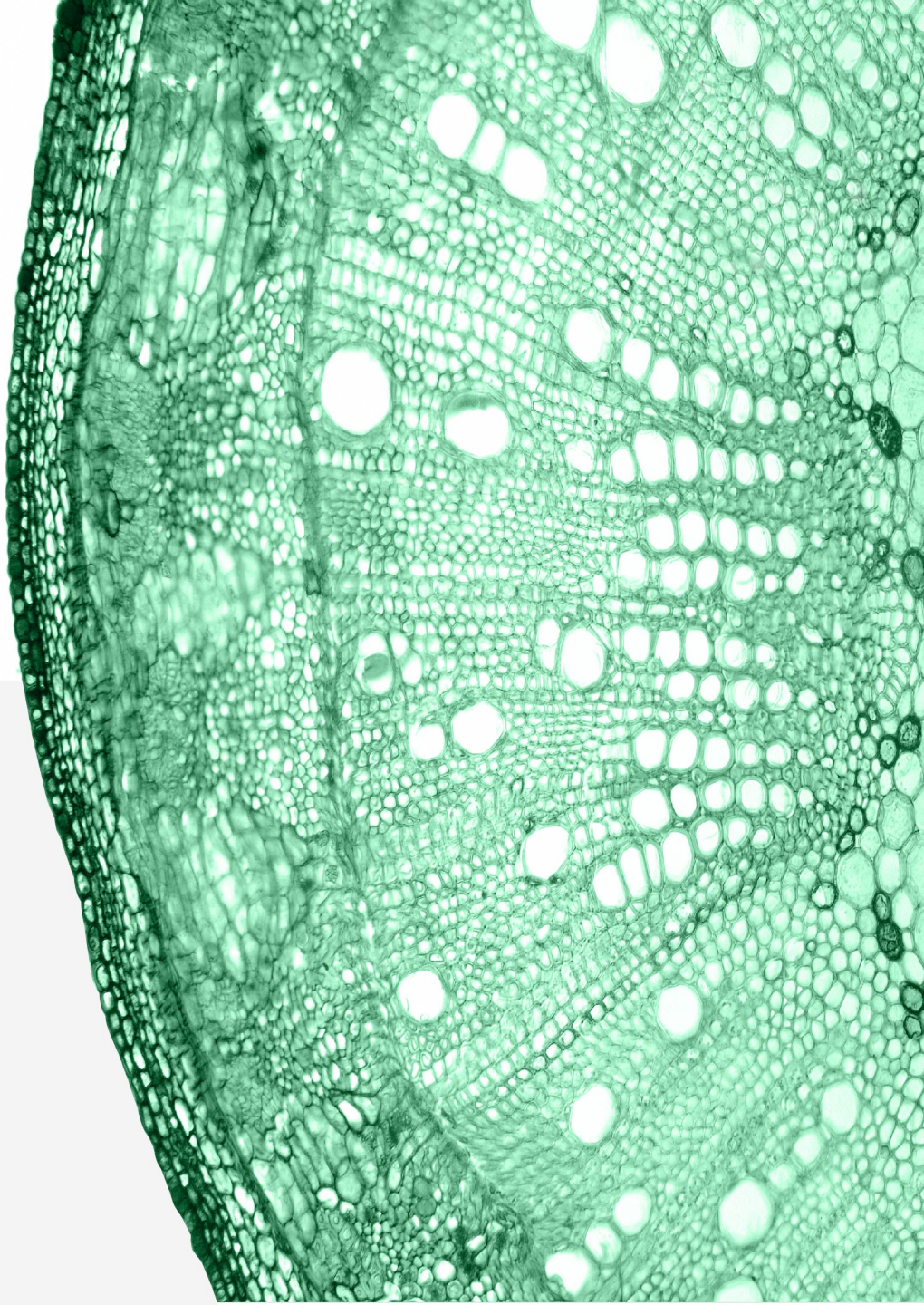
Gut-Brain Axis and The Microbiome

Depression and The Microbiome



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Depression and the Microbiome: Why Look at Depression?

Within a one-month period of the COVID-19 pandemic, there was:

- A 34.1% increase in prescriptions for anti-anxiety medications,
- An 18.6% increase in antidepressant prescriptions, and
- A 14.8% increase in anti-insomnia drugs

During such a short period of time, this steep rise hints at the magnitude of COVID-19's immediate and widespread effect on mental health.

A SingleCare survey showed that 59% reported that their mental health was affected by COVID-19

The Substance Abuse and Mental Health Services Administration (SAMHSA)-Disaster Distress Helpline reported:

- A 338% increase in the call volume for individuals experiencing emotional distress from February 2020 to March 2020, and
- An 891% increase compared to the call volume from March of 2019

The WHO urged governments to put tribulations regarding the upsurge and severity of mental health illnesses at the "front and center" of their COVID-19 response

AMERICA'S STATE OF MIND: U.S. trends in medication use for depression, anxiety and insomnia. 2020. https://www.express-scripts.com/corporate/americas-state-of-mind-report.

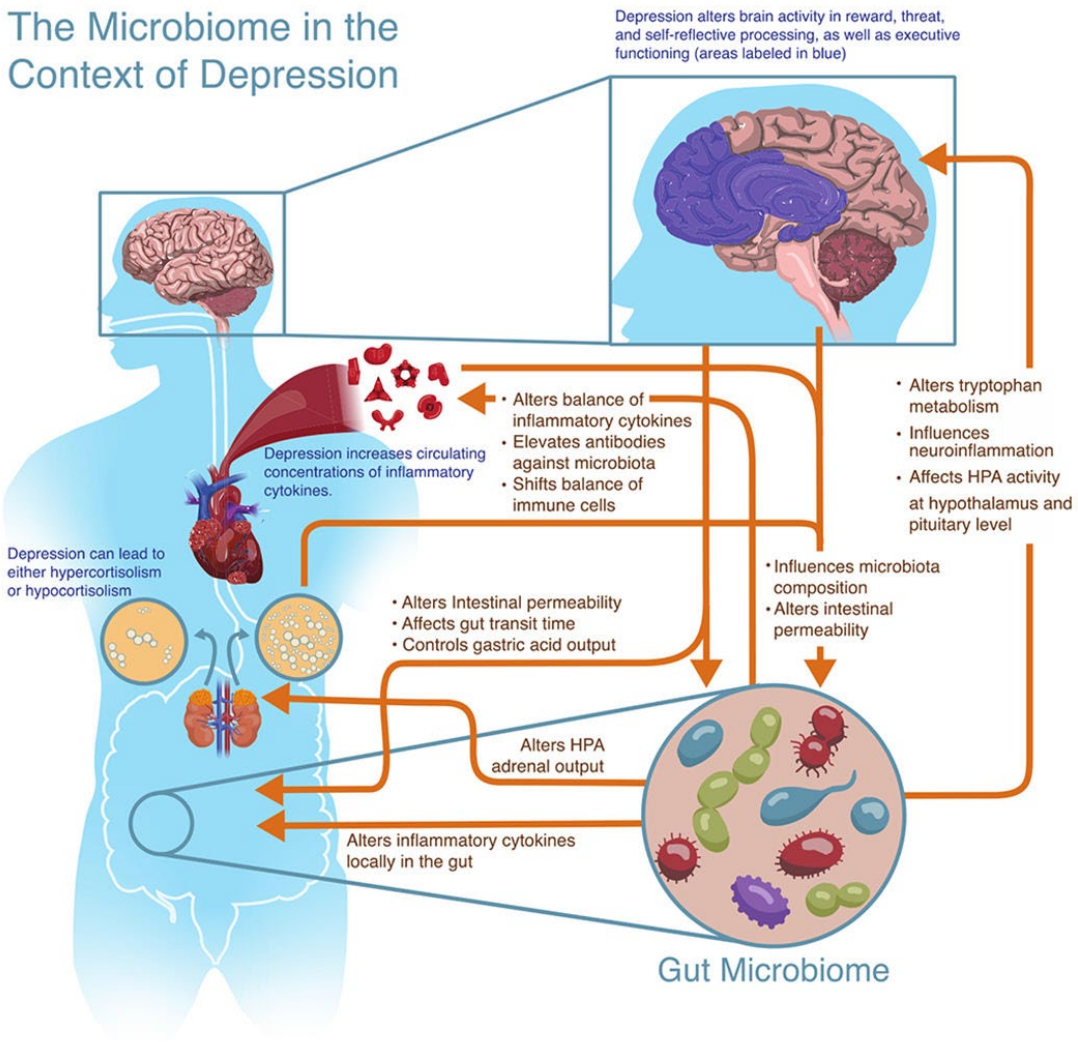
Mental Health Survey 2020. https://www.singlecare.com/blog/news/mental-health-survey/.

A crisis mental-health hotline has seen an 891% spike in calls. 2020. https://www.cnn.com/2020/04/10/us/disaster-hotline-call-increase-wellness-trnd/index.html.

Microbiome and Depression

- Studies have shown that depression is related to intestinal microbiota imbalance inducing changes in brain chemistry and behavior
- 60% of patients with depression may have intestinal function disturbance (e.g. IBS)
- Depressed patients have a reduced microbiome abundance and microbial diversity
- Level of *Bifidobacterium* and *Lactobacillus* in feces of patients with major depression was less compared to healthy subjects
- Gut microbiota in bipolar disorder patients contained fewer *Faecalibacterium* (an anti-inflammatory) relative to healthy control subjects

Bidirectional communication between the central nervous system, endocrine system, immune system and the microbiome, which contribute to depression



Fluxa and Lowry. 2020. Neurobiol Dis.; 135: 104578. doi:10.1016/j.nbd.2019.104578.

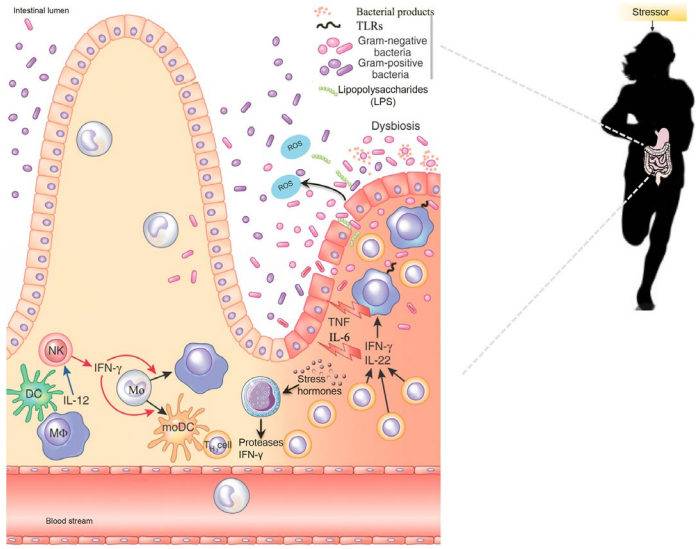
Effect of gut microbiota on mood disturbance, fatigue, insomnia and risk of depression during exercise

How bacteria connects with the brain and influence behavior:

- Bacterial products gain access to the brain via:
  - The bloodstream
  - Cytokine release from mucosal immune cells,
  - The release of gut hormones (e.g. 5-hydroxytryptamine (5-HT) from enteroendocrine cells, or
  - Afferent neural pathways, including the vagus nerve.

How stress during exercise influences gut microbiome?

- Through the release of stress hormones or sympathetic neurotransmitters that influence gut physiology and alter the microbiota.
- **Alternatively,** host stress hormones (e.g. noradrenaline) might influence bacterial gene expression or signaling between bacteria, and this might change the microbial composition (dysbiosis) and activity of the microbiota.



Clark, A and Mach, N. J. International Society of Sports Nutrition (2016) 13:43 DOI 10.1186/s12970-016-0155-6

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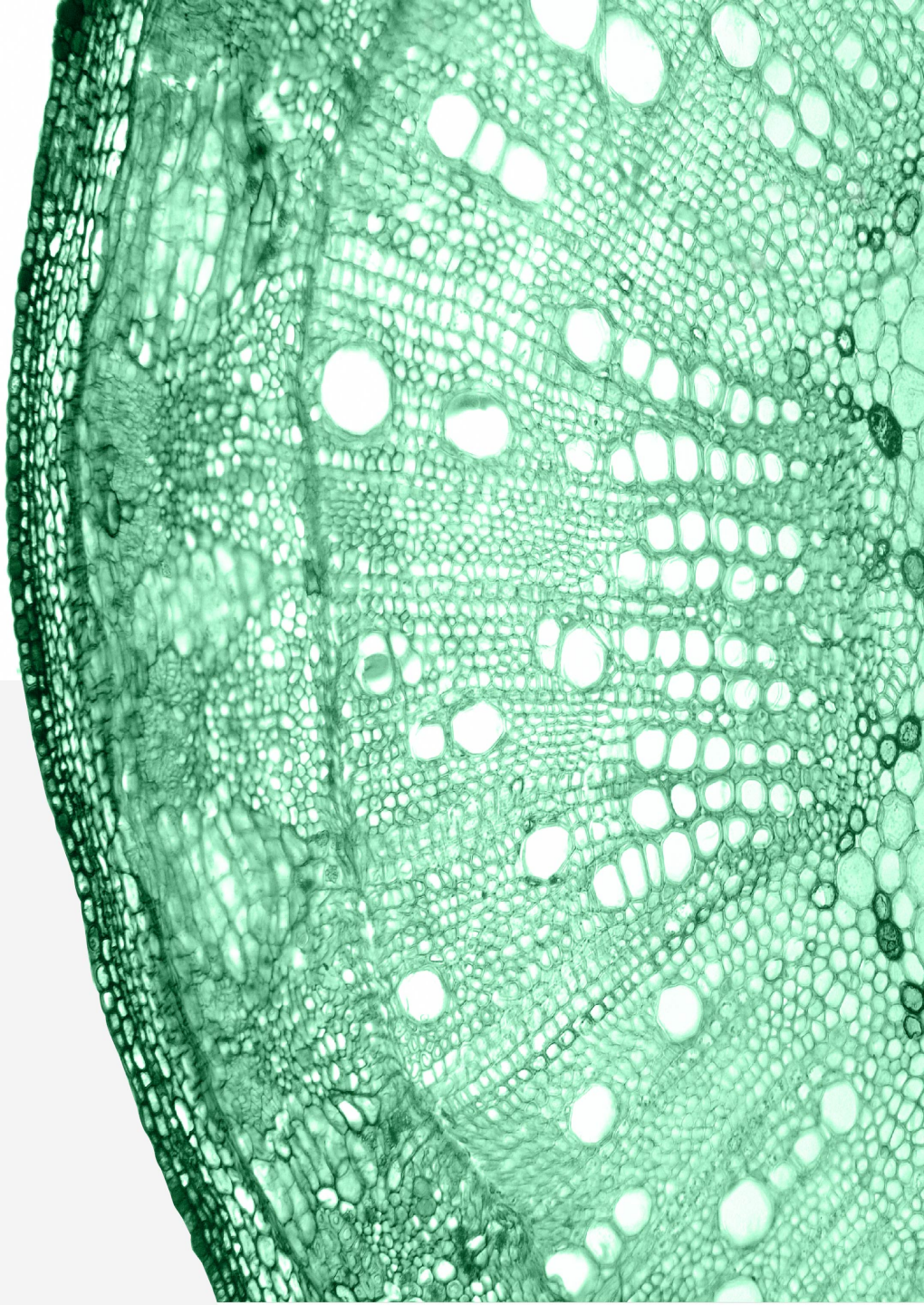
Gut-Brain Axis and The Microbiome

Autism and The Microbiome



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Autism Spectrum Disorder (ASD)

ASD is a group of **neurodevelopmental disorders** that begin in early childhood, characterized by **problems in communication and social behavior.**

ASD includes: autism (AD), Asperger's syndrome and pervasive developmental disorder–not otherwise specified (PDD-NOS).

Prevalence of ASD has dramatically increased from 4.5 in 10,000 children in 1966 to 1 in 110 in 2006 and to 1 in 68 children in 2010[1].

No one factor, except in rare cases, may be enough to trigger an ASD.

Triggers are believed to work in concert and be additive, such **that each additional trigger** increases the risk of developing ASD.

1. http://www.cdc.gov/ncbddd/autism/data.html

Autism Spectrum Disorders

Associated Factors

A number of factors have been implicated with ASDs:

- **Genetic factors (main focus initially)**
- **Environmental factors (suggested recently)** including:
    - Pre- or postnatal exposure
    - Chemicals and drugs
    - Air pollution
    - Stress
    - **Gut microbiota and dietary factors***

*Dietert RR et al. Environmental risk factors for autism. Emerg Health Threats J2011

Autism Spectrum Disorders

Associated Factors

- **Microbiome imbalance:** Children with PDD-NOS and, especially, AD shows dysbiosis in their fecal microbiota, manifested as an overgrowth of some organisms and loss of others compared to healthy controls[1].

- These studies provide evidence supporting a role for the GI microbiota in the pathogenesis of ASDs.

- GI disturbances (abdominal pain, diarrhea and bloating) and metabolic disorders typical of microbial dysbiosis are frequently described in infants with an ASD.

1.De Angelis et al. PLoS One 2013; 8:e76993; PMID:24130822; http://dx.doi.org/10.1371/journal.pone.0076993

Fecal metabolomic profiling in ASDs provide insight into the involvement of the microbiome

Children with AD had significantly lower levels of SCFAs (normally produced by beneficial microbes) compared with healthy controls.

SCFAs represent **the primary fuel for** colonocytes and are involved in water and electrolyte absorption by the colonic mucosa.

Butyric acid, one of the SCFAs, has specific effects:

- Regulate trans-epithelial transport,
- Positively modulate the inflammatory and oxidative states of the intestinal mucosa,
- Reinforce the mucosal barrier and
- Modulate visceral sensitivity and motility

Therefore, having a low level of SCFAs metabolites has negative implications for children ASD and links the microbiome to autism.

Microbiome Profile of Autistic Patients Clinical Trial Demographic Information of Participants

Description	Autistic Individuals	Healthy Controls
Number = 81	51 (100%)	30* (100%)
Males	44 (85.4%)	13 (43.3%)
Diagnosed at age <3 years	29 (56.9%)	N/A
Diagnosed at age >3 years	22 (43.1%)	N/A
BMI <18	26 (50.9%)	12(40%)
BMI 18-25	20 (39.2%)	14(46.6%)
BMI >25	5 (0.9%)	4(13.3%)
White/Caucasians	41 (80.4%)	25 (83.3%)
Rural	11 (21.6%)	6 (20%)
Suburban & Urban	40 (78.4%)	24 (80%)
Breast Fed	41 (80.4%)	25 (83.3%)
History of antibiotic during pregnancy	9 (17.6%)	4 (13.3%)
Delivered vaginally	36 (70.6%)	21 70%)
Delivered by C-section	15 (29.4%)	9 (30%)

Gastrointestinal Symptoms of the Participants

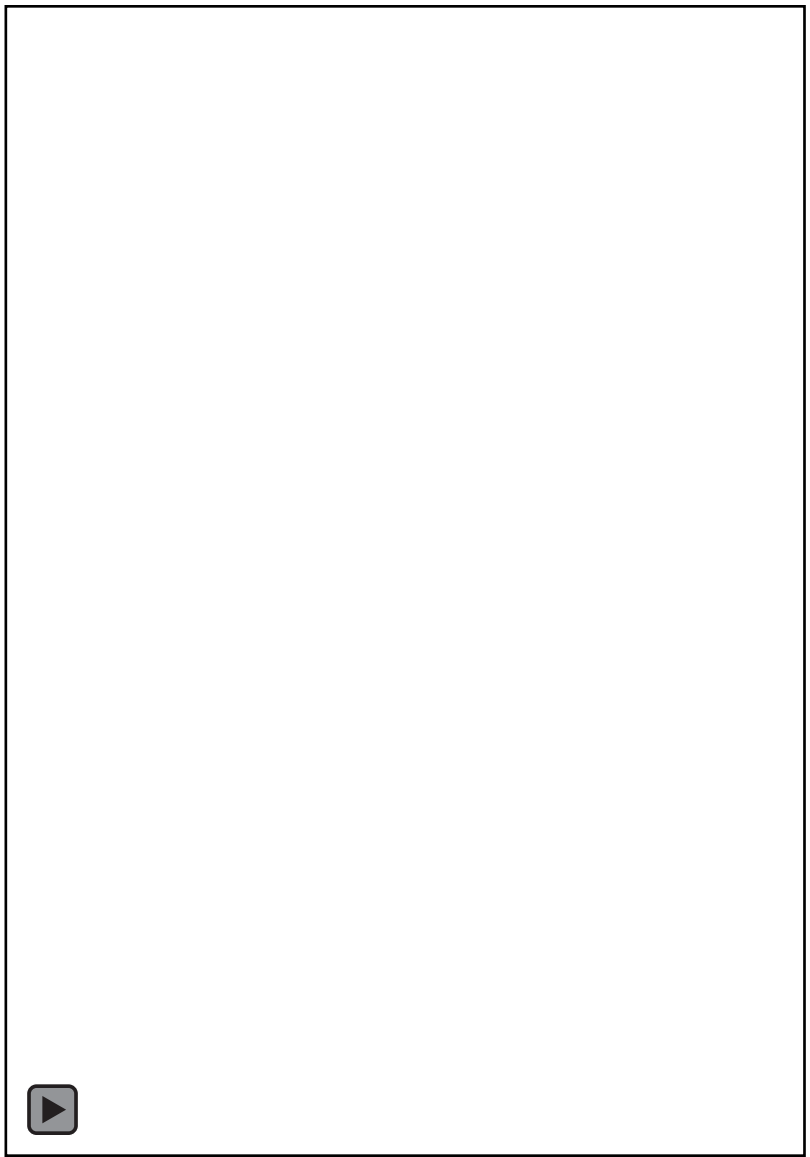
	Cases	Controls
Upper GIT symptoms*	9 (17.6%)	2 (6.7%)
Lower GIT symptoms*	30 (58.8%)	15 (50%)
GIT comorbidities*	22 (43.1%)	10(33.3%)
History of PPI*	7 (13.7%)	3 (10%)
Probiotics or dietary supplement	38 (74.5%)	11 (36.7%)

***Digestive health issues captured include:** Acid reflux, constipation & diarrhea

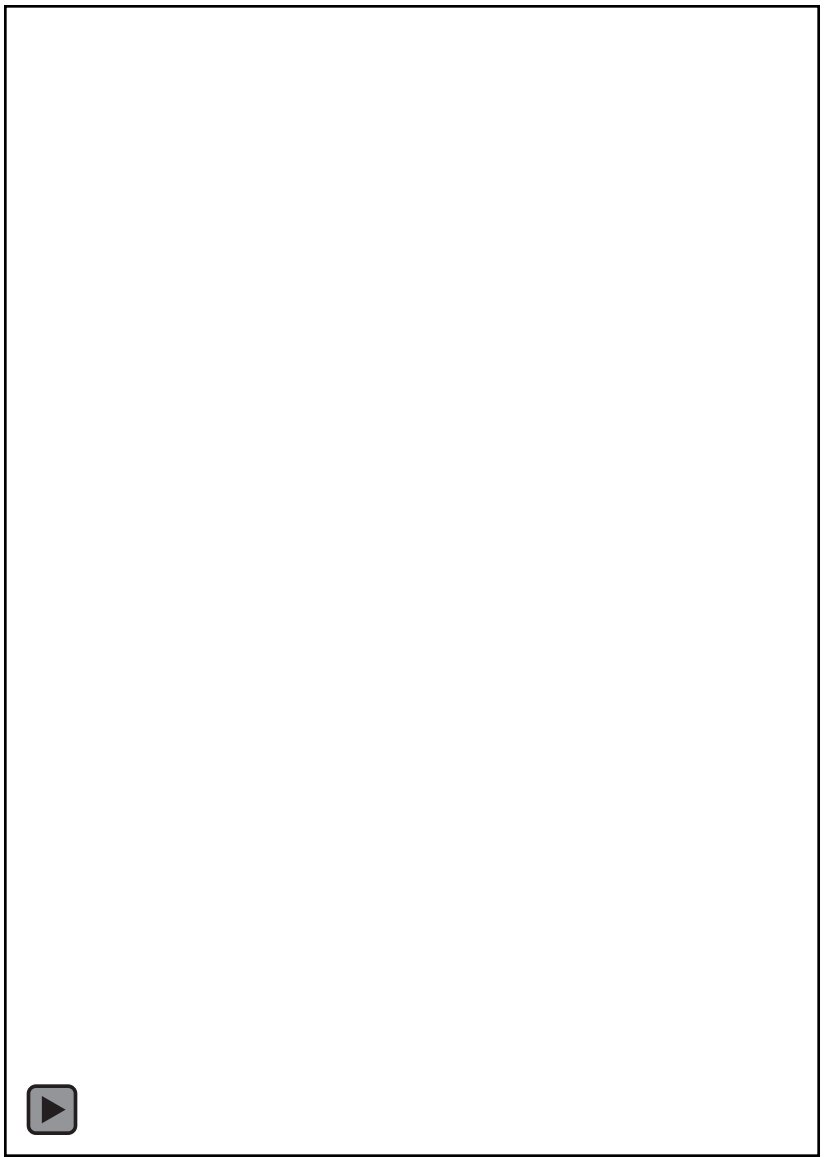
***GIT comorbidities include:** Lactose intolerance, Celiac disease, and Irritable Bowel Disease

***Proton Pump Inhibitors**

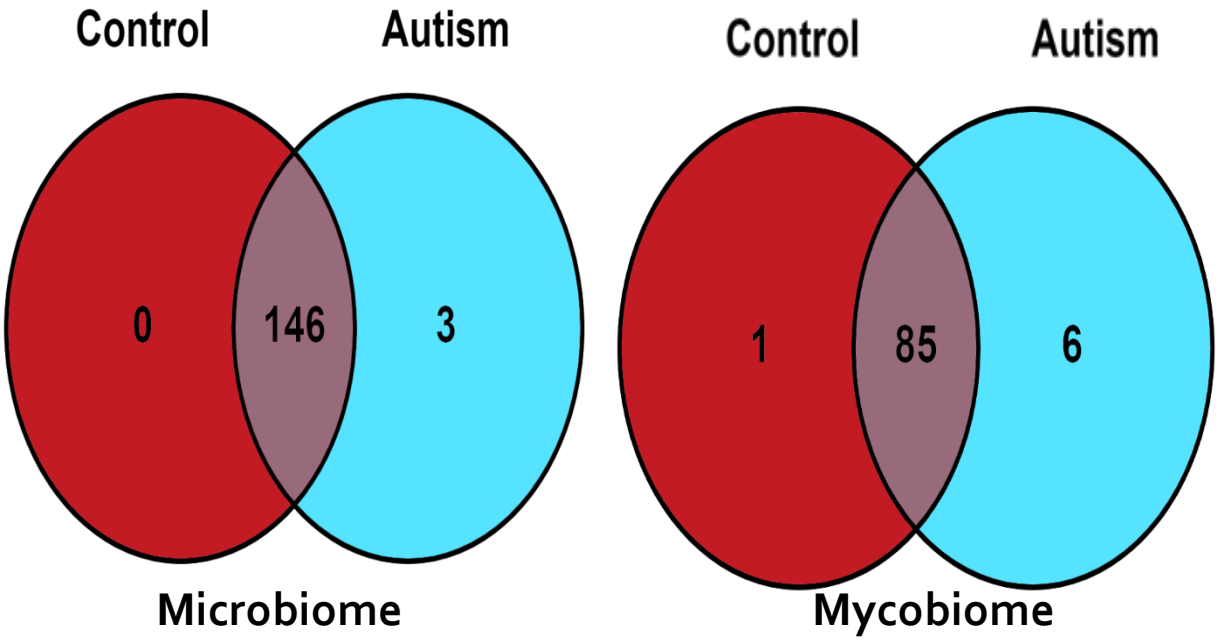
Autism Vs Sibling Control Mycobiome PCA



Autism Vs Sibling Control Microbiome PCA



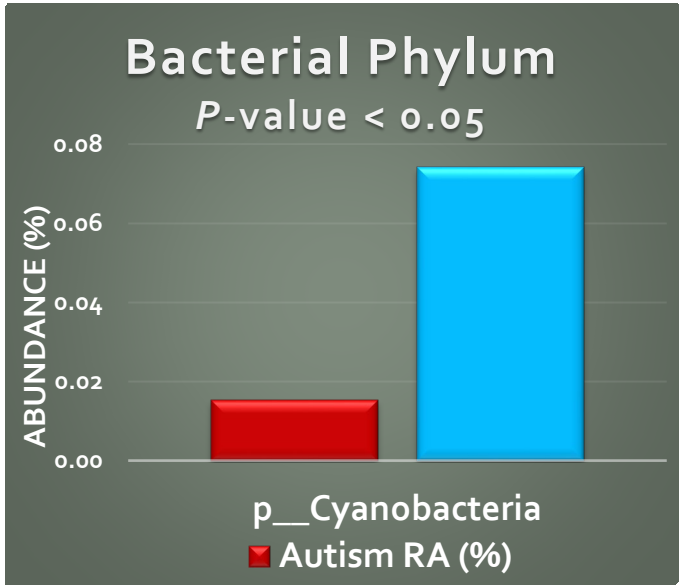
Microbiome
Unique to Autism
Bacillus humi
Roseomonas mucosa
Lactobacillus ruminis
Mycobiome
Unique to Autism
Thermomyces lanuginosus
Issatchenkia terricola
Candida ethanolica
Pichia manshurica
Aspergillus candidus
Aspergillus oryzaeAspergillus oryzae
Unique to Control
Trichosporon montevideense

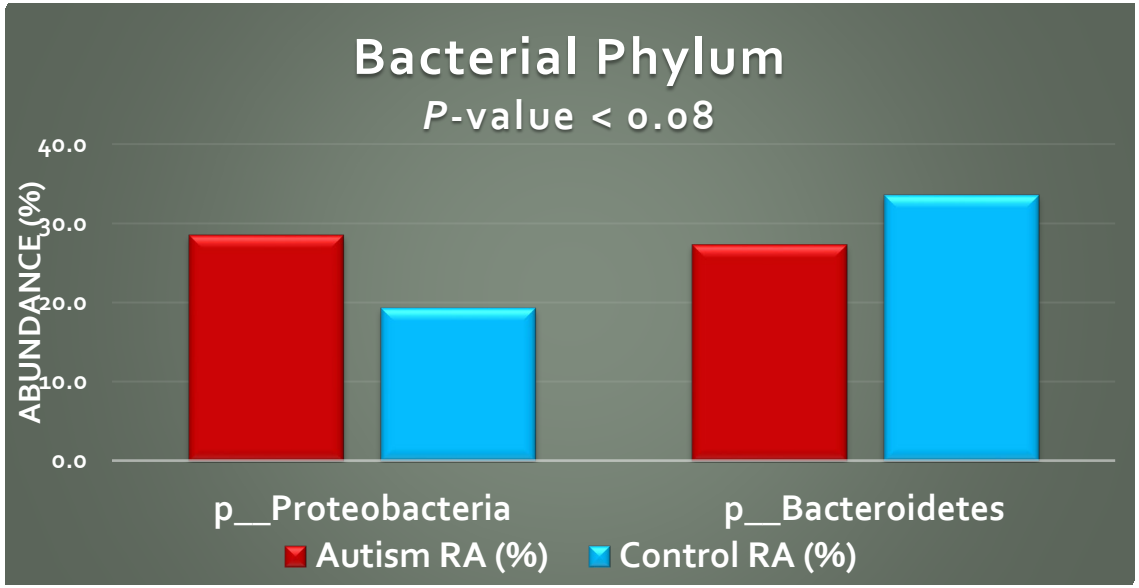


Conclusion: Richness/diversity is greater in Autism cases when compared to controls.

Trichosporon montevideense: Produces a potent biosurfactant that prevents adhesion of *Candida albicans* and *Candida krusei* cells to human buccal epithelial cells. In addition, it is known to interfere with biofilm formation in many *Candida* species (therapeutic candidate).

Roseomonas mucosa (bacteria) and Thermomyces lanuglinosus (*a fungus*), have been associated with infective endocarditis and systemic lupus erythematosus (SLE). Both are opportunistic pathogen that has been reported in human infections.



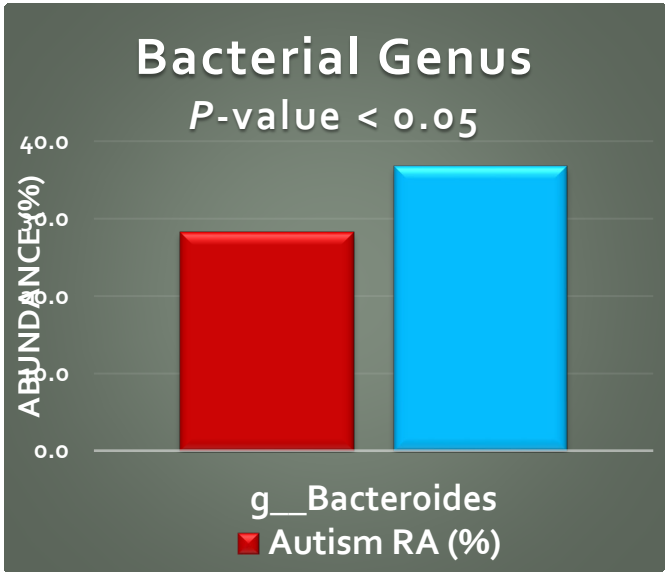


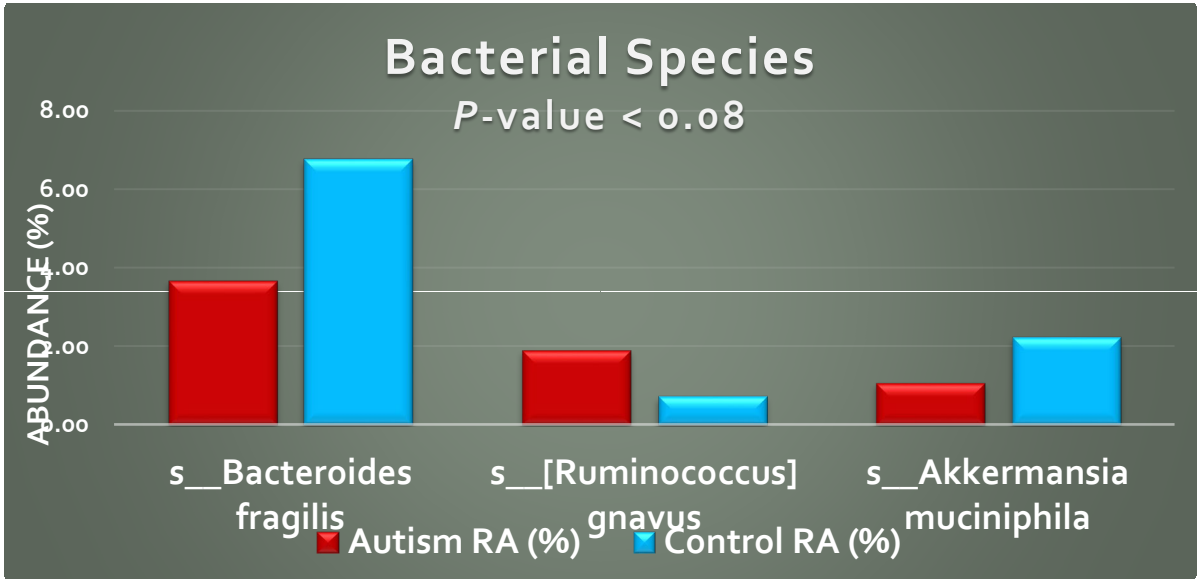
Phyla Decreased in Autism:

- ***Cyanobacterium:*** Involved in fermentation of plant derived food, mainly fiber. Contributes to a balanced gut by production of vitamins K and B.
- **Bacteroidetes:** In the gut it's a primary producers of butyrate, via colonic fermentation thus plays a role in maintaining a healthy gut.

Phylum Increased in Autism:

- **Proteobacteria:** a marker of potential dysbiosis and inflammation when elevated.





Genus Decreased in Autism:

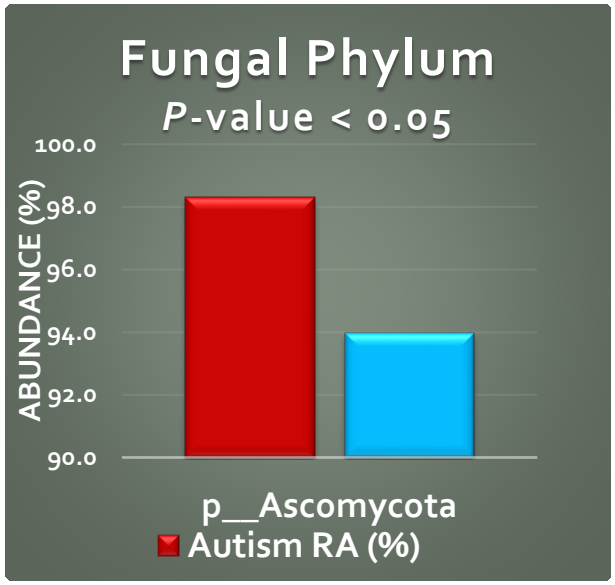
- ***Bacteroides:*** is a **beneficial bacteria** associated with anti-inflammatory effects.

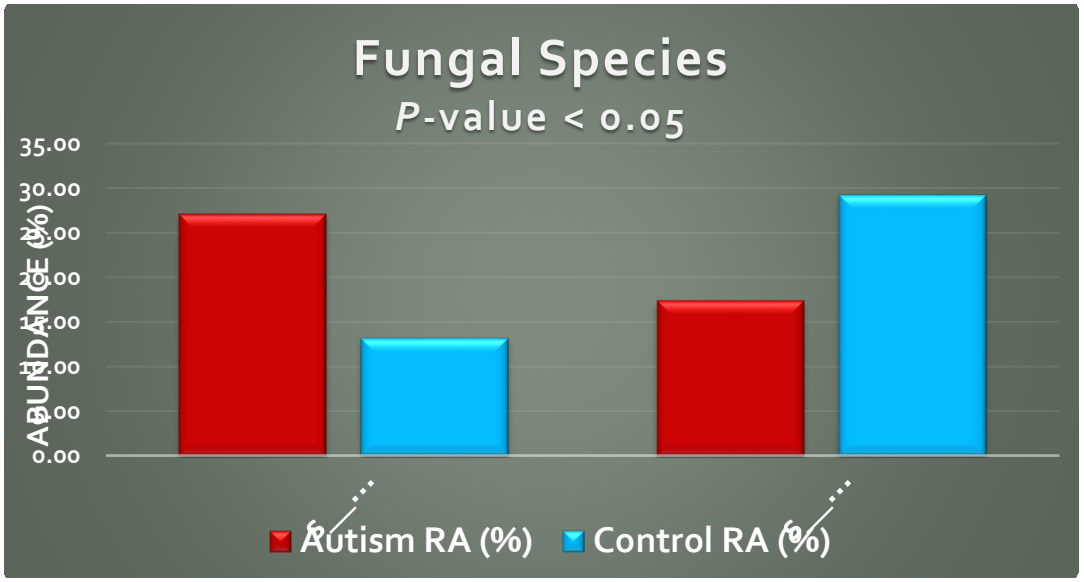
Species Decreased in Autism:

- ***Akkermansia muciniphila:*** is a **beneficial bacteria** associated with anti-inflammatory effects and with balanced gut health.
- ***Bacteroides fragilis:*** *Bacteroides fragilis*, given as a probiotic, helped to alleviate Autism-like symptoms in mice

Species Increased in Autism:

- ***Ruminococcus gnavus***: A pathogen associated with dysbiosis in Crohn's patients often co-occurring with increased disease activity. Produces complex polysaccharide leading to activation of the pro- inflammatory cascade.





Phylum Increased in Autism:

- ***Ascomycota:*** Dominates the fungal ecology of most human guts.
- *Ascomycota* are elevated contributing to the statistically significance increase in the Autism cases.

Species Increased in Autism:

- ***Candida albicans*:** Is considered pathogenic.
- High levels of *Candida* has been linked to high carbohydrate diets, as well as, increase sugar cravings.

Species Decreased in Autism:

- ***Galactomyces geotrichum:*** Elevated in the healthy sibling controls. Considered a **good commensal fungi** due to its role as a primary fermenter of complex sugars.

Conclusion

- Identified bacterial and fungal species associated with gut microbiome imbalance in autistic individuals
- **Factors influencing imbalance in Autism**:
  - Male gender
  - Low fish diet
  - Seizures
  - Upper GI issues
- **Goal: Identify and implement** strategies to modulate the microbiome

How to Address G-B-A Disorders?

- Diet
- Lifestyle
- Nutritional Supplements

Gut-Brain Axis: Good News for the Future

Going forward, we are learning how dysbiosis of the microbiome influences neurodegenerative diseases and conditions.

This will put us in a great position to conduct more research, using new tools, that will allow us to better understand the gut-brain axis.

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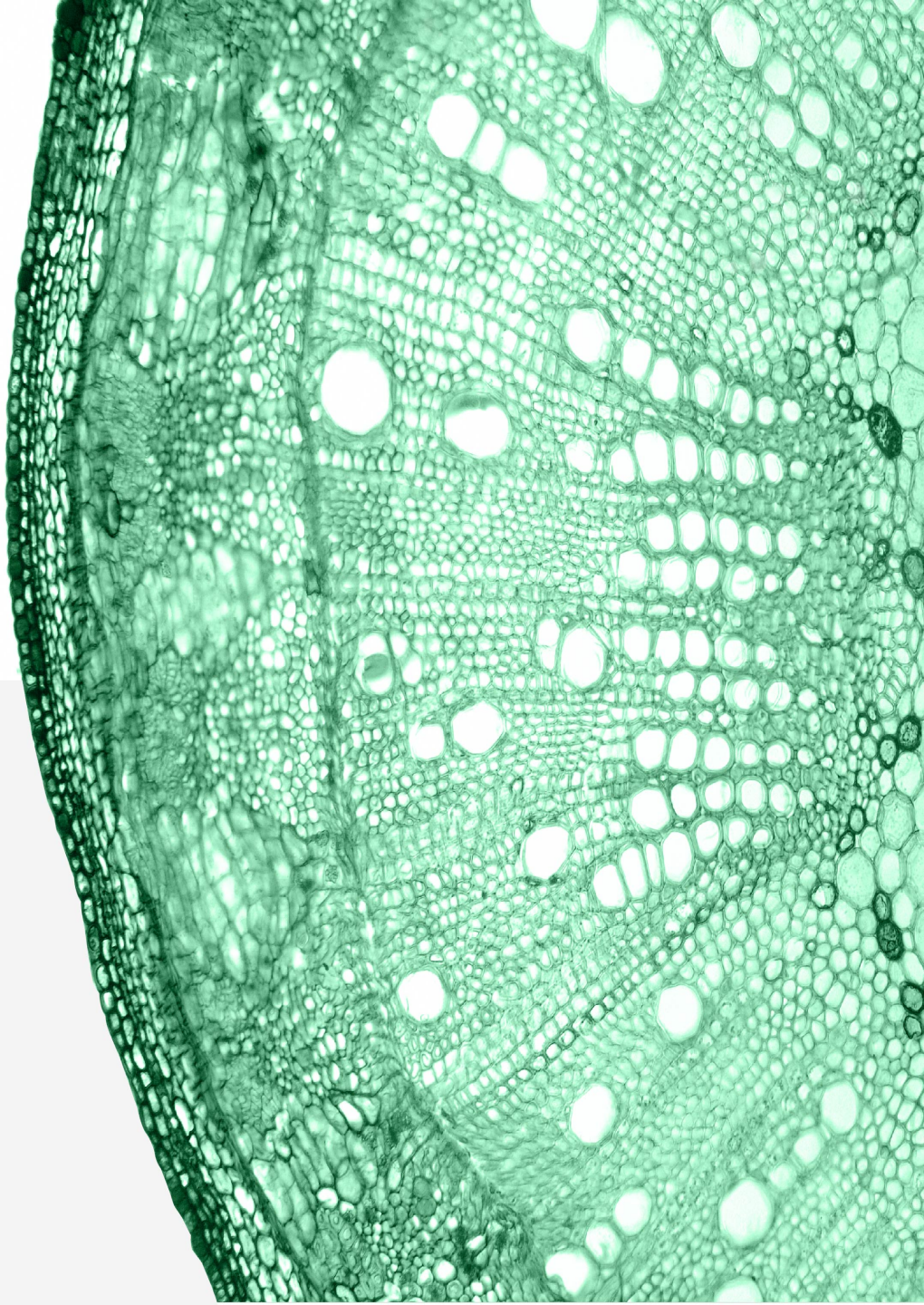
Diet as a Modifier of the Microbiome



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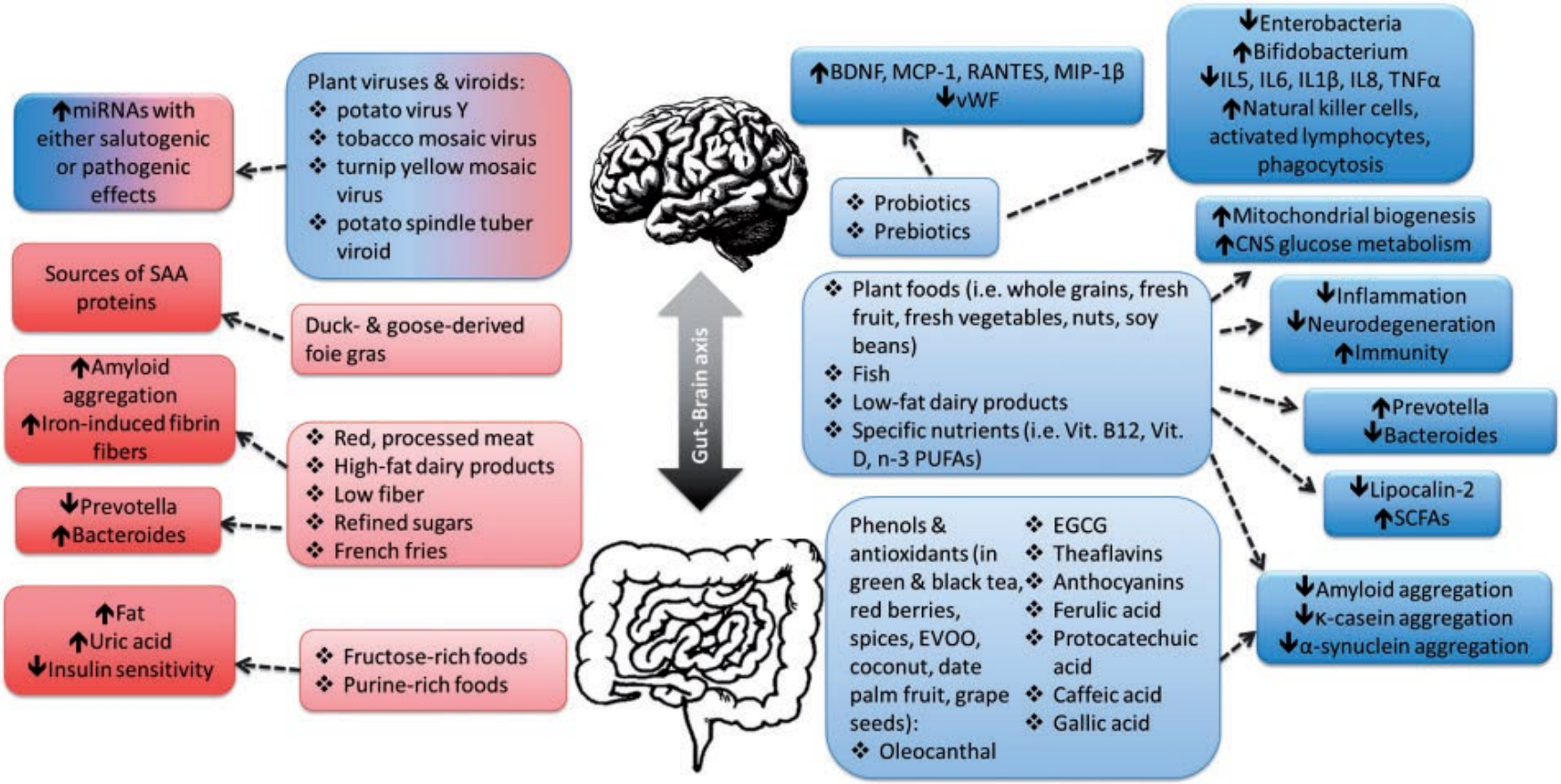
Effects of Nutrients/Foods on Gut Microbiota or Amyloid Formation

Pathogenic effects

Protective effects



Pistollato *et al.* Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. Nutrition ReviewsVR Vol. 74(10):624–634

Relationship Between Diet and Depression

A large number of promising studies have examined the relationship between diet and depression.

Firth *et al.* examined 16 eligible randomized controlled trials with outcome data for > 45,000 participants.

Their findings indicated that dietary intervention significantly reduced depressive symptoms in high-quality trials (g = 0.321, 95% CI = 0.12 to 0.53, p = .002).

Other studies have revealed an association between diet quality and the probability of and risk for depression.

Additionally, pro-inflammatory dietary patterns have specifically been linked to a greater likelihood of developing depression and depressive symptoms.

Firth J, et al. Psychosom Med 2019;81:265-80. Li Y, et al. Psychiatry Res 2017;253:373-82.
Oddy WH, et al. Brain Behav Immun 2018;69:428-39.
Phillips CM, Clin Nutr 2018;37:1485-91.
Lassale C,, et al. Mol Psychiatry 2019;24:965-86.
Li Y,, et al. Psychiatry Res 2017;253:373-82.
Shivappa N,, et al. J Affect Disord 2018;235:39-44.

Diet and Depression

Relationship between the **prevalence of depressive symptoms** and certain **eating habits** is established:

- Depressive symptoms have been associated with a high glycemic index due to a high intake of sugars/refined carb
- Conversely, healthy eating habits are associated with a decreased risk of depression
- Considerable improvements in depression levels were observed in people who followed the "Mediterranean Diet"

Phillips CM, Shivappa N, Hebert JR, Perry IJ. Dietary inflammatory index and mental health: A cross-sectional analysis of the relationship with depressive symptoms, anxiety and well-being in adults. Clin Nutr 2018;37:1485-91.

Shivappa N, Hebert JR, Veronese N, et al. The relationship between the dietary inflammatory index (DII((R))) and incident depressive symptoms: A longitudinal cohort study. J Affect Disord 2018;235:39-44.

Gangwisch JE, Hale L, Garcia L, et al. High glycemic index diet as a risk factor for depression: analyses from the Women's Health Initiative. Am J Clin Nutr 2015;102:454-63.

Francis HM, Stevenson RJ, Chambers JR, Gupta D, Newey B, Lim CK. A brief diet intervention can reduce symptoms of depression in young adults - A randomised controlled trial. PLoS One 2019;14:e0222768.

Jacka FN. Nutritional Psychiatry: Where to Next? EBioMedicine 2017;17:24-9.

Parletta N, Zarnowiecki D, Cho J, et al. A Mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: A randomized controlled trial (HELFIMED). Nutr Neurosci 2019;22:474-87.

Diet and Depression: Underlying Mechanism/s

- **Several pathways diet can affect depression, they are related to:** oxidative stress, inflammation, and mitochondrial dysfunction, which are disrupted in people with mental health disorders
- **A healthy diet includes** various compounds that beneficially engage these pathways.
- **For example,** fruits and vegetables contain fiber, vitamins, minerals, and a large number of polyphenols.
- These factors are associated with decreased depression rates, **due to their anti-inflammatory, neuroprotective, and prebiotic properties.**
- **Unhealthy diets**, rich in nutritional factors that negatively engage these pathways.
- **Specifically,** processed foods ingredients (e.g. artificial sweeteners, emulsifiers, saturated fatty acids) may change the gut microbiome's composition and activate inflammatory pathways

Morris G, Walder K, McGee SL, et al. A model of the mitochondrial basis of bipolar disorder. Neurosci Biobehav Rev 2017;74:1-20.

Chang SC, Cassidy A, Willett WC, Rimm EB, O'Reilly EJ, Okereke OI. Dietary flavonoid intake and risk of incident depression in midlife and older women. Am J Clin Nutr 2016;104:704-14.

González R, Ballester I, López-Posadas R, et al. Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 2011;51:331-62.

Dietary Approach to Reducing Clinical Depression

A study investigated the effects of dietary improvement on symptoms of depression and anxiety found that:

- Weight loss, nutrient boosting and fat reduction diets can all reduce the symptoms of clinical depression
- Adopting a healthier diet can boost people's mood.
- Specifically, eating more nutrient-dense meals which are high in fiber and vegetables, while cutting back on fast-foods & refined sugars help avoid the potentially negative psychological effects of a 'junk food" diet.
- Change in lifestyle (particularly exercise) will also help tackle low mood and depression.
- Critically, combining dietary approach and exercise results in greater improvement in depressive symptoms.

Dietary Approach to Reducing Clinical Depression, Continued

Take Home Message:

Eating a healthier diet and **participating in regular exercise** could be a viable treatment that help people with low mood.

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Nutritional Supplements as a Modifier of the Microbiome

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Probiotics are an essential part of the holistic approach to treating depression

- Probiotics plays an important role in alleviating depression
- They help to serve the critical function of rebalancing the microbiome
- Published evidence suggests that probiotics will play a promising role in combating depression
- Studies conducted on humans and animals indicated that probiotics are associated with a reduction in anxiety and depression
- These studies indicated that probiotic therapy reduced depressive symptoms and improved the functionality of both the HPA axis and the antidepressant, Citalopram

Studies Supporting the Role of Probiotics in Depression Reductions

A double-blind, placebo-controlled randomized study examining the effects of probiotics (administered for 4 weeks) on behavior, brain function, and gut microbiome in 45 healthy volunteers showed that:

- **Probiotic use resulted in a shifts in gut microbiome profiles and brain activation patterns**

- The probiotic used contained 9 bacterial strains: ***Lactobacillus*** *casei* W56, *Lactobacillus acidophilus* W22, *Lactobacillus paracasei* W20, ***Bifidobacterium lactis*** W51, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, *Bifidobacterium lactis* W52, *Lactobacillus plantarum* W62 and *Bifidobacterium bifidum* W23

- **Using multi-strain probiotics may result in a synergistic activity thereby providing better benefits than using single strain probiotic as they differentially:**
  - Help probiotics to establishment themselves in the gut
  - Antagonize pathogens
  - Produce more beneficial short chain fatty acids

How Probiotics Reduce Depression: Underlying Mechanism/s

Multi-strains probiotics reduce depression through two potential mechanisms:

1. Exerting immunomodulatory effects, and
2. Decreasing intestinal translocation of microbial constituents across the gut intestinal barrier

Immunomodulatory effects is indicated by a **decrease of stress-induced:**

1. **Interleukin (IL)-18** levels in the serum
2. **Interferon gamma (IFNg)** levels in the small intestine

Additionally, probiotic treatment led to a reduction of:

- Corticosterone in stool and catecholamines in the hypothalamus, as well as anxiety-like behavior
- Total and low-density lipoprotein (LDL) cholesterol levels

Based on this, we believe that probiotics are an important part of the holistic approach to rebalancing the microbiome and treating depression.

Lifestyle as a Modifier of the Microbiome

The last element of our holistic approach to combating depression focuses on lifestyle habits

The gut microbiota is an extremely dynamic system that constantly changes over time due to a combination of various factors and lifestyle habits

We believe that these two lifestyle habits: (**exercise and sleep**) are critical as a result of the existing evidence that demonstrates their unique benefits in improving gut health and decreasing depression

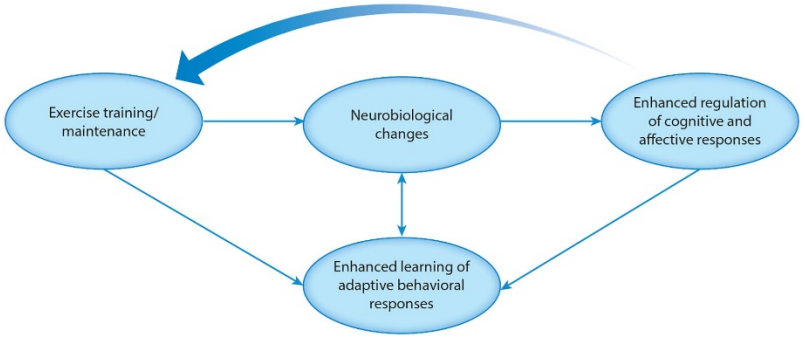
Exercise and Depression

- Exercise is a significant lifestyle habit to consider due to its relationship with the microbiome and depression
- Exercising is associated with various benefits that are directly or indirectly related to microbiome health. For example:
    - Exercise can reduce many inflammatory conditions that influence depression
    - Exercise can improve intestinal barrier dysfunction

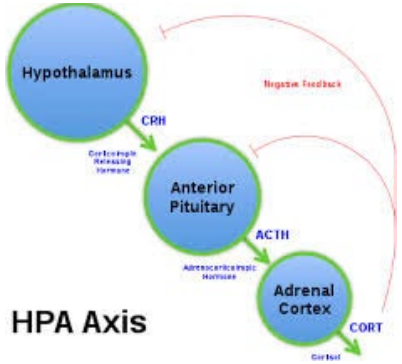
Mechanism/s Responsible for Depression Reduction Associated with Exercise

- Exercise alleviates depression through common neuro-molecular mechanisms including:
  - Regulation of HPA-axis activity,
  - Increase expression of neurotrophic factors [i.e., Brain derived neurotropic factor (BDNF)],
  - Increase availability of serotonin and norepinephrine, and
  - Reduce systemic inflammatory signaling.

These activities stimulate the development of: new neurons, increase cerebral vasculature and synaptic connections thereby boosting cognitive function with the ultimate result of reducing depression symptoms.



A model integrating neurobiological and behavioral mechanisms by which exercise training improves mental health outcomes.

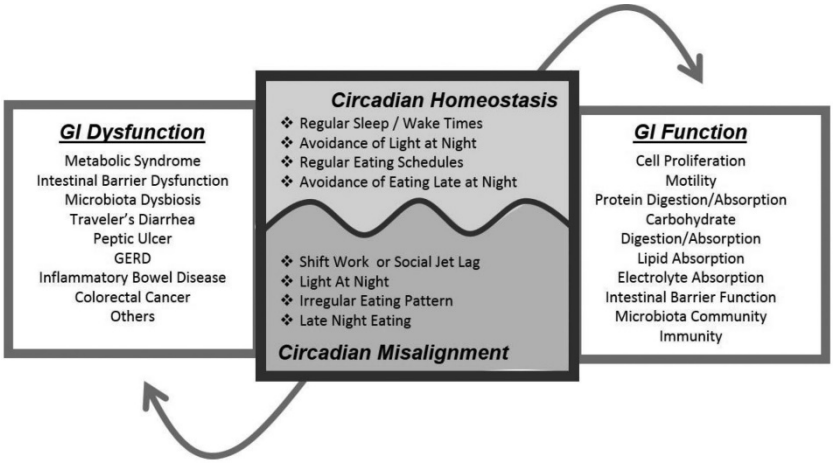


Patrick J. Smith P, and Merwin R. 2021. Annu Rev Med. 2021 January 27; 72: 45–62. doi:10.1146/annurev-med-060619-022943.

Sleep, the Microbiome and Depression

- Sleep has been shown to have a direct impact on the microbiome in many ways.
- Studies have demonstrated the link between sleep deprivation or disruption and microbial dysbiosis.
- Lack of sleep can lead to and exacerbate depression and depressive symptoms. Individuals with depression experience trouble falling or staying asleep,
- Circadian homeostasis permits optimal gastrointestinal function. Conversely circadian misalignment including irregular schedules and late night eating promote disease and dysfunction in the GI tract.

Circadian regulation of gastrointestinal function and dysfunction.



Voigt R *et al*. PLoS One 2014;9:e97500.
Voigt R. 2019. Expert Rev Gastroenterol Hepatol. 13(5): 411–424. doi: 10.1080/17474124.2019.1595588

Sleep and the Microbiome: A Bidirectional Relationship

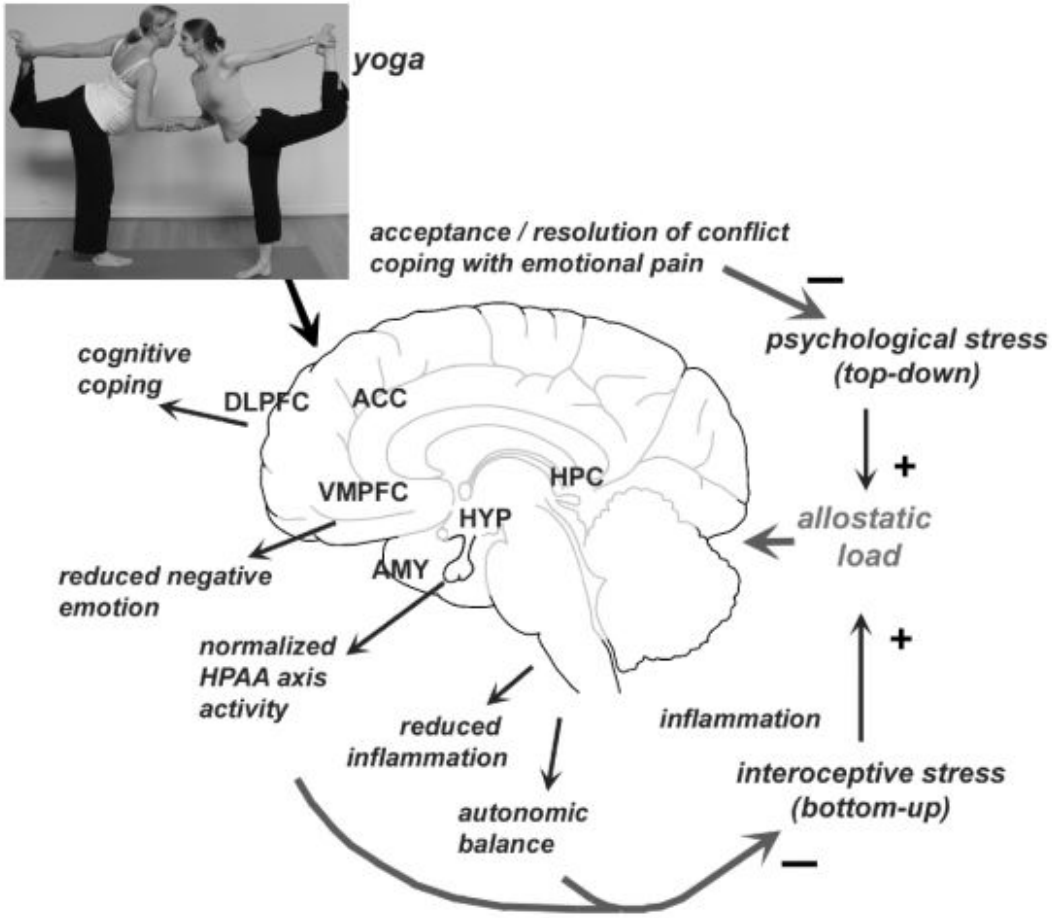
- The relationship between sleep and the microbiome is bi-directional where the microbiome can affect our sleep pattern and vice versa
- Microbiome diversity is positively correlated with sleep efficiency, and total sleep time, while negatively correlated with sleep fragmentation
- Suggesting that gut microbiome diversity promotes healthier sleep
- Specifically, richness within the phyla *Firmicutes* and *Bacteroidetes* were positively correlated with sleep efficiency
- An increase or decrease in F/B ratio is often regarded as dysbiosis
- *Actinobacteria* phylum was shown to negatively correlate with the number of awakenings. i.e., increased richness within this phylum contributes to high sleep quality
- Positive effect of the microbiome on sleep is attributed to their ability to secret γ-aminobutyric acid, a neurotransmitter known to promote sleep

Brain substrates by which yoga may mitigate the effects of both "top-down" and "bottom-up" stress effects on "Wear and Tear on the Body" allostatic load.

In response to breathing and postural feedback:

Prefrontal cortical areas (PFC), notably VMPFC, modulate stress- responsive brain regions including the amygdala (AMY), hippocampus (HPC), and hypothalamus (HYP), to improve HPA-axis activity, autonomic balance, and inflammation, reducing drive on bottom-up stress pathways.

Meditative/mindfulness aspects of yoga encourage positive coping mediated by PFC structures including DLPFC and dorsal ACC, thereby reducing drive on top-down stress.



Explore (NY). 2012 March 1; 8(2): 118–126. doi:10.1016/j.explore.2011.12.005.
How Might Yoga Help Depression? A Neurobiological Perspective
Patricia Anne Kinser, PhDc, WHNP-BC, MS, RN1,2,*, Lisa Goehler, PhD1, and Ann Gill Taylor, EdD, RN, FAAN1

Holistic Approach to Balancing the Gut Microbiome and Gaining Wellness

Our Recommendations

Eating a healthier diet, taking regular exercise, and sound sleep could be a viable treatment that will support a balanced microbiome and harmonious gut-brain axis communication.

LN02114 09/21

Adopting a Total Gut Balance

Nutritionally balanced
Whole-food-based
Low-glycemic
Rich in fiber and resistant starches
Low in sugar
Full of good mono- and polyunsaturated plant fats
Low in saturated fat and animal fat
Rich in lean plant protein and animal protein primarily from seafood, with a bit of poultry thrown in
Ultimately flexible, diverse, and customizable

Mahmoud Ghannoum, Eve Adamson, 2019. Total Gut Balance, The Countryman Press, New York

Total Gut Balance (Mycobiome Diet) is Designed To:

Works on all parts of the **microbiome** (bacteria and fungi) and **lifestyle practices**

Limit the growth of pathogenic fungi (e.g. *Candida*): with a diet designed to stifle its growth:

1. vitamins A, C, and B s,
2. low-carb,
3. a good balance of plant-based protein and fatty acids.

Enhance the growth of beneficial bacteria: by providing prebiotic food thereby keeping fungal growth under control and at the same time crowding out pathogenic bacteria that cooperate with *Candida* in biofilm-making efforts.

Break down existing biofilms using particular kinds of vegetables, fruits (garlic, apple cider vinegar).

Reduce overall inflammation with potent anti-inflammatory and antioxidant foods rich in targeted vitamins, minerals, and other beneficial constituents.

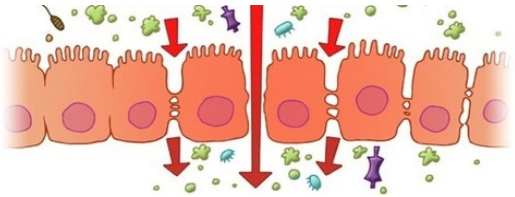
Overview of the Total Gut Balance Diet, also referred to as the Mycobiome Diet

Frequency	Food/Food Type	Quantity
Daily	• Coconut or extra-virgin olive oil • Resistant starch foods • Cruciferous vegetables • Mycobiome-friendly vegetables • Apple cider vinegar <u>OPTIONAL</u> • Eggs • Poultry (Ex: chicken and turkey) • Low-fat or non-fat dairy products • Tofu and tempeh • Edamame	• At least 1 tablespoon • At least 1 cup but no more than 2 cups • At least 1 cup with no maximum • At least 2 cups • At least 1 tablespoon • 2 • 6 ounces • 1 cup • 4-6 ounces • 1 cup, 2 servings maximum
3-7 times per week	• Fish/seafood • Ground turmeric • Ginger • Garlic • Pistachios and/or walnuts • Green Tea • Fermented Foods	• Up to 6 ounces • At least 1 teaspoon • At least 1 teaspoon dried and ground or 1 tablespoon fresh grated • 1 to 2 cloves • ¼ cup • At least 1 cup • ½ cup
Limited consumption	• Alcohol • Maple syrup or raw honey • Coffee and/or black tea	• No more than 3x/week or exclude completely • No more than 1 tablespoon per day, less is better • Prioritize organic varieties
Never	• No added sugar sweeteners (except for maple syrup and raw honey) • No refined grains • No processed, cured meat • No processed or packaged food with more than three ingredients • No oils or fats, other than those allowed above, including butter • No full-fat dairy products	

Ghannoum and Adamson, 2020. Total Gut Balance, The Countryman Press, A Division of W Norton and Company

Lifestyle Recommendations

Chronic Stress → **Inflammation** → **Weakens Gut Lining + Alters Microbiome**



Toxins Enter Blood Stream → **Further Decline in Immune System**

Activity
- At least 15-20 minutes a day
- Moderate activity especially when stressed

Limit Stress
- Prioritize
- Take time for yourself each day
- Meditation/yoga

Sleep
- At least 7-8 hours a day

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WholisticMatters™

MICROBIOME, INFLAMMATION AND LEAKY GUT

- Inflammation is the root of all diseases
- Diseases connected to the microbiome
- Relationship between the microbiome and inflammation
- Gut microbiota modulate inflammation at distant sites
- Mechanisms mediating host-microbiota interactions
- Associations between the intestinal microbiota and autoimmune disorders and neuroinflammation
- Leaky Gut and its causes and symptoms
- Approaches to support immunity and lower inflammation

Inflammatory Diseases are Connected to the Microbiome

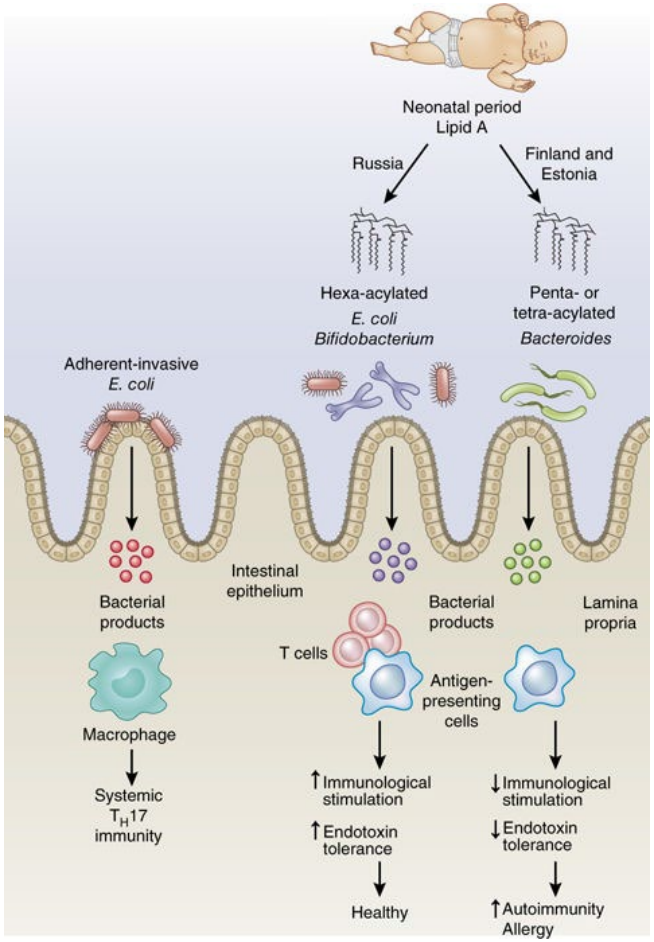
- At the forefront of how your gut microbiome determines whether or not you'll deal with various illnesses is inflammation
- Inflammation is the root of most diseases
- Studies show that an anti-inflammatory lifestyle is protective over brain neurons, balances hormones, fights the formation of tumors and has mood-enhancing benefits
- Beneficial bacteria can help manage neurotransmitter activity making them natural antidepressants and anti-anxiety organisms

What diseases are connected to the microbiome and inflammation?

Microbiome imbalance and poor gut health are tied to dozens of diseases:

- **Autoimmune diseases (arthritis, inflammatory bowel disease, Hashimoto's disease, etc.):** Autoimmune disorders develop when the body's immune system goes awry and attacks its own healthy tissue.
- Inflammation largely stems from an overactive immune system and poor gut health. Leaky gut syndrome can develop, which results in small openings in the gut lining opening up, releasing particles into the bloodstream and kicking off an autoimmune cascade.
- **Brain disorders/cognitive decline (Alzheimer's, dementia, etc.): Inflammation** is highly correlated with cognitive decline, while an **anti-inflammatory** lifestyle led to better memory retention, longevity and brain health. **Differences in our microbial communities** might be one of the most important factors in determining if we deal with cognitive disorders in older age.
- **Cancer:** Studies have shown **a link between gut health and better protection from free radical damage**, which causes brain, breast, colon, pancreatic, prostate and stomach cancers.
- Microbes influence our genes, which means they can either promote inflammation and tumor growth or raise immune function and act as a natural cancer treatment.

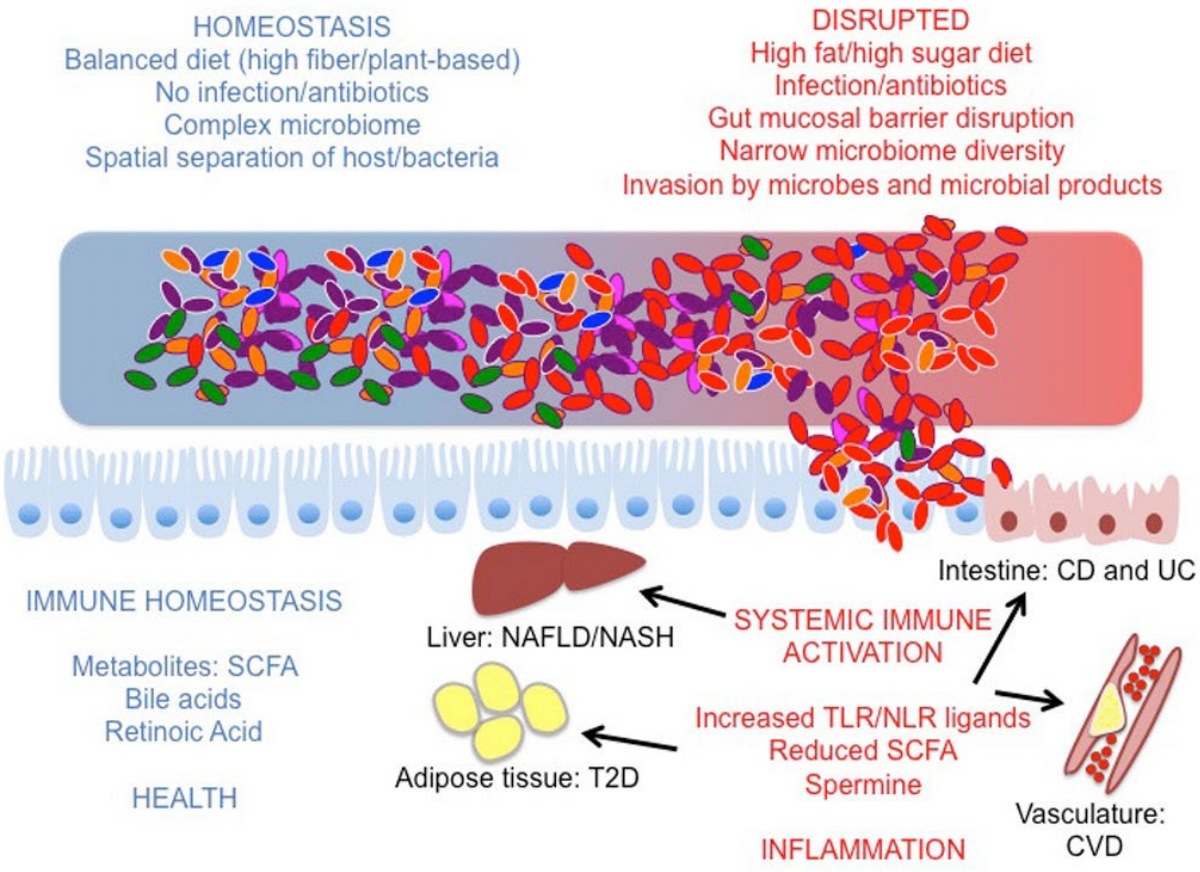
Gut Microbiota Modulates Inflammation at Distant Sites



- Relative to Russia, Finland has an incidence 2-6 fold higher for allergies and 5-6 fold higher for type 1 diabetes
- Infants from Russia have more abundant ***E. coli*** spp. **expressing stimulatory hexa-acylated LPS**, whereas infants from Finland and Estonia have more abundant **Bacteroides spp**. **expressing the less stimulatory tetra- and penta-acylated LPS82**
- Hexa-acetylated LPS induces greater Immunological stimulation but also endotoxin tolerance thought to dampen the capacity for immunological education
- However, the less stimulatory LPS from Bacteroides spp. impairs LPS tolerance, thus increasing susceptibility to immunological disease

Blander J *et al.* Nat Immunol. 2017; 18(8): 851–860. doi:10.1038/ni.3780

Potential linkages between the environment, microbiota, immune system and chronic inflammatory disease



Hand *et al.* 2016. Trends Endocrinol Metab.; 27(12): 831–843. doi:10.1016/j.tem.2016.08.003.

Potential linkages between the environment, microbiota, immune system and chronic inflammatory disease

Homeostasis: the microbiota assists the host in converting the diet into metabolites

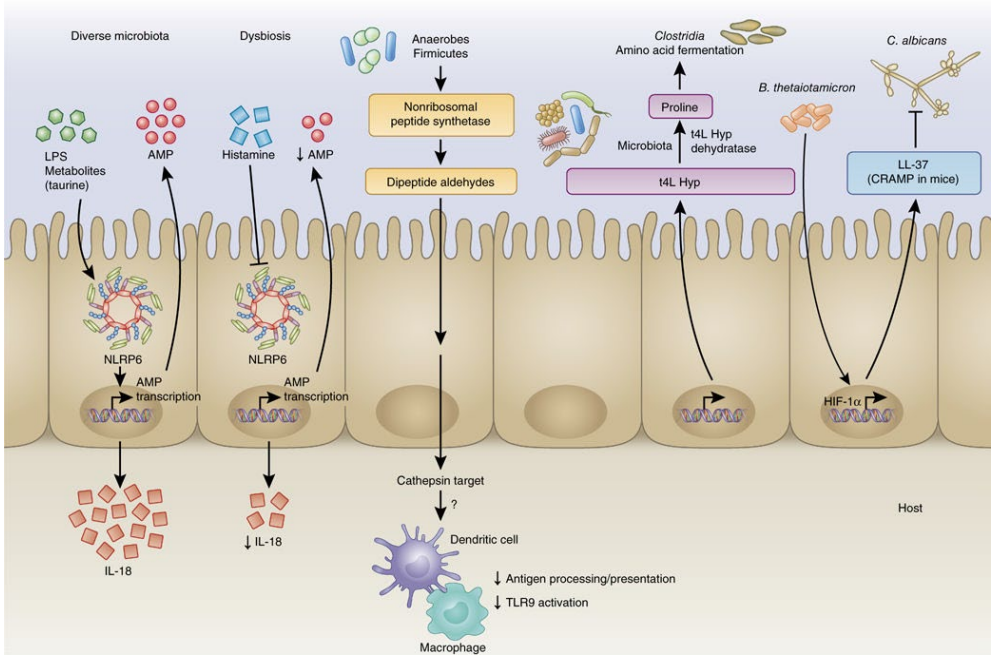
- These metabolites bolster the barrier between the host and the microbiota, preventing systemic immunity and inflammation.

Dysbiosis: leads to reducing microbial diversity and a shift in the microbial metabolites

- This shift can lead to increased invasion of host tissue by bacteria and their products.
- Contributing to immune activation at the core of chronic inflammatory disease
  - e.g. IBD = Irritable Bowel Disease; CVD = Cardiovascular Disease; T2D = Type II Diabetes; NASH/NAFLD = Non-alcoholic Steatohepatitis/ Non-alcoholic Fatty Liver Disease)

Hand et al. 2016. Trends Endocrinol Metab.; 27(12): 831–843. doi:10.1016/j.tem.2016.08.003.

Mechanisms mediating host–microbiota interactions



- The microbiota provides two signals for NLRP6 inflammasome activation in intestinal epithelial cells:

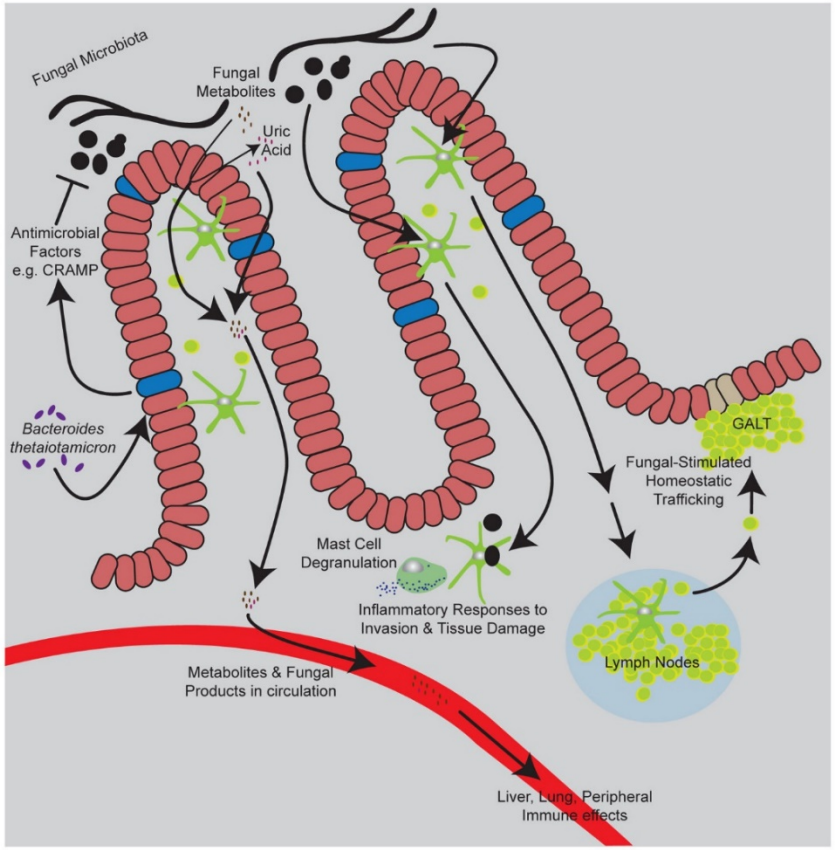
  Signal 1 is in the form of LPS, and

  Signal 2 is in the form of metabolites such as the **bile-acid conjugate taurine**.
- These signals activate the NLRP6 inflammasome in intestinal epithelial cells and lead to the production of epithelial IL-18 and antimicrobial peptides (AMP).
- Under dysbiotic conditions, microbiota-derived histamine, putrescine and spermine are increased, thus suppressing NLRP6 inflammasome signaling, decreasing production of epithelial IL-18 and AMP in the colon and promoting intestinal inflammation.

Blander J *et al.* Nat Immunol. 2017; 18(8): 851–860. doi:10.1038/ni.3780

Diverse effects of commensal fungi

- Bacterial microbiota and the intestinal epithelium influence the ability of fungi to colonize the gut.
- Commensal fungi produce metabolites and products that influence immunity and inflammation at local and distal sites.
- Immune cells in the gut respond to commensal fungi to influence inflammation and immune homeostasis.



Marissa J. Paterson*, **Seeun Oh***, and **David M. Underhill. Host-microbe Interactions: Commensal Fungi in the Gut.** Curr Opin Microbiol. 2017 December ; 40: 131–137. doi:10.1016/j.mib.2017.11.012.

Influence of fungal dysbiosis on inflammation and immunity at distal sites

Studies showed that high-level GI colonization with *C. albicans* in antibiotic-treated mice can exacerbate pathology in allergic airway disease models

Others reported that inducing alterations in the existing fungal microbiome with antifungal drugs can similarly alter the course of house dust mite (HDM)-induced allergic airway disease

Perturbing fungal homeostasis by oral treatment with the antifungal fluconazole reduced the prevalence of some intestinal fungi but allowed for increased growth of specific fungi including *Aspergillus, Wallemia* and *Epicoccum*

Antifungals-induced fungal dysbiosis or oral exposure to Aspergillus, Wallemia and Epicoccum caused increases in allergy-related antibodies (IgE and HDM IgG1), Th2 cytokine producing T cells (IL-4, IL-5, IL-10) and infiltration of eosinophils into the lungs upon HDM immunization

Wheeler et al. 2004. Infect Immun; 72:4996–5003.

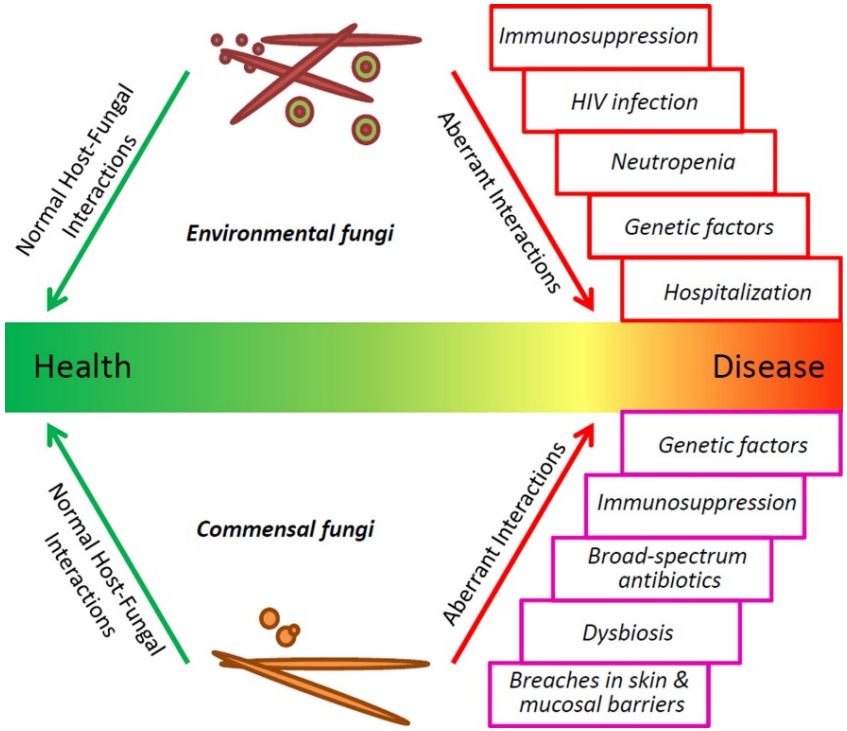
Noverr et al. 2004. Infect Immun; 72:4996–5003.

Interaction Between Fungi and Immune System

Highlights

- The immune system is constantly exposed to fungi living at host mucosal surfaces or coming from the environment.
- Interactions between fungi and host immunity have typically been studied in the context of fungal disease.
- Many opportunistic fungi can also be commensals.
- The immune system must tolerate colonization with commensal fungi but defend against fungal invasion.

Balance between fungi and the host

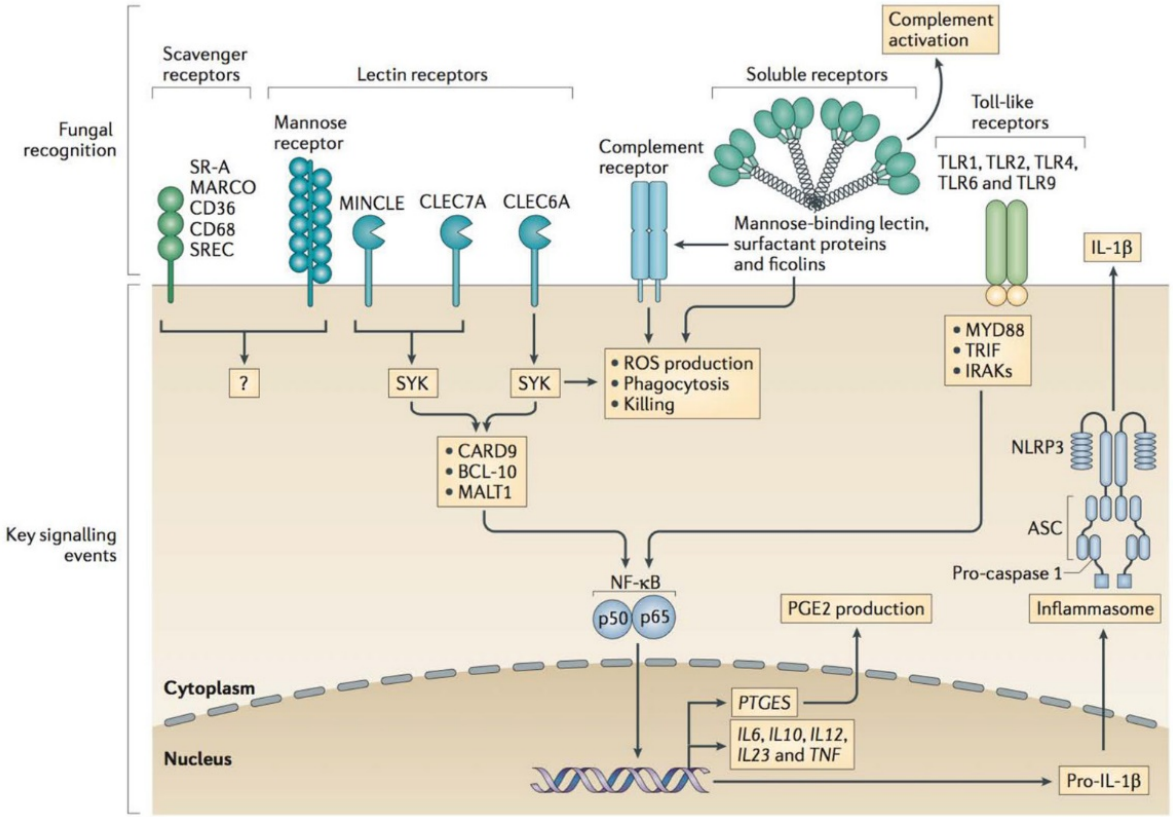


- Environmental and commensal fungi are in constant contact with the host providing myriads of fungal antigens with which to interact.
- During homeostasis commensal fungi are kept at the mucosal surfaces, while environmental fungi are either killed or tolerated upon contact with the host.
- Under aberrant conditions when mucosal and immune barriers are compromised, fungi invade tissues leading to dissemination, inflammation and disease.

Jose et al. 2017. Cell Host Microbe. 09; 22(2): 156–165. doi:10.1016/j.chom.2017.07.002.

Immune receptors and signaling pathways involved in recognition of fungi

- **Innate immune cells utilize a wide variety of membrane-bound and soluble receptors to recognize fungi.** Membrane-bound receptors such as lectin receptors (that recognize fungal polysaccharides), Toll-like receptors (TLRs), and scavenger receptor family members can recognize a variety of fungi or their soluble products.
- These receptors trigger phagocytosis, respiratory burst and intracellular signaling leading to activation of transcription factors (e.g. NF-κB) that mediate production of inflammatory cytokines and chemokines that are important for host defense against fungi.
- Fungi may also be recognized by soluble receptors such as the mannose-binding lectin (MBL) that can direct complement activation for killing and release of inflammatory mediators as well as opsonize fungi for recognition by additional membrane-bound receptors such as complement receptors.



David M. Underhill and Iliyan D. Iliev . 2014. **The mycobiota: interactions between commensal fungi and the host immune system** *Nat Rev Immunol.* 2014 June ; 14(6): 405–416. doi:10.1038/nri3684.

Bacteriome-Mycobiome Interactions in Crohn's Disease

- Crohn's Disease (CD) is an inflammatory disease that causes chronic inflammation of the gastrointestinal tract and most often involves the small intestine and colon.

- CD is mediated by multitude of factors:
  - Host genotype (genetic factor),
  - Dysregulated immune responses, and
  - The intestinal microbiota: Dysbiosis of the intestinal flora plays a key role in modulating CD-associated inflammatory processes.

Characterization of the Mycobiome and Bacteriome in Crohn's Patients and Healthy Relatives in Multiplex Families

Subjects Demographics

Variable	CD	NCR	NCU	Total
Families	9	9	4	13
Individuals	20	28	21	69
Female	12	13	13	38
Male	8	15	8	31
Age (mean, yrs)	44.5	48.4	41.3	45.1

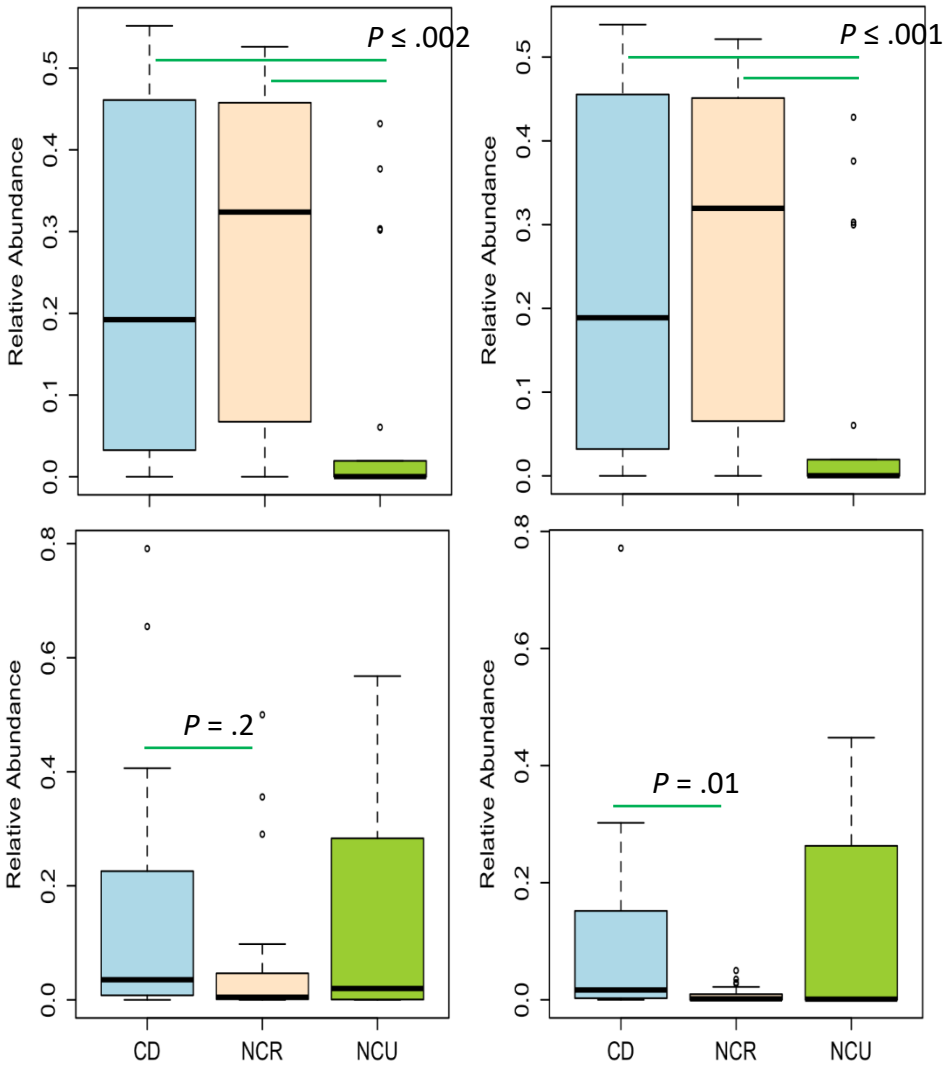
CD – Crohn's disease patients
NCR – Non-Crohn's, Related individuals
NCU – Non-Crohn's, Unrelated individuals

Clinical Details of CD Patients

Age Category	A1 (<=16 yr)	0
	A2 (17-40 yr)	8
	A3 (>= 40 yr)	12
Location	L1 (Terminal Ileum)	11
	L2 (Colon)	2
	L3 (Ileum-Colon)	6
	L4 (Upper GI Tract)	0
Behavior	B1 (Nonstenotic)	3
	B2 (Stenotic)	4
	B3 (Penetrating)	12
Disease Status	Active	3
	Remission	8

The level of beneficial fungi decrease in Crohn's Disease, while pathogenic ones increase

Saccharomyces spp



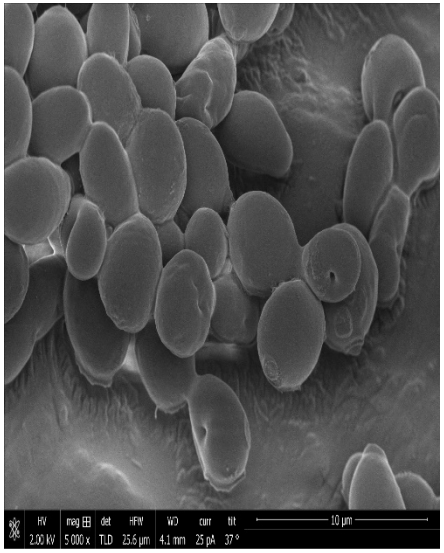
S. cerevisiae

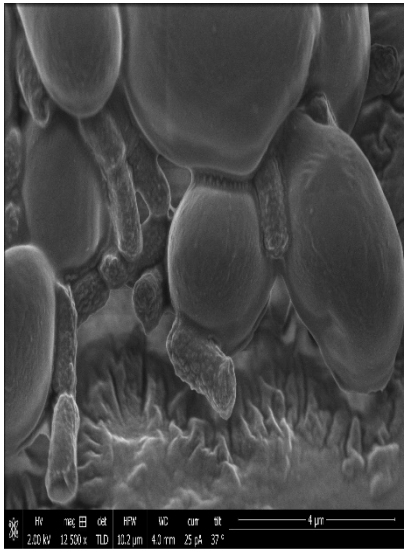
Candida spp.

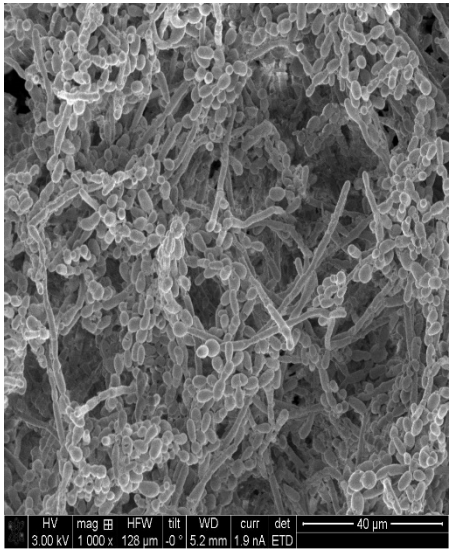
C. tropicalis

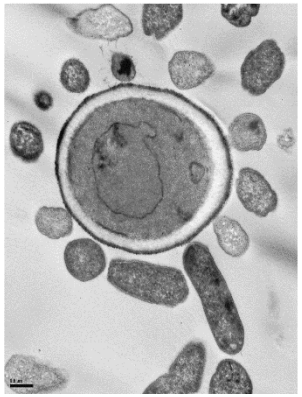
- ***S. marcescens*** **and** ***E. coli*** **significantly correlated with** ***C. tropicalis and they*** **were significantly increased in CD patients**

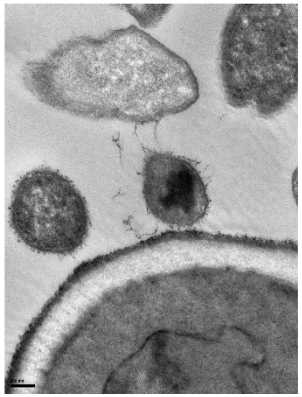
Since microbes in the gut exist as biofilms, *C. tropicalis*, *S. marcescens and E. coli* were evaluated for their ability to form biofilms.





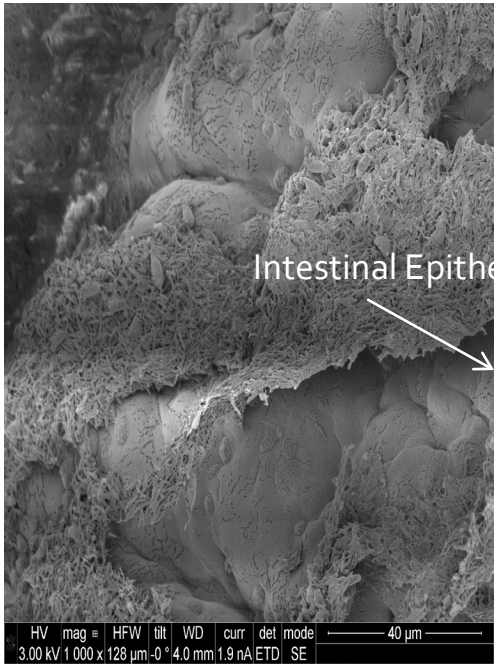


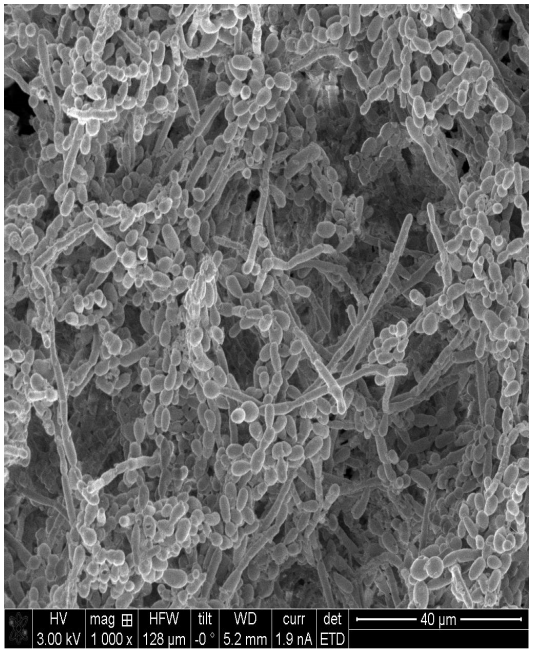




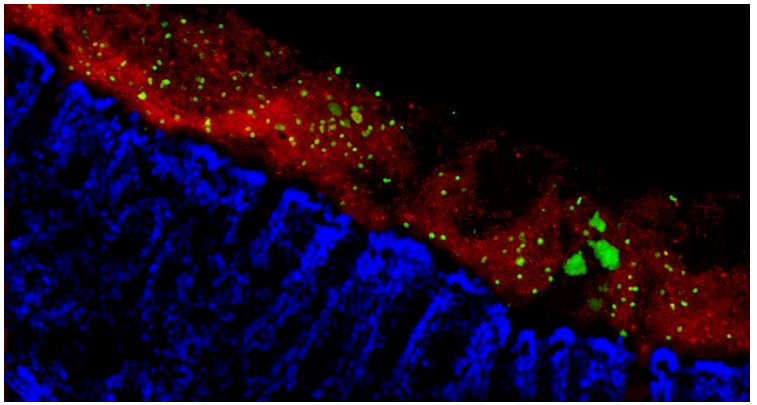
Fungi and bacteria cooperate together to form thick biofilms

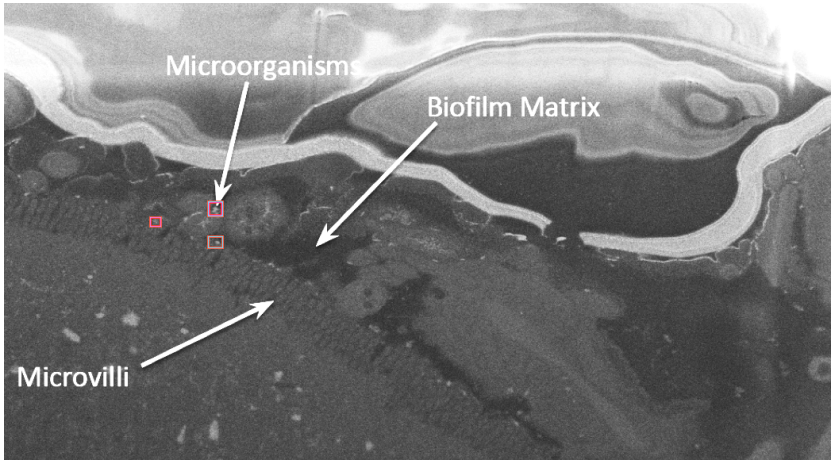
Fungi and bacteria cooperate together to form mixed species biofilms in the gastrointestinal tract

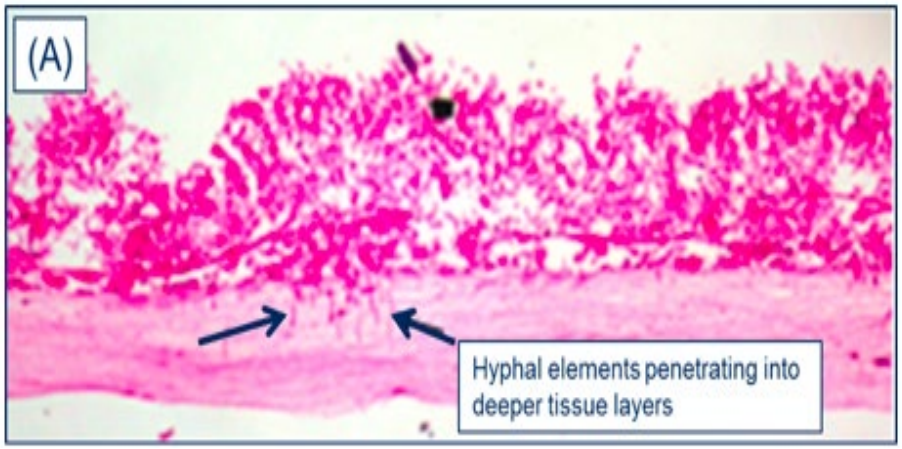




Fungus Green, Bacteria Red





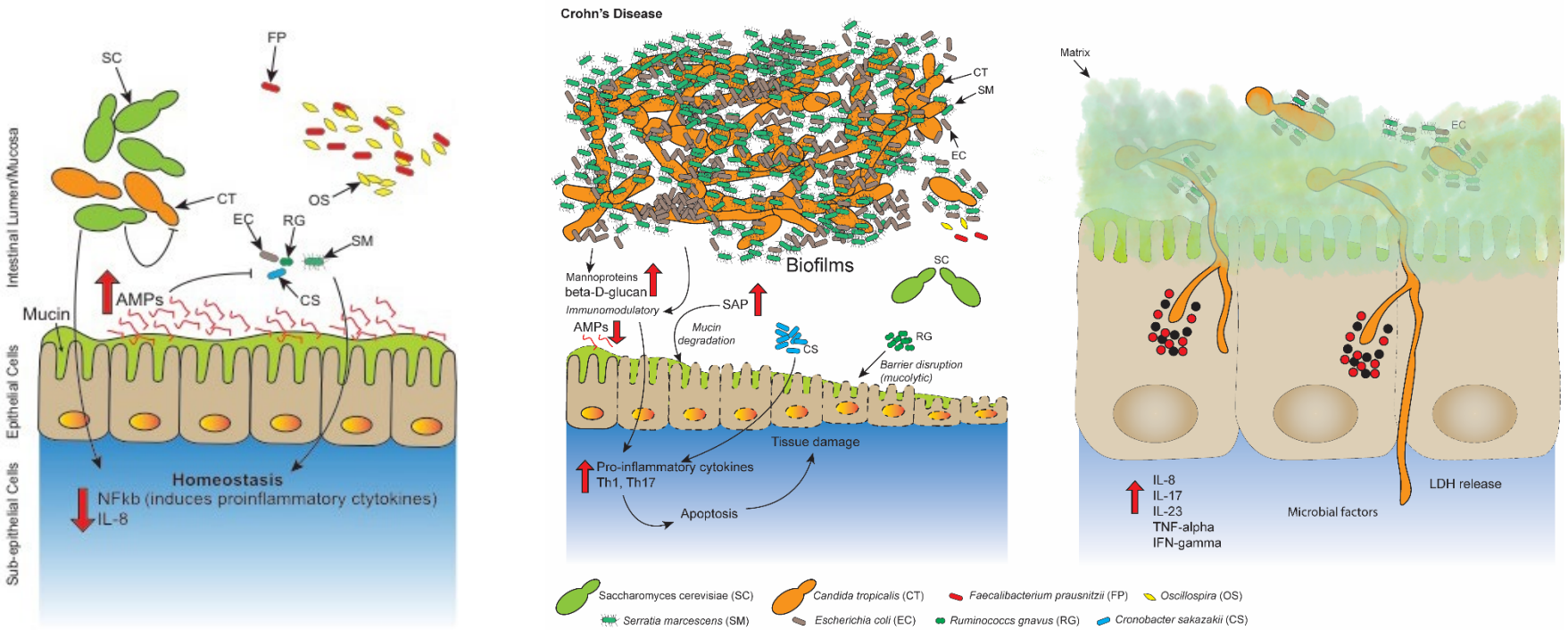


Treatment with an Antifungal Reduced Inflammation

- To determine whether the altered fungal burden during colitis contributes to disease severity
- Fungal growth was suppressed using the antifungal fluconazole
- Fluconazole treatment during colitis led to reduced weight loss, and milder histological disease
- Decreased Th1 and Th17 responses and decreased production of inflammatory cytokines
- Taken together, these results support the conclusion that an inability to control fungi in the gut leads to more severe colitis

Iliev et al. 2012. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science. 2012 June 8; 336(6086): 1314–1317. doi:10.1126/science.1221789.

Fungal and Bacterial Dysbiosis Promotes Inflammation



Hoarau, ... and Ghannoum, M. 2016. Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's Disease. mBio 7(5):e01250-16. doi:10.1128/mBio.01250-16.

Natural foods can lower inflammation and help balance the microbiome

Many natural foods can lower inflammation and help increase gut beneficial microbes.

Anti-inflammatory foods that should be the base of your diet include:

- **Fresh vegetables:** rich in phytonutrients that are shown to lower cholesterol, triglycerides and symptoms of rheumatoid arthritis, Alzheimer's disease, cancer, cardiovascular disease and diabetes.
- Aim for variety and a minimum of 4-5 servings per day, including: beets; carrots; cruciferous vegetables (broccoli, cabbage, cauliflower and kale); dark, leafy greens (kale, spinach); onions; peas; salad greens; and squashes.
- **Whole pieces of fruit (not juice):** Fruit contains various antioxidants like resveratrol and flavonoids, which are tied to cancer prevention and brain health (3-4 servings/ day is optimal); especially apples, blackberries, blueberries, cherries, nectarines, oranges, pears, plums, pomegranates, red grapefruit or strawberries.
- **Herbs, spices and teas:** turmeric, ginger, basil, oregano, thyme, plus green tea
- **Wild-caught fish, cage-free eggs and grass-fed/pasture-raised meat:** high in omega-3 fatty acids and great sources of protein, healthy fats, and essential nutrients (zinc, selenium and B vitamins).
- **Healthy fats:** grass-fed butter, coconut oil, extra virgin olive oil, nuts/seeds.
- **Ancient grains and legumes/beans:** 2-3 servings per day or less is best (all sorts of beans (black beans, black-eyed peas, chickpeas, lentils, black rice, amaranth, buckwheat, quinoa)

Eating to Support Immune Health and Lower Inflammation

Diet plays a critical role in establishing gut health and supporting beneficial members of the microbiome.

Foods that promote inflammation include:

- **Refined vegetable oils:** e.g., canola, corn and soybean oils, which are high in pro-inflammatory omega-6 fatty acids
- **Pasteurized dairy products:** common allergens
- **Refined carbohydrates and processed grain products**
- **High fat red meat**: high in omega-6s due to feeding the animals corn and ingredients that negatively affect their microbiomes
- **Added sugars:** found in the majority of packaged snacks, breads, condiments, canned items, cereals, etc.
- **Trans fats/hydrogenated fats:** used in packaged/processed products and often to fry foods

How Else Can You Establish a Balanced Microbiome?

1. Avoid Antibiotics as Much as Possible

- Broad spectrum antibiotics not only inhibit pathogens, they also eliminate beneficial bacteria.
- While antibiotics can save lives when they're truly needed, they're often are overprescribed and misunderstood.
- Over time, dangerous bacteria can become resistant to antibiotics, making serious infections harder to fight.

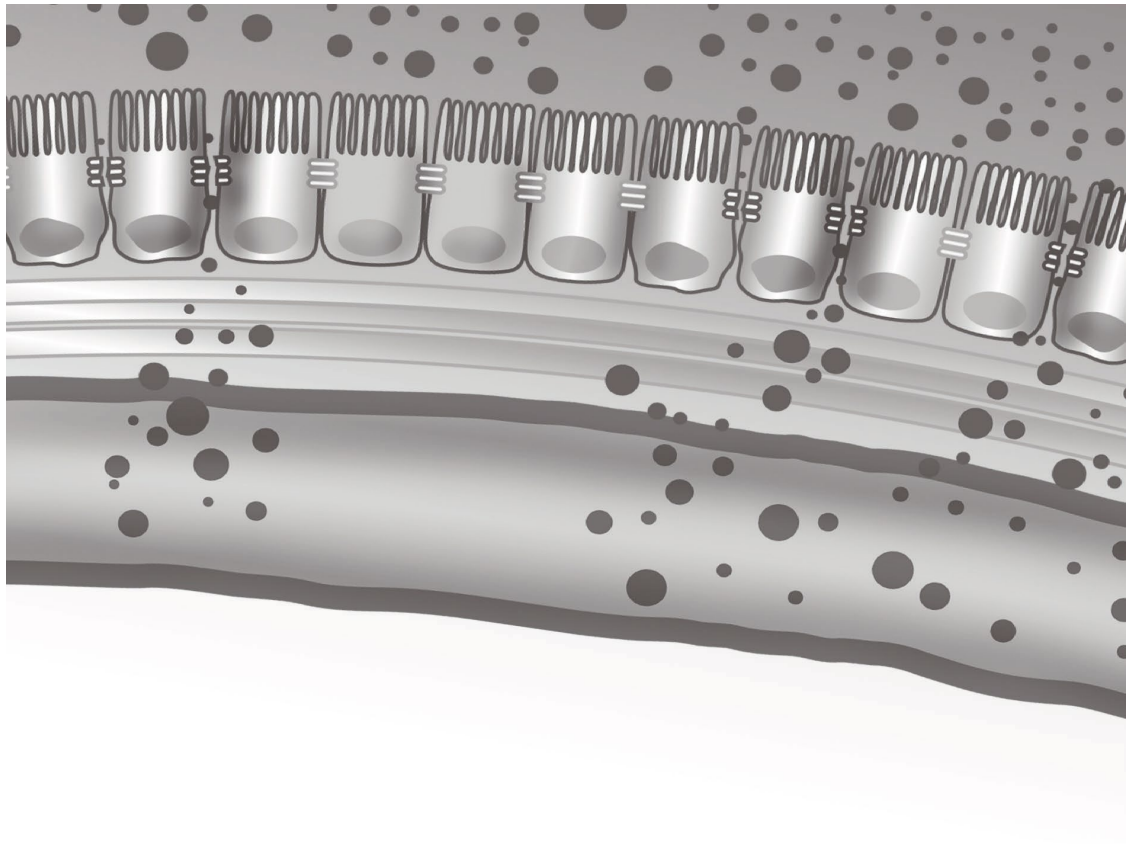
2. Lower Stress, Sleep Well, and Exercise More

- These are three lifestyles shown to affect our microbiome balance. Stress enhances the release of pro-inflammatory cytokines that damages healthy cells.
- Exercise is a natural stress reliever that can help lower inflammation, balance hormones and strengthen the immune system.

3. Add Supplements

- Co-enzyme Q10, carotenoids, omega-3 fish oil, selenium and antioxidants (vitamins A, B, C, D and E) can help keep free radical damage from disturbing microbiota gut health.

Leaky Gut



- Intestinal permeability from inflammation due to toxic waste leaks through the intestinal wall into the blood stream
- This chronic condition is known as **Leaky Gut Syndrome**

The gut epithelium is the first line of defense

Against numerous unwanted microbes and antigens entering the body.

Dysfunction of this barrier, referred to as "leaky gut," and the inflammatory response it perpetuates, is associated with numerous disorders.

Increased gut permeability is a risk factor for gut diseases (e.g., **Crohn's, celiac, and irritable bowel syndrome)**, as well as **inflammatory and autoimmune diseases** in other parts of the body (e.g., type 1 diabetes, asthma, multiple sclerosis, and chronic fatigue syndrome).

In addition, inflammatory responses initiated in the gut are thought to have flow-on effects on the brain, via the gut–brain axis, as shown by the association between a leaky gut and impaired brain function in people with depression, anxiety, and schizophrenia.

FasanoA, Shea-DonohueT. Nat Clin Pract Gastroenterol Hepatol 2005;2:416–22.

Groschwitz KR, Hogan SP. J Allergy Clin Immunol 2009;124:3–20; quiz 1-2.

TurnerJR. Nat Rev 2009;9:799–809. Fasano A. Clin Rev Allergy Immunol. 2012;42:71–8. Schmidt C. Nature 2015;518: S12–5.

What is Leaky Gut?

The term leaky gut syndrome, or leaky gut is a health disorder in which the lining of the small intestine is more permeable than it should be and becomes subject to inflammation by various irritants.

The abnormally large spaces allow entry of toxic material into the bloodstream that would, in healthier circumstances, be repelled and eliminated.

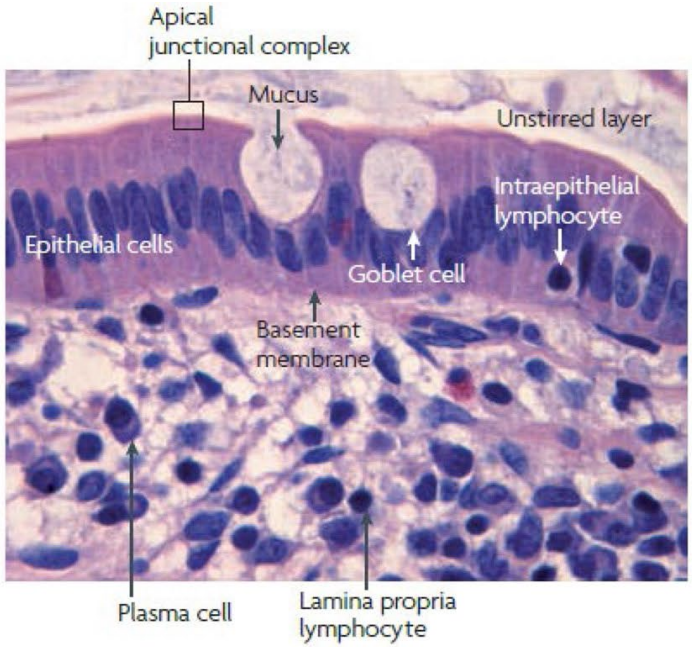
The gut becomes "leaky" in the sense that bacteria, viruses, fungi, parasites and their toxins, and undigested food pass through a damaged, hyperpermeable, porous or leaky gut.

When these foreign substances enter the bloodstream, the immune system goes into reaction mode and begins creating antibodies against its own tissues.

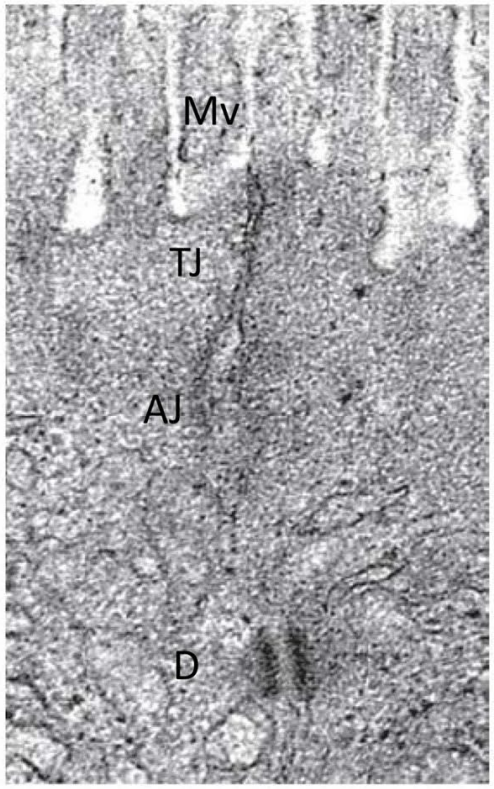
Chronic overstimulation of the immune system leads to chronic inflammation and disease.

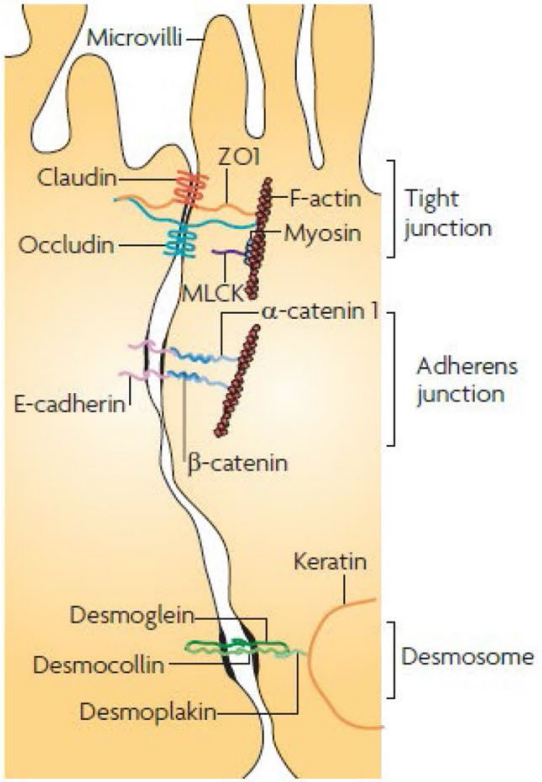
Anatomy of the mucosal barrier: Key elements of the tight junction

Mucosal Barrier



TEM and Line drawing of Junctional Complex





Camilleri M. Gut 2019;68:1516–1526. doi:10.1136/gutjnl-2019-318427

Leaky Gut is Not Only Present in Disease States

The gut barrier takes up to 2 years from birth to fully mature.

There are differences in gut physiology between breastfed and formula-fed infants, including gut permeability, which makes formula-fed infants more susceptible to gut infections and at higher risk of leaky gut-associated illnesses in later life.

Conversely, in later life the gut barrier naturally deteriorates.

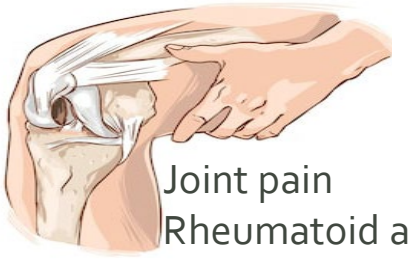
A simple treatment to support appropriate gut barrier maturation and help maintain its resilience throughout life could be of great benefit.

Le Huerou-Luron I, *et al*. Nutr Res Rev 2010;23:23–36.

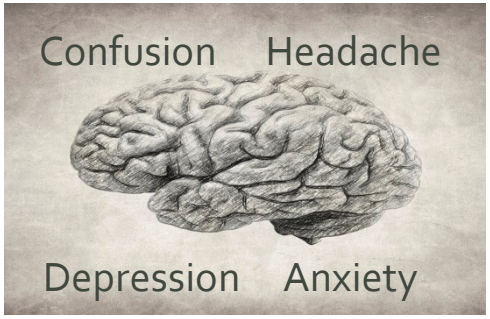
Schreiber RA, Walker WA. Ann Allergy 1988;61:3–12.

Parrish AR.. Tissue Barriers 2017;5:e1343172.

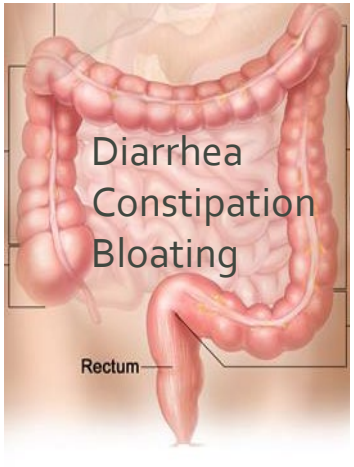
Leaky Gut affects the whole body

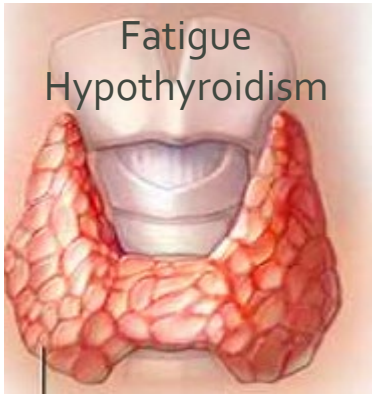


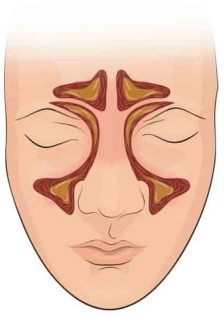
Joint pain
Rheumatoid arthritis
Fibromyalgia



Acne
Rosacea
Eczema
Psoriasis



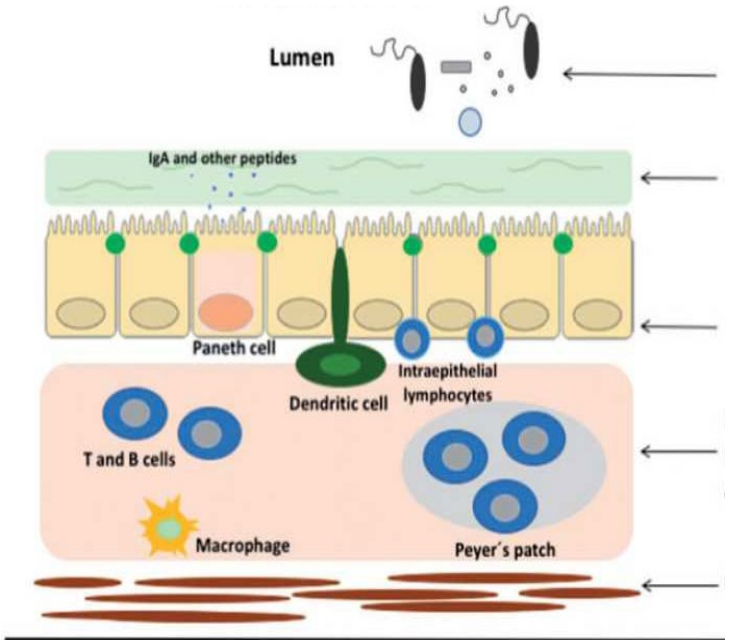




Frequent cold
Food sensitivity

Difference Between Normal Gut and Leaky Gut

Normal Gut



Healthy microbes

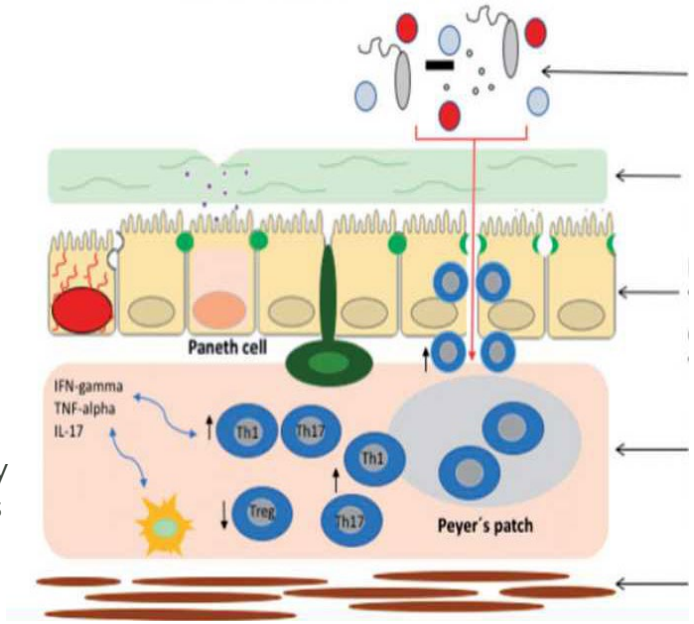
Normal Mucus layer

Normal Junctions

Anti-inflammatory immune cells

Muscle layer

Leaky Gut



Pathogens

Altered Mucus layer

Leaky Junctions

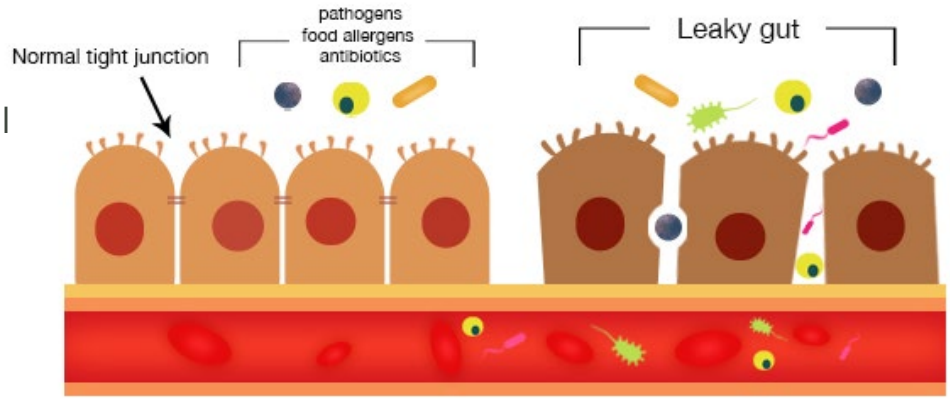
Pro-inflammatory immune cells

Muscle layer

Camara-Lemarroy *et al.*, 2018

Underlying Causes of Leaky Gut

GI Infections
- *Candida*
- Small intestinal Bacterial Overgrowth



Pro-Inflammatory Diet
- Meat rich in fat
- Refined sugar
- Artificial sweeteners

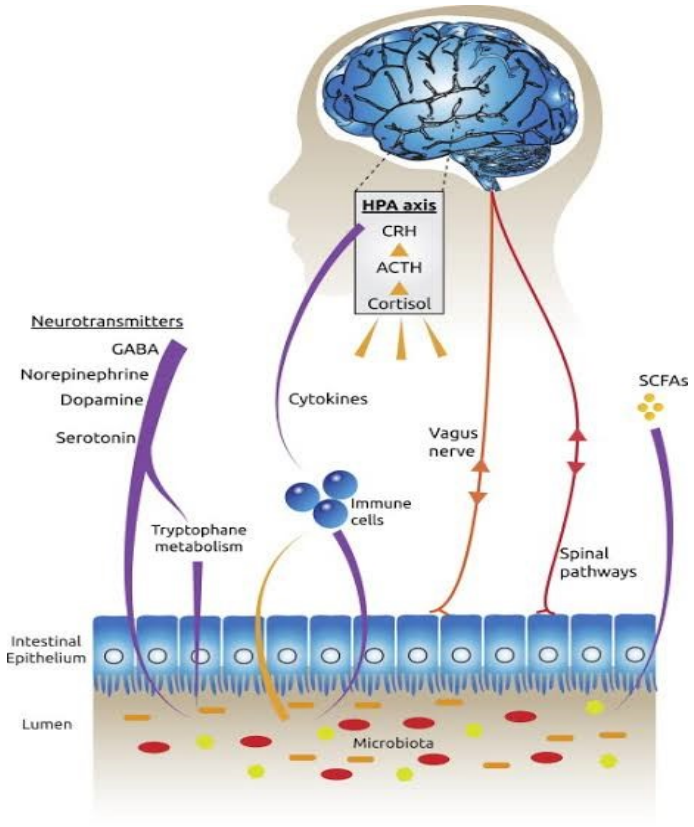
GI Diseases
- Crohn's Disease
- IBD

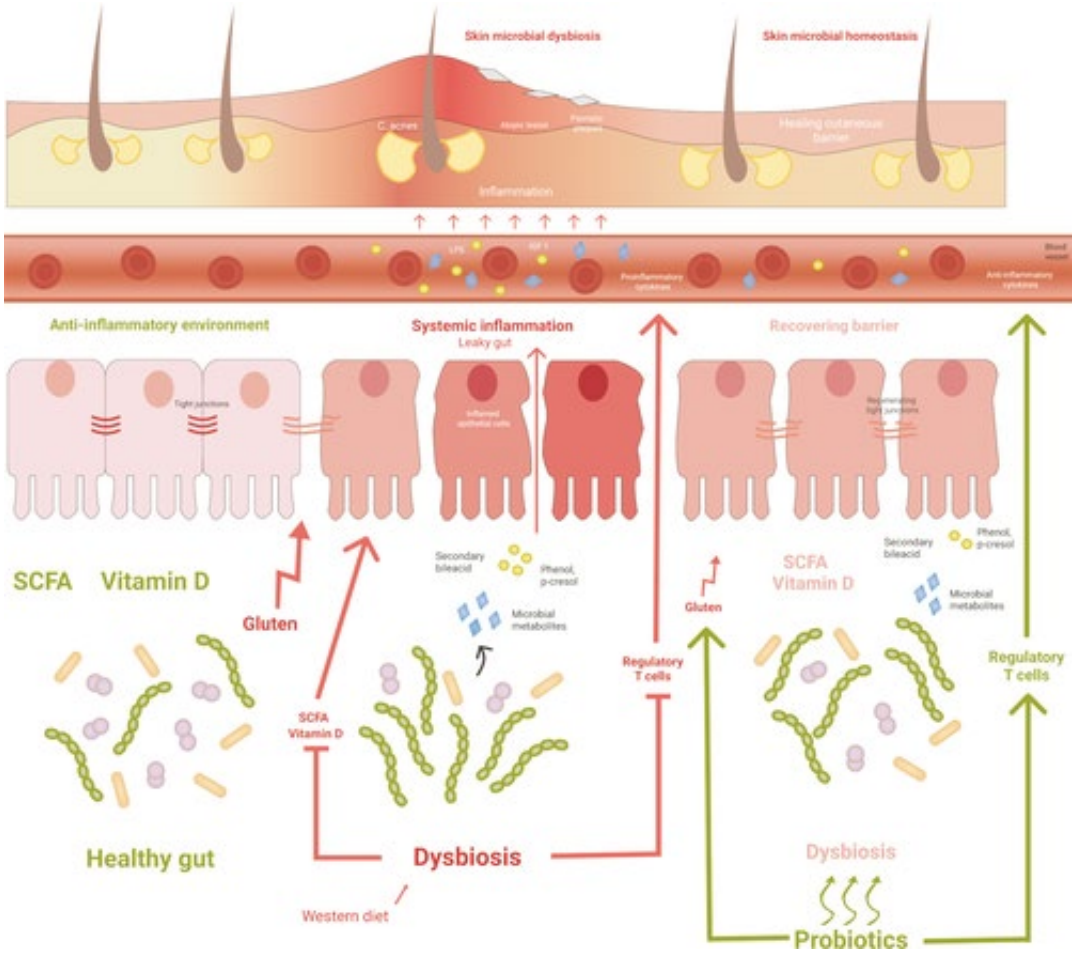
Therapeutics
- Broad spectrum antibiotics
- Steroids
- Anti-acids

Causes of Leaky Gut:

- Host genotype (genetic factor),
- Dysregulated immune responses, and
- The intestinal microbiota: Dysbiosis of the intestinal flora

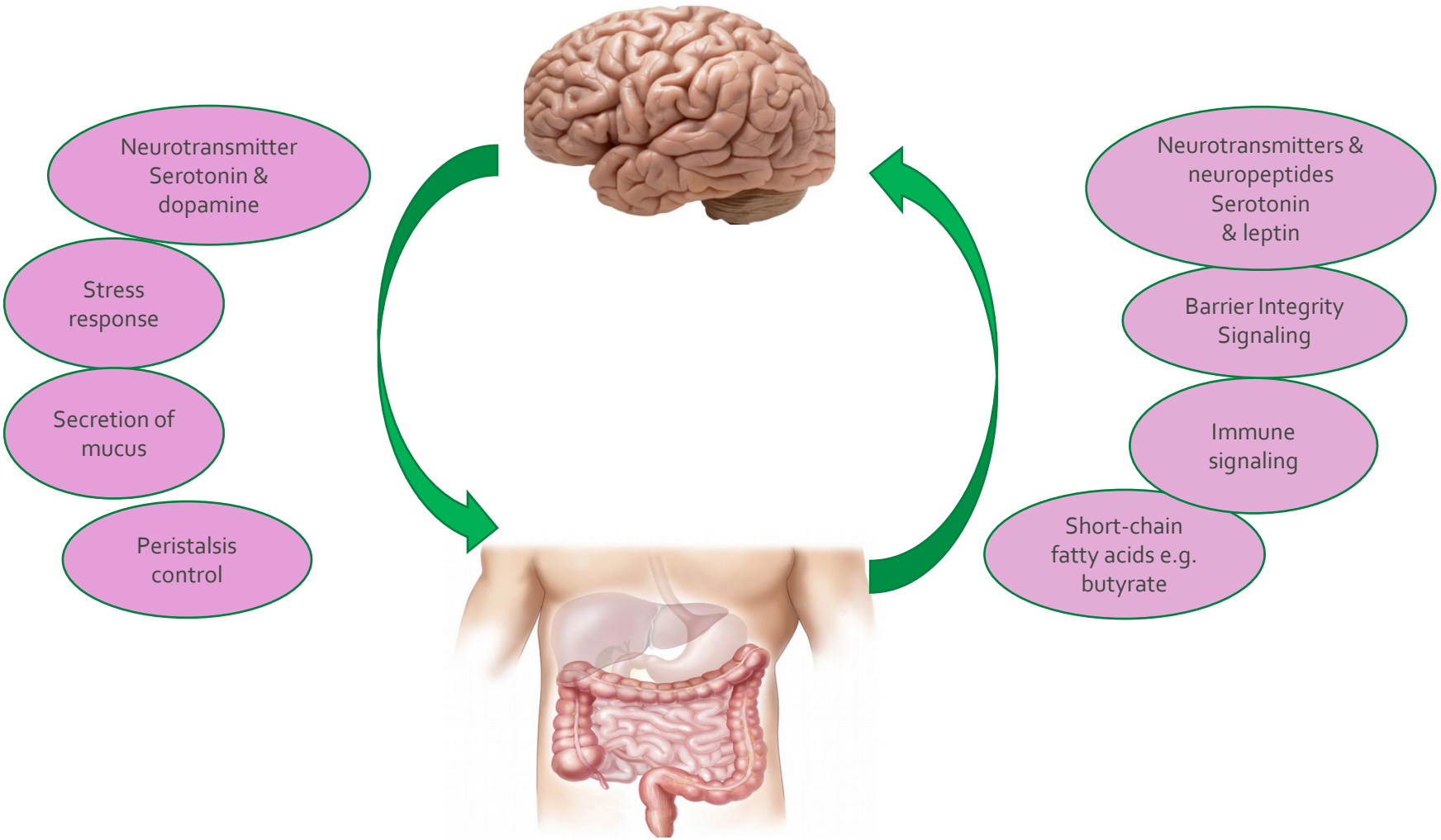
Leaky Gut can affect various parts of our body from the gut to the brain and even the skin through the gut-brain-skin axis. All are connected.





Szántó, M *et al.* Experimental Dermatology. 2019;28:1210–1218. https://doi.org/10.1111/exd.14016

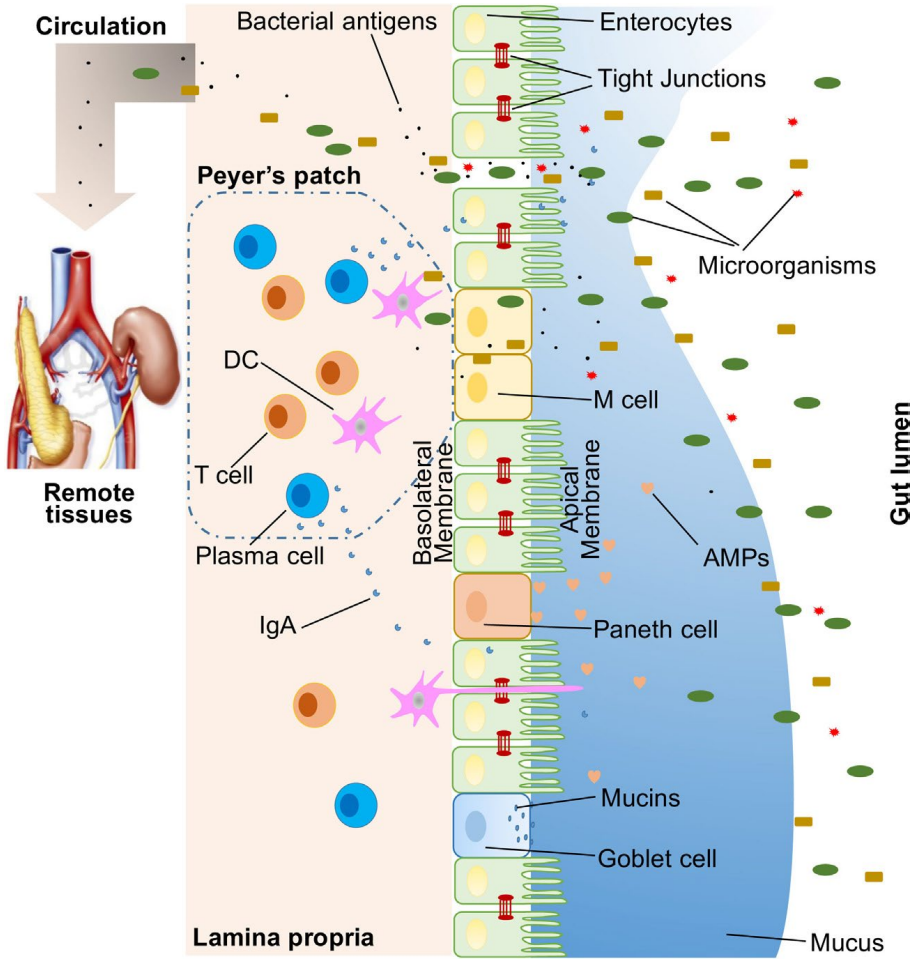
Gut-Brain Axis



Host Barriers to Leaky Gut

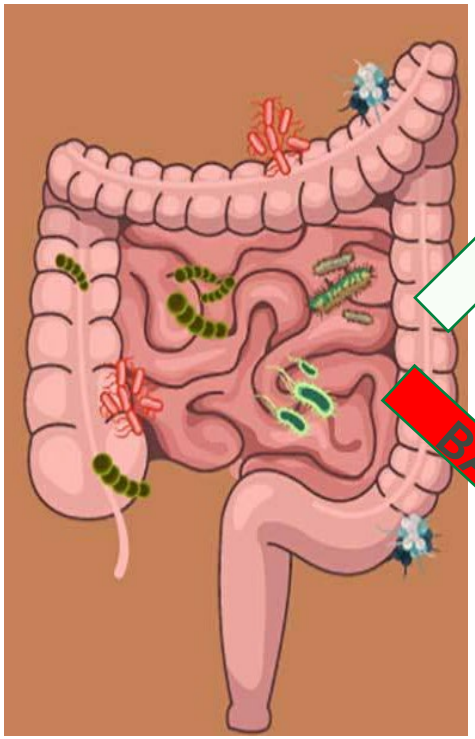
Physical barrier (epithelium, tight junctions, mucus, commensal bacteria), biomedical barrier [AMPs, and immunological barrier (lymphocytes and igA).

Microbial translocation to remote tissues (kidney, pancreas) in the presence of a leaky gut.



Mu Q, *et al.*: 2017. Leaky Gut As a Danger Signal for Autoimmune Diseases. Front. Immunol. 8:598. doi: 10.3389/fimmu.2017.00598

Unbalanced Microbiome in Leaky Gut

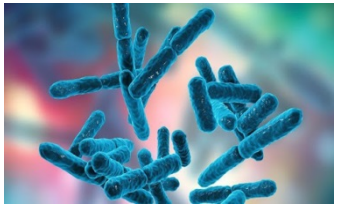


GOOD

BAD

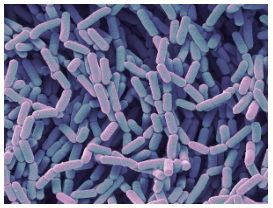
Bifidobacteria

Control levels of other bacteria
Inhibit tumors
Boost immunity
Produce vitamins



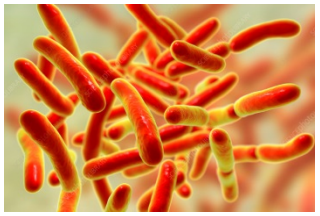
Lactobacilli

Produce vitamins & nutrients
Protect against tumors
Anti-inflammatory



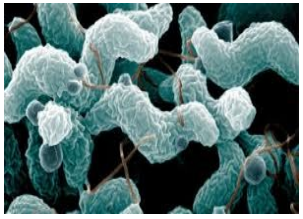
Faecalibacterium prausnitzii

Anti-inflammatory
Decrease intestinal permeability



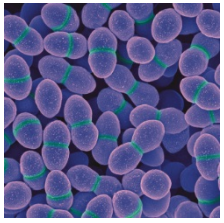
Campylobacter

Bloody diarrhea
Acquired from contaminated food



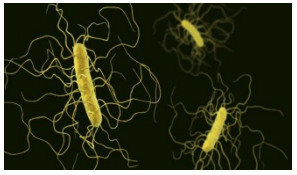
Enterococcus faecalis

Cause postsurgical infections



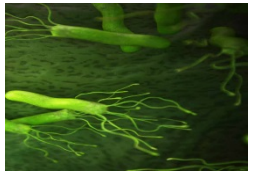
Clostridium difficile

After antibiotics watery diarrhea
Toxic megacolon

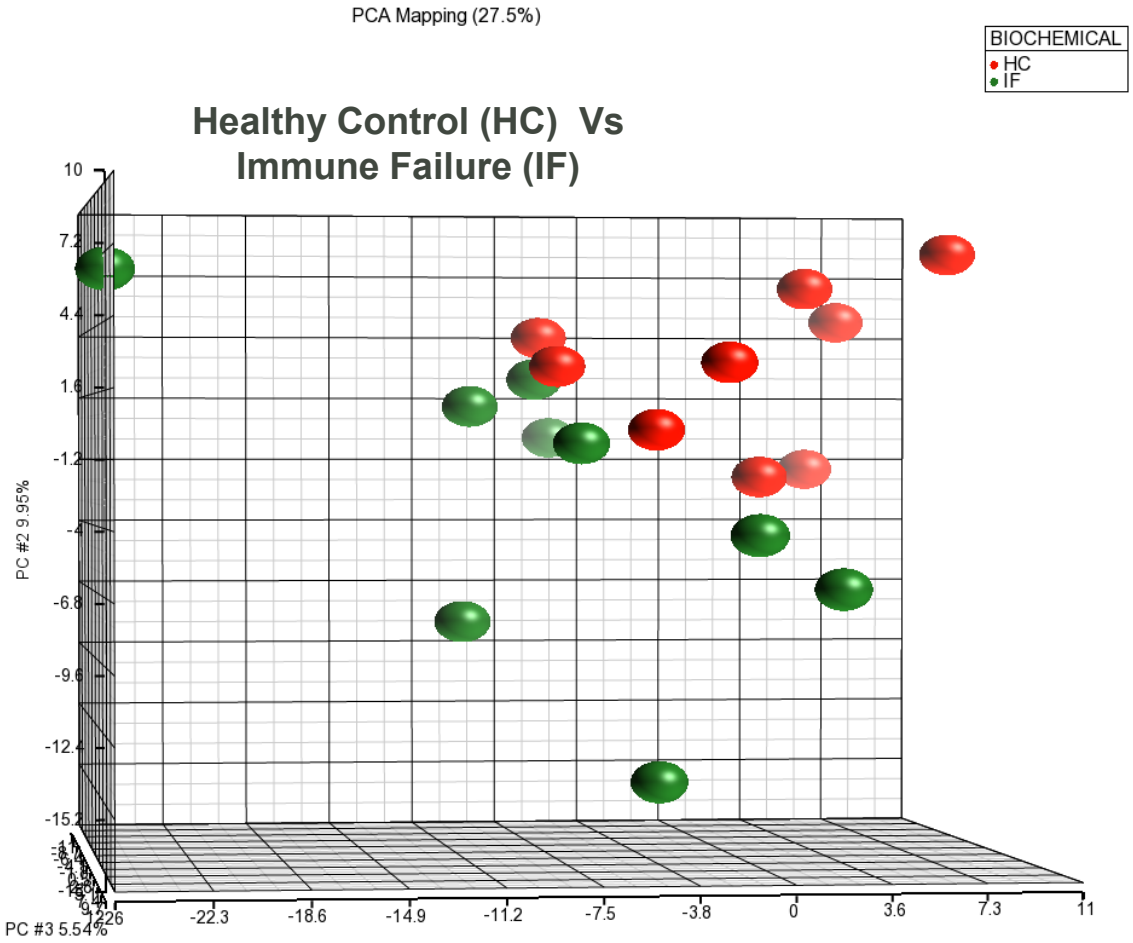


H.Pylori

Most common bad bacteria
Causes ulcer & cancer



Intestinal Metabolite Imbalance in HIV-Infected Patient vs Healthy Controls



Metabolites Cluster Differently

Ghannoum, M. Unpublished data

The Vicious Cycle of Leaky Gut

Diseases Associated with Leaky Gut

Gut Diseases	Autoimmune Diseases	Neuropsychiatric Diseases
Celiac disease	Systemic Sclerosis	Alzheimer's Disease
Crohn's disease	Diabetes	Multiple Sclerosis
Irritable bowel disease	Ankylosing Spondylitis	Parkinsonism
Ulcerative colitis		Autism
		Schizophrenia

Symptoms

- Chronic diarrhea, constipation, or bloating
- Nutritional deficiencies
- Fatigue
- Headaches
- Confusion
- Difficulty concentrating
- Skin problems, such as acne, rashes, or eczema
- Joint pain

Foods Exacerbating Leaky Gut

Pro-inflammatory foods

- Eliminate gluten (pastas, noodles, breads, pastries, cereals, beer/malted beverage)
- Avoid corn (similar sensitivity to gluten)

Allergenic foods

- Dairy – difficulty metabolizing casein, whey, lactose (milk, cheese, ice cream, yogurt)

Foods to Avoid with Leaky Gut

Cell junction 'gap' inducers

- Lectin-containing foods found in pro-gluten and Nightshade members (i.e. potato, tomato, pepper)
- Stimulate release of compounds found to open gaps in barrier wall

Dysbiosis promoters of unhealthy flora

- Sugar – refined, high-fructose, alcohol
- Soy – tofu, edamame, protein bars

4 Rs program for Treatment of Leaky Gut

Remove the Bad

- Remove processed food, saturated fat, soy, gluten, eggs, dairy
- Quit smoking
- Decrease caffeine
- Stop alcohol
- Decrease stress

Replace the Good

Healthy Fats and Proteins

- Nuts, seeds, avocado, olive & coconut oils
- Free range poultry, grass-fed beef, & wild-caught fish (w/ higher levels of omega-3 fatty acids)

Hypoallergenic Protein Sources

- Rice, peas, hemp, chia

Collagen-Containing (Bone Broth)

- Gelatin in broth protects and heals mucosal lining
- Rich source of glutamine, a preferred source of energy for the cells of the small bowel shown to reduce intestinal permeability

Replace the Good

Fiber-rich, Low-glycemic Carbs

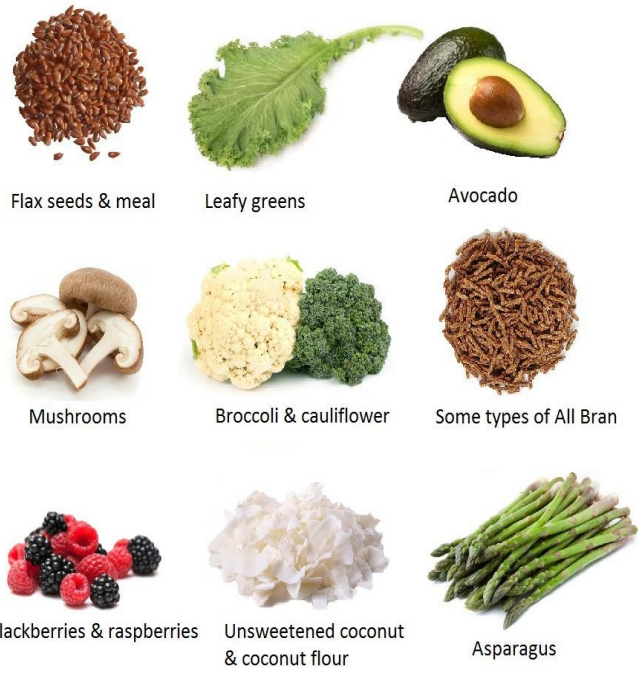
- Non-starchy, leafy, and cruciferous vegetables provide prebiotic fiber and promote probiotic bacteria
- Cauliflower, cabbage, broccoli, Brussels sprouts, collard greens, arugula

Complex Carbs

- Minimally processed, low-sugar
- Butternut squash, sweet potato, apples, berries

Fermented Foods

- Good source of probiotics to protect lining
- Unpasteurized sauerkraut, kimchi, pickles



Repair Leaky Gut with Supplements

SUPPLEMENT		BENEFIT
Quercetin		Mast cell stabilization, no histamine release
Slippery Elm		Increase mucus production & promote healing
Omega-3s		Balance bacterial level in the gut
Sweet Wormwood		Anti-microbial properties
Cat's Claw		Anti-inflammatory & antioxidant
Apple Cider Vinegar		Stimulate gastric acid production kill bacteria & help digestion
Magnesium		Help gut muscle relaxation
Marshmallow Root		Coat the damaged area and protect it
Turmeric/Curcumin		Anti-inflammatory
Licorice		Anti-inflammatory & promote healing

Can Probiotics Help?

Diseases associated with gut barrier dysfunction are known to be concomitant with alterations in gut microbiota composition.

Therefore, it is conceivable that probiotic bacteria could be used to alter microbiota composition to improve gut barrier function and in turn reduce inflammatory responses.

Indeed, there are some promising probiotics candidates to do just this.

However, those most in need of such treatments are vulnerable populations who are most at risk from any potential unwanted consequences of treatment with a live microbe:

- the very young and old,
- individuals with abnormal immune functions

Well-characterized postbiotics with specific effects may be the solution to this issue.

Fukui H. 2016. Intest Dis ;1:135–45. Bron PA, 2017. Br J Nutr ;117:93–107.

Mechanism of Probiotics

Alleviating intestinal inflammation

Normalization of gut mucosal dysfunction

Down-regulation of hypersensitivity reactions

Probiotics: Probiotic foods contain "good bacteria" that populate your gut and fight off bad bacterial strains. Try to include probiotic foods like yogurt, kombucha, kvass, kefir or cultured veggies in your diet daily.

Do Postbiotics Help Leaky Gut? The Promise

Postbiotics Definition: By-products of the fermentation process (feeding on fibers) carried out by probiotics in the intestine.

- Cui et al. investigated the ability of the cell-free supernatant of the probiotic *Lactobacillus reuteri* ZJ617, to overcome LPS- induced acute liver injury in mice.

Results: showed that the supernatant reduced the detrimental effect of LPS on the liver where it stopped LPS from getting into the body to have a detrimental effect.

- In contrast, an increase in LPS permeability was noted in the control group.
- This barrier dysfunction then enabled the LPS to translocate to the liver where it activated hepatic TLR4 signaling, which is known to lead to liver damage.

Conclusion:

- Treatment with the probiotic strain supernatant prevented the breakdown of the gut barrier, stopping the impact of LPS before it began.
- This ability to maintain gut barrier function during an immune assault is perhaps the most important finding in this paper.
- It indicates that postbiotics could have benefits well beyond those in the liver reported here.

Cui et al. 2019. *Lactobacillus reuteri* ZJ617 culture supernatant attenuates acute liver injury induced in mice by lipopolysaccharide. J Nutr;149:2046–55.

Do Postbiotics Help Leaky Gut? The Promise

- Further work is needed, of course, to determine whether the benefits reported here using a rodent model translate into relevant outcomes in humans.
- Process development is also needed to ensure that the ZJ617-derived postbiotic can be produced in a cost-effective way and remain stable for a suitable timeframe.
- However, if a postbiotic product is able to promote gut barrier resilience it could have far-reaching benefits.

Re-inoculating with Probiotics Will Lead to a Number of Benefits

BENEFIT
Decrease *E.coli*-induced increase in permeability
Decrease binding of bad bacteria to gut mucosa
Increase mucus and decrease allergy
Decrease adherence and translocation of bacteria & has anti-inflammatory actions
Anti-inflammatory properties
Increase junction proteins and decrease permeability
Anti-inflammatory properties
Modulate gene expression, increase nutrient absorption, fortify mucosal barrier and blood vessels

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MICROBIOME TESTING

- Health implications of the microbiome
- Learn about the different communities that make up the microbiome and how that influences health conditions
- How the microbiome affects our body positively and negatively
- Understand what exactly microbiome testing is and how it can be useful to help rebalance and maintain a healthy microbiome

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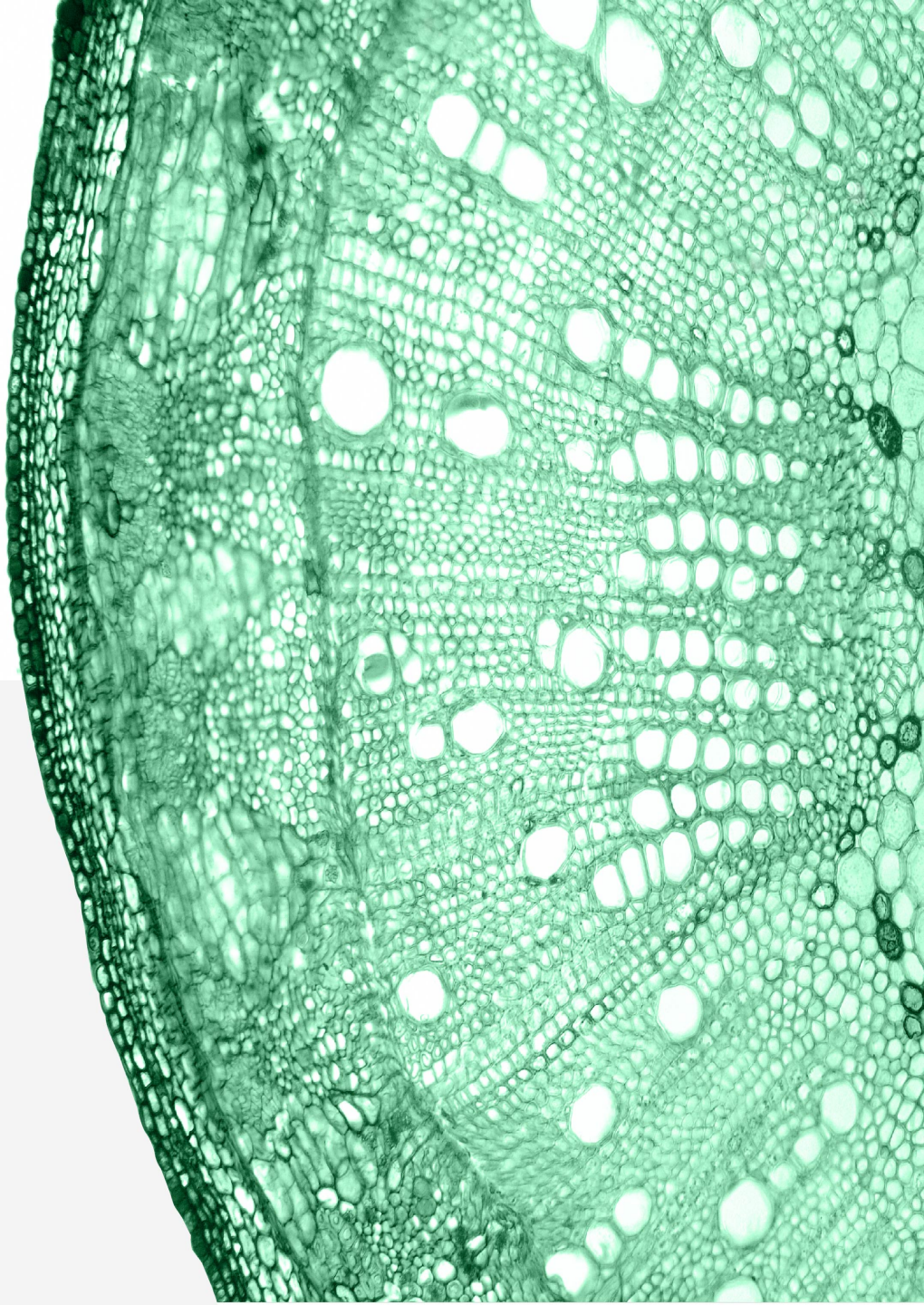
NEED FOR TESTING

How do you determine if a person has dysbiosis?



Health Implications of the Microbiome

- Recent research has recognized that the intestinal microbiome may play a potential role in the pathogenesis of common health conditions.

- This has led to a **growing interest in personalized microbiome analysis among healthcare practitioners and their patients.**

- Based on the many roles the microbiome plays in the host's health, an opportunity exists to support a balanced microbiome through diet, lifestyle and supplements, for promotion of a healthy lifestyle.

Health Implications of the Microbiome

A diverse microbiome may help support various aspects of health:

- **Digestive Health**: An imbalanced microbiome may contribute to many health conditions, including digestive issues.

- **Brain Health**: The gut-brain axis consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions.

- **Overall Health**: Recent recognition that the intestinal microbiome plays potential roles in the pathogenesis of multiple common diseases has led to a growing interest in personalized microbiome analysis among clinical investigators and patients.

Health Implications of the Microbiome

- Clinical publications have shown that dysbiosis of the microbiome has been associated with an array of negative general side effects, as well as specific disorders and diseases.
- In this regard, dysbiosis can cause inflammation and a wide range of gastrointestinal symptoms like:
  - Diarrhea
  - Gas
  - Bloating
  - Nausea
  - Indigestion
  - Nutrient absorption issues

Health Implications of the Microbiome

Specifically, studies have shown that dysbiosis is associated with the development and/or progression of a broad spectrum of health conditions or diseases, including the following:

- Inflammatory Bowel Disease
- Irritable Bowel Syndrome
- Celiac Disease
- Systemic Lupus Erythematosus
- Type 1 Diabetes
- Type 2 Diabetes
- Rheumatoid Arthritis
- Atopic disease such as food allergies and childhood allergic asthma
- Obesity
- Hypertension

See Appendix Slides for supporting references

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How do you determine if a person has dysbiosis?

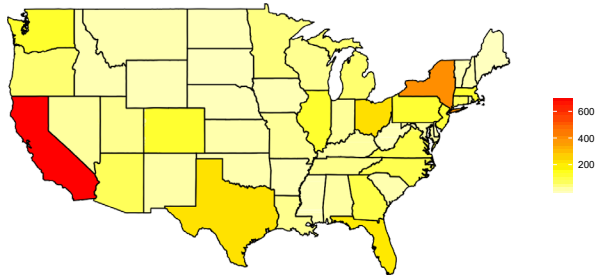
There are two ways to determine whether a patient has microbiome dysbiosis.

- The first is by using a standardized assessment tool (or quiz) for an introductory health assessment.
- The second metric is to perform an at-home gut microbiome health test for a more in-depth assessment of a patient's microbiome.
- Answers to the standardized health assessment tool will provide a possible idea of whether the individual has a potential microbiome imbalance, patient's microbiome.
- While the at-home test will provide a personalized answer with a quantitative measure of the bacterial and fungal communities that resides in an individual’s gut.

NEED FOR TESTING

Defining a Healthy Microbiome

- To identify the distinct microbiome profile representing the "healthy" microbiome enterotype, we analyzed fecal samples from thousands of individuals across the United States, each of whom provided informed consent.
- All individuals filled out a standardized instrument (questionnaire) regarding his/her dietary habits, lifestyle, sleeping durations, stress levels, weight gain or loss, and gastrointestinal symptoms (constipation, bloating, diarrhea, etc.).
- Individuals provided information on any medications or if a health professional had diagnosed them with any specific diseases or conditions.
- Human Microbiome Project (HMP) guidelines were followed for inclusion and exclusion criteria (e.g. excluded individuals with chronic diseases (e.g. diabetes, heart disease, and obesity (having BMI > 35 kg/m2), and those taking medications (e.g. antibiotics, antifungals, acid reflux medications).



Defining a Healthy Microbiome

- Our healthy population was characterized by having a higher level of *Bacteroidetes* when compared to *Firmicutes*.
- Closely resembles the microbial profile of normal/healthy subjects reported in the Human Microbiome Project (HMP), https://hmpdacc.org.
- **Individuals cluster together into three different groups sharing a similar microbiome profile:**
- Red dots represent individuals that share a healthy microbiome profile (Group 1).
- Blue dots represent individuals that exhibit elevated levels of harmful proinflammatory bacteria (*Proteobacteria*, Group 2).
- Green dots represent individuals that exhibit elevated levels of *Firmicutes* (Group 3).

Principal component analysis of cumulative data of all individuals tested

NEED FOR TESTING

Defining Dysbiosis: Using a standardized assessment instrument to presumptively detect dysbiosis

- Although a large number of factors have been identified to correlate/cause microbiome imbalance, these factors have been reported using different sequencing methodologies, generally enroll a small number of subjects, and involve various patient cohorts.
- These differences make it difficult to identify a standardized list of underlying predisposing conditions/issues that can help a person determine whether s/he has dysbiosis.
- To identify microorganisms that can lead to dysbiosis, we collected data from thousands of subjects using a standardized microbiome test.
- Comparing the microbiome profile of these individuals allowed us to categorize them into individuals with dysbiosis versus those with a balanced microbiome. Our groups were in concordance with the Human Microbiome Project.
- This analysis allowed us to devise a scoring schema. i.e., Gut Score. A gut score of ≥ 7 indicates a healthy microbiome while a score between 5 and 6 indicate mild dysbiosis, and finally a score of ≤ 4 suggests significant dysbiosis.

NEED FOR TESTING

Defining Dysbiosis: Using a standardized assessment instrument to presumptively detect dysbiosis

- Using the developed schema and the collected metadata we then reanalyzed each individual to identify conditions that were implicated in microbiome imbalance.
- **Table shows** many conditions/situations that can put a person at increased risk for microbial imbalance.
- While the MDAC instrument is not designed to diagnose dysbiosis in and of itself, it allows a quick gauge of potential dysbiosis-risk-level.
- For every box checked, the likelihood of dysbiosis increases. Three or more checked boxes significantly increases the risk of dysbiosis.
- This checklist results is not meant to indicate that an individual is currently suffering from dysbiosis.
- The MDAC checklist is just the beginning and merely puts one on a path towards evaluating their microbiome and the resulting aspects of their overall health.

Common Microbiome Dysbiosis Associated Conditions (MDAC)

Factors/Conditions	
○ Lactose Intolerance	○ Antibiotics
○ Obesity	○ Asthma
○ Acid reflux	○ Vegetarian
○ Arthritis	○ Fruit per day
○ HTN/HBP	○ Dairy Free
○ IBS	○ Steroids

NEED FOR TESTING

Taking an at-home microbiome gut health test:

- A number of companies have started to offer at-home gut health microbiome tests due to the recognition that each person has a specific profile of microorganisms residing in their body (i.e., their **microbiome fingerprint**), along with the increased understanding of the critical role the microbiome plays in overall health and wellness.
- **Objective** of a gut health microbiome test is to provide an individual with a snap-shot of the composition of their gut microbiome.
- Having a personalized microbiome profile allows one to:
    - Determine if his/her microbiome is balanced and how it compares to a healthy microbiome.
    - Knowing the specific microbiome profile as well as any personal metadata including lifestyle (e.g., exercise, sleep, stress level, diet, health/disease status, medications, etc.) will allow one to **receive actionable** diet, lifestyle, and supplement recommendations based on their microbiome's specific makeup, as well as a person's lifestyle.

NEED FOR TESTING

Taking an at-home microbiome gut health test

- **Interpretation of a microbiome test** relies on available scientific findings pertaining to individual identifiable patterns of associations/correlations between the microbiome profile/metadata and symptoms to provide recommendations to improve one's gut health and overall wellness.
- The proposed recommendations do not guarantee that following these suggestions will result in the improvement of a person's symptoms. It is important to recognize that microbiome testing is based on recent advances that are exponentially expanding using DNA-based next-generation sequencing that offers new opportunities for personalized precision medicine and nutrition.
- The science behind the testing and recommendations are novel and will continue to evolve as advances in tis area continue to appear.
- It is also important to know that at-home microbiome health tests are **not FDA-approved** and are **not intended to diagnose, cure, mitigate, prevent, or treat any disease or condition**. Such tests solely provide informational insights into the microorganisms residing in one's gut.
- Based on recent discoveries linking the microbiome and the lifestyle a person practices, suggestions for improvement of overall health based on gut data may be possible. The provided insight may help individuals adopt new lifestyle/ dietary approaches that will help rebalance their microbiome and help in improving their gut health and overall wellness.
- It is recommended that individuals taking the test should consult their healthcare practitioners prior to initiation of any health related changes.
- If symptoms persistent, subjects should not rely on gut health tests to diagnose, cure, mitigate, prevent, or treat them.

NEED FOR TESTING

Designing at-home microbiome gut health test

The aim of a microbiome test is to provide a patient with a personalized snapshot of their microbiome and to inform if his/her gut microbiome is balanced or imbalanced (i.e., in dysbiosis).

Two critical components are necessary to achieve this aim:

1) Having personal metadata, which can be collected using a standardized instrument (questionnaire) that a patient completes when registering his/her at-home test, and,

2) Obtaining a personalized microbiome dataset that is generated using Next-Gen Seq of the bacteriome and mycobiome obtained from a stool sample submitted by that individual.

Based on these two pieces of information a digital report is created using bioinformatic analyses.

Various reports have different formats; however, most reports compare the individual results collected from the individual's sample to the profile of a "healthy" individual and provide a set of personalized recommendations to rebalance and/or maintain a balanced microbiome.

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Thank you!