The Role of the Microbiome and GI Function in Maintaining Host Health

(latest discoveries and therapeutic horizons)

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Objectives

- Understand the role of gastrointestinal health and metabolic, circadian, immune, brain, mitochondrial and overall host health
- Explore mechanisms of microbial influence on human health
- Causes and consequences of dysbiosis
- Treatments and protocols for gut health

If the GI system is unable to digest, absorb and assimilate nutrients effectively, while providing a barrier system to selectively prohibit invasion of unwanted micro-organisms, and maintain a healthy balance of commensal microorganisms, **health will forever be out of reach, and disease the prevailing norm of society**.

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GI problems on the rise

- 70 million Americans suffer from GI disease (many more have gut dysfunction)
- 5 million hospitalizations each year
- 75 million office visits
- 250,000 deaths annually
- GI diseases cost an estimated 142 billion dollars
- Hospitalizations for nonalcoholic fatty liver disease (NAFLD) have doubled since 2000.
- Almost 2 million Colorectal cancers DX annually

GI problems on the rise

- 150,000 hospitalizations due to IBD, UC and Crohn's
- Inflammatory bowel disease and functional/motility disease both cost almost $1 billion per year in inpatient costs and has increased over 50% in the last decade
- Morbid obesity has tripled since 2000
- Total number of bariatric procedures > 100,000 annually
- Celiac disease has almost doubled 2000-2009

Dysbiosis

- Change in the composition of intestinal microbiota, both commensal and pathogenic, is known as dysbiosis; this condition may affect homeostasis, leading to non-specific inflammation and disease. Dysbiosis implies an imbalance in microbial metabolite composition (Belizário et al., 2018) and is mainly the result of an “unhealthy” diet, the use of antibiotics, and lifestyle factors (Dudek-Wicher et al., 2018), it can also be caused by emotional and physiological stress (Li et al., 2018). Dysbiosis may result in epigenetic changes in adjacent intestinal cells, as well as in hepatocytes and adipocytes (Belizário et al., 2018; Qin and Wade, 2018).
- Rapid changes in feeding habits over the last century may have contributed to our current enterotypes and general health (Moeller et al., 2014). Bowel disease, irritable bowel syndrome, obesity, diabetes, and cancer have been associated with specific bacterial dysbiosis (Clemente et al., 2012; Belizário et al., 2018).

Front Cell Dev Biol. 2020; 8: 51.

- Infants born by Cesarean delivery or from mothers that have used antibiotics and thus harbor a particular enterotype, have a higher risk of developing asthma, type I diabetes, and celiac disease (Mueller et al., 2015).

- There is evidence of a time-of-day-specific intestinal microbiota taxonomic composition associated with rhythmic food intake, dietary structure, gender, and the host biological clock (Liang et al., 2015; Thaiss et al., 2016; Li et al., 2018), and there is a clear role for the intestinal microbiota in the regulation of metabolism, the immune system, and circadian rhythmicity.

- One of the mechanisms of communication between the intestinal microbiota and the other three biological domains involves microbial metabolites.

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Stress Effects on Brain And Digestion

Neurotransmitter Metabolism

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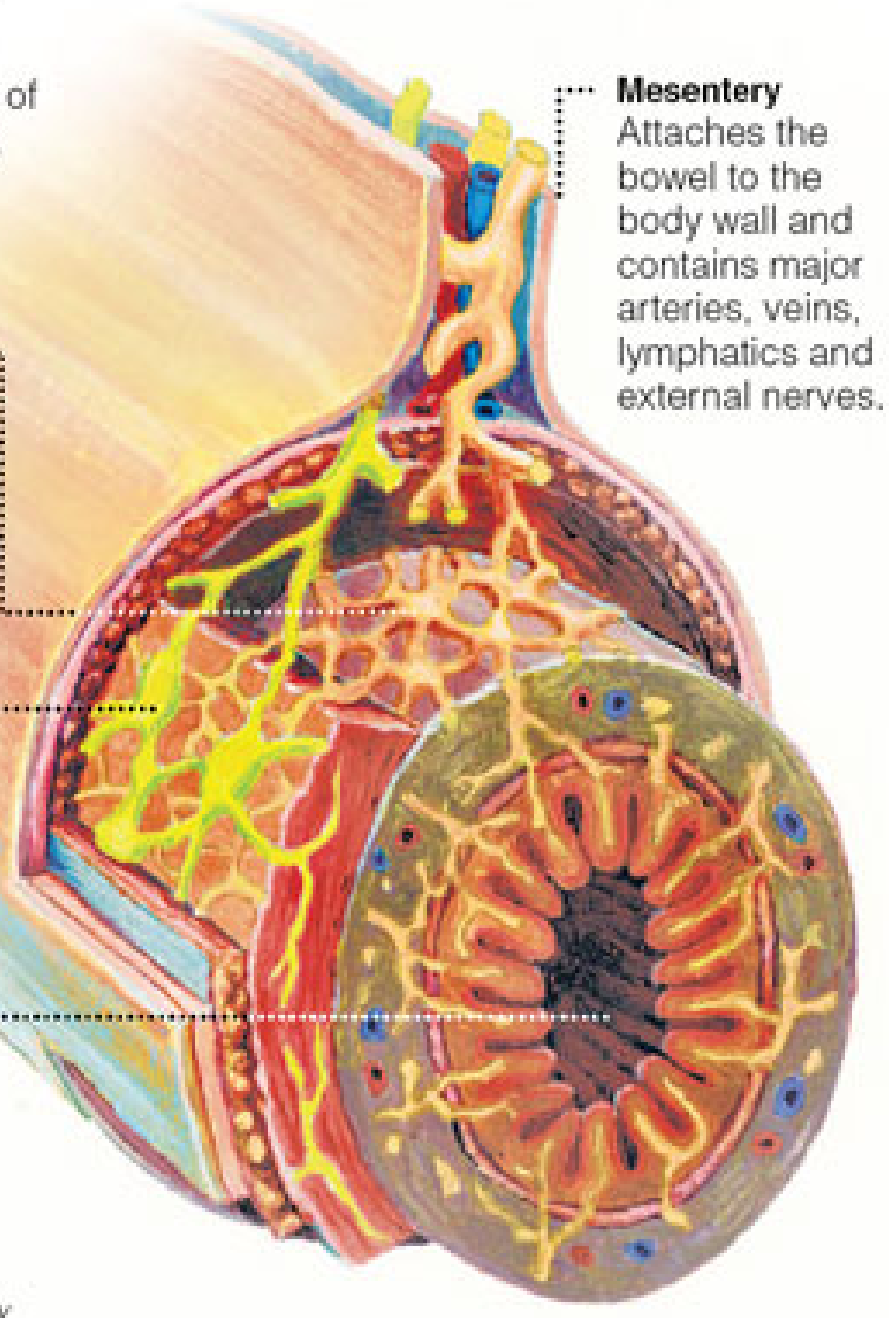
The Brain in Your Gut

The gut's brain, known as the enteric nervous system, is located in sheaths of tissue lining the esophagus, stomach, small intestine and colon.

SMALL INTESTINE CROSS SECTION



Mesentery Attaches the bowel to the body wall and contains major arteries, veins, lymphatics and external nerves.

Submucosal plexus Layer contains sensory cells that communicate with the myenteric plexus and motor fibers that stimulate the secretion of fluids into the lumen.

Myenteric plexus Layer contains the neurons responsible for regulating the enzyme output of adjacent organs.

Lumen No nerves actually enter this area, where digestion occurs. The brains in the head and gut have to monitor conditions in the lumen across the lining of the bowel.

Source: Dr. Michael D. Gershon, Columbia University

General Ways to Improve Digestion

- Eat slowly and CHEW, CHEW, CHEW
- **Decrease stress – Relaxation Response, Buteyko Breathing. Laughing has been shown to increase GI secretions**
- Incorporate AI diet with plenty of herbs/spices
- Take enzymes with meals
- Walk after eating, or gentle rebounder
- Exercise regularly
- Avoid food allergies/sensitivities – get tested
- Do not overeat

Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions.

Compr Physiol. 2014 Oct;4(4):1339-68.

- Although the gastrointestinal (GI) tract possesses intrinsic neural plexuses that allow a significant degree of autonomy over GI functions, the central nervous system (CNS) provides extrinsic neural inputs that regulate, modulate, and control these functions. While the intestines are capable of functioning in the absence of extrinsic inputs, the stomach and esophagus are much more dependent upon extrinsic neural inputs, particularly from parasympathetic and sympathetic pathways. The sympathetic nervous system exerts a predominantly inhibitory effect upon GI muscle and provides a tonic inhibitory influence over mucosal secretion while, at the same time, regulates GI blood flow via neurally mediated vasoconstriction. The parasympathetic nervous system, in contrast, exerts both excitatory and inhibitory control over gastric and intestinal tone and motility. Although GI functions are controlled by the autonomic nervous system and occur, by and large, independently of conscious perception, it is clear that the higher CNS centers influence homeostatic control as well as cognitive and behavioral functions.

Peripheral apelin mediates stress-induced alterations in gastrointestinal motor functions depending on the nutritional status.

Clin Exp Pharmacol Physiol. 2018 Sep 17.

- Exposure to stress induces gastrointestinal (GI) dysmotility. In rodents, acute restraint stress (ARS) inhibits gastric emptying (GE) and intestinal transit (IT) via central and peripheral corticotropin-releasing factor (CRF)-mediated pathways. Peripherally-administered apelin-13 was shown to inhibit GI motor functions, moreover, stress-induced upregulation of gastric apelin content was demonstrated in rats suggesting that peripheral apelin may mediate stress-induced alterations in GI motility.
- GE and IT were delayed by CRF and ARS. ARS-induced changes were attenuated by F13A, whereas astressin was ineffective. CRF-induced alterations in GE and CT were restored completely by astressin, while they were diminished by F13A. Antral phase III-like contractions were disturbed following ARS which were preserved by preadministration of astressin, but not F13A. CRF impaired gastric and duodenal fasting contractions, while these changes were not altered by F13A. ARS increased apelin expression in stomach and duodenum. Apelin immunoreactivity was detected in mucosa, smooth muscles and myenteric plexi, whereas dense Apelin Receptor (APJ) receptor expression was observed within tunica muscularis. APJ receptor was downregulated in rats fasted overnight. These results suggest that enteric apelin acts as an inhibitor stress mediator in the postprandial state.

frontiers in Cellular Neuroscience

MINI REVIEW
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Epigenetic Regulation of Enteric Neurotransmission by Gut Bacteria

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The Human Microbiome Project defined microbial community interactions with the human host, and provided important molecular insight into how epigenetic factors can influence intestinal ecosystems. Given physiological context, changes in gut microbial community structure are increasingly found to associate with alterations in enteric neurotransmission and disease. At present, it is not known whether shifts in microbial community dynamics represent cause or consequence of disease pathogenesis. The discovery of bacterial-derived neurotransmitters suggests further studies are needed to establish their role in enteric neuropathy. This mini-review highlights recent advances in bacterial communications to the autonomic nervous system and discusses emerging epigenetic data showing that diet, probiotic and antibiotic use may regulate enteric neurotransmission through modulation of microbial communities. A particular emphasis is placed on bacterial metabolite regulation of enteric nervous system function in the intestine.

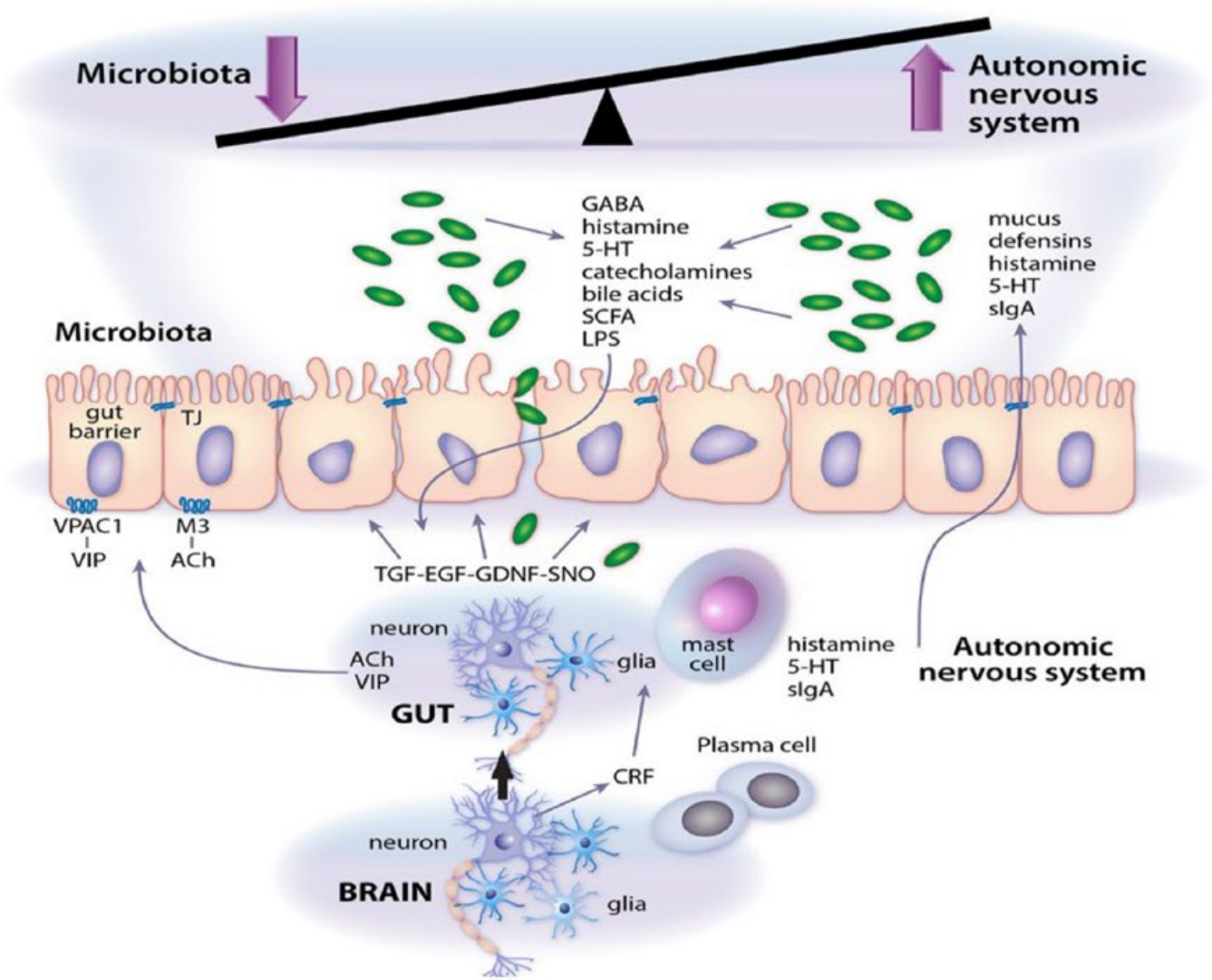


FIGURE 1 | Microbial neurotransmitter crosstalk with the autonomic nervous system. As outlined in the article, a system of checks and balances operate to regulate gut function. Abbreviations: 5-HT, serotonin; Ach, acetylcholine; CRF, corticotrophin releasing factor; EGF, epidermal growth factor; GDNF, glial cell line-derived neurotrophic factor; LPS, lipopolysaccharide; M3, M3 muscarinic receptor; SCFA, short chain fatty acids; sIgA, secretory IgA; SNO, s-nitrosothiol; TGF, transforming growth factor; VIP, vasoactive intestinal peptide; VPAC1, VIP and PACAP receptor 1.

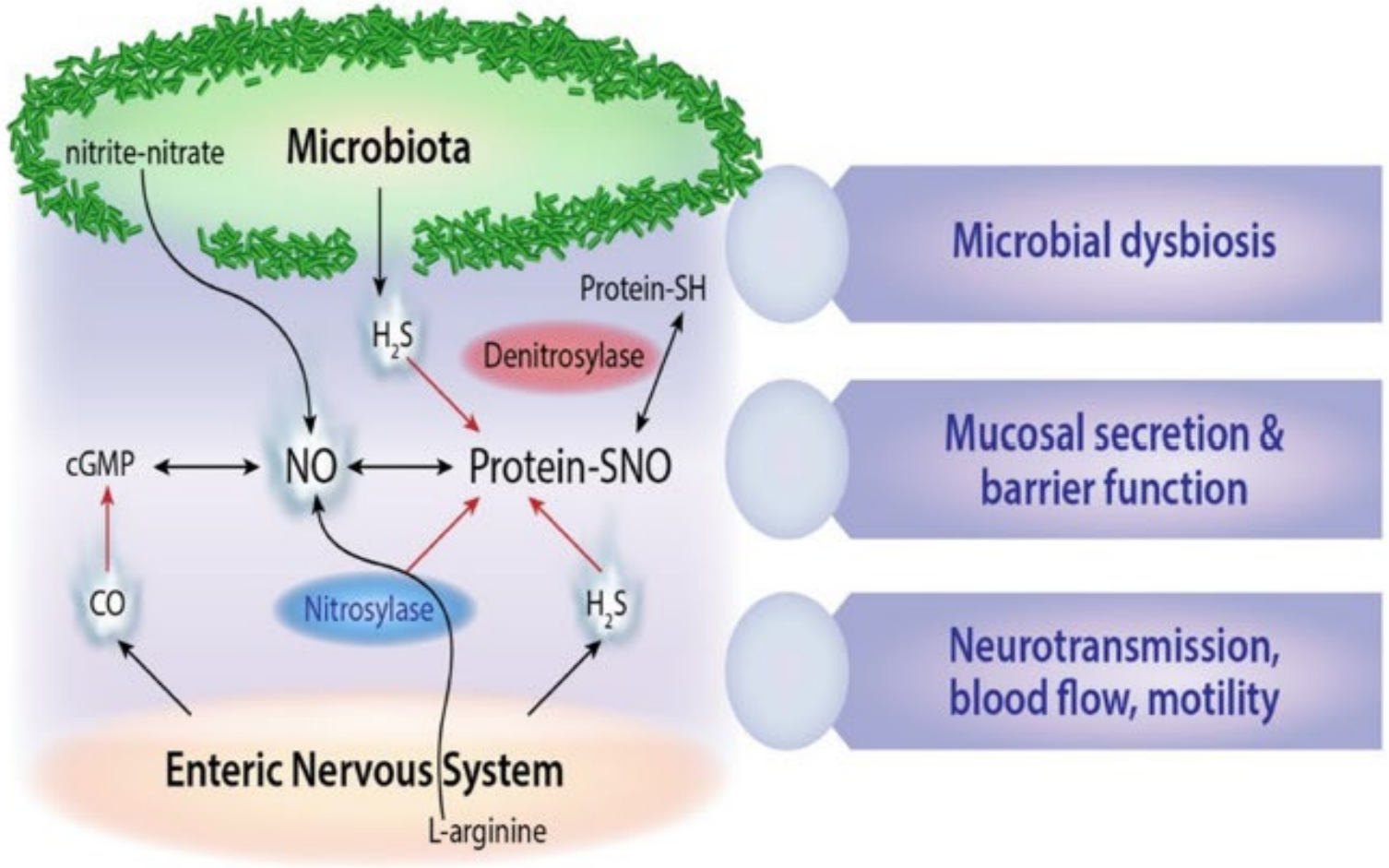


FIGURE 2 | Microbial gaseous neurotransmitters. Schematic outline of microbial derived nitric oxide (NO), S-nitrosothiol derivatives (SNO), and hydrogen sulfide (H_2S) signals and their cross-interactions with carbon monoxide (CO) neurotransmitters in the enteric nervous system.

Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders.

Front Psychiatry. 2018 Mar 13;9:44. doi: 10.3389/fpsyt.2018.00044. eCollection 2018.

- The vagus nerve represents the main component of the parasympathetic nervous system, which oversees a vast array of crucial bodily functions, including control of mood, immune response, digestion, and heart rate. It establishes one of the connections between the brain and the gastrointestinal tract and sends information about the state of the inner organs to the brain *via* afferent fibers. In this review article, we discuss various functions of the vagus nerve which make it an attractive target in treating psychiatric and gastrointestinal disorders. There is preliminary evidence that vagus nerve stimulation is a promising add-on treatment for treatment-refractory depression, posttraumatic stress disorder, and inflammatory bowel disease. Treatments that target the vagus nerve increase the vagal tone and inhibit cytokine production. Both are important mechanism of resiliency. The stimulation of vagal afferent fibers in the gut influences monoaminergic brain systems in the brain stem that play crucial roles in major psychiatric conditions, such as mood and anxiety disorders. In line, there is preliminary evidence for gut bacteria to have beneficial effect on mood and anxiety, partly by affecting the activity of the vagus nerve. Since, the vagal tone is correlated with capacity to regulate stress responses and can be influenced by breathing, its increase through meditation and yoga likely contribute to resilience and the mitigation of mood and anxiety symptoms.

Afferent and efferent connections

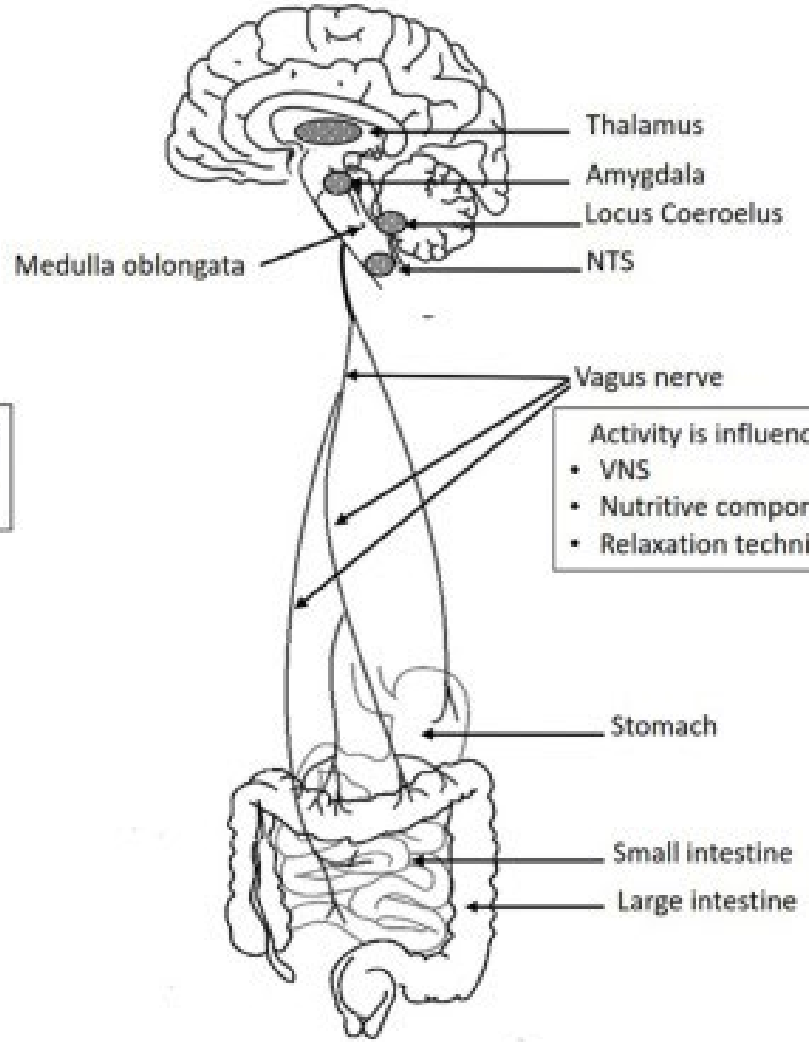
90% of vagus nerve fibers

- Imflammation
- Satiety (Hunger)
- Satiation (Fullness)
- Energy Metabolism

- Secretion of gastric acid and digestive enzymes
- Gastric capacity

10% of vagus nerve fibers

Anatomy



Activity is influenced by:
- VNS
- Nutritive components
- Relaxation techniques

Disorders

Psychiatric disorders
- Major depression
- PTSD

Inflammatory GI Disorders
- Ulcerative Colitis
- Crohn`s Desease

The Effects of Stress and Meditation on the Immune System, Human Microbiota, and Epigenetics.

Adv Mind Body Med. 2017 Fall;31(4):10-25.

- Globally, more than 25% of individuals are affected by anxiety and depression disorders. Meditation is gaining popularity in clinical settings and its treatment efficacy is being studied for a wide array of psychological and physiological ailments. An exploration of stress physiology is an essential precursor to delineation of the mechanisms underlying the beneficial effects of meditation practices.
- The review outlines a model of interconnected physiological processes that might support the continued inclusion and expansion of meditation in the treatment of diverse medical conditions and to investigate the role that gut microbiota may play in realizing well-being through meditation.
- Psychological stress typically triggers a fight-or-flight response, prompting corticotropin-releasing hormone and catecholamine production in various parts of the body, which ultimately disturbs the microbiota. In the absence of stress, a healthy microbiota produces short-chain fatty acids that exert anti-inflammatory and antitumor effects. During stress, an altered gut microbial population affects the regulation of neurotransmitters mediated by the microbiome and gut barrier function. Meditation helps regulate the stress response, thereby suppressing chronic inflammation states and maintaining a healthy gut-barrier function. Conclusions • The current research team recommends the integration of meditation into conventional health care and wellness models. Concurrently, studies to explore the effects of meditation on human microbiota are warranted.

Stress and Brain

- Stress is probably one of the pervasive factors in brain health
  - increases cortisol which damages hippocampus and hypothalamus
  - increases IL-6, IL-10 and TNF, stimulating microglia and inflammatory interruption of neurotransmitters
  - incr IGF-1 a known cancer initiator
  - increases insulin resistance which is associated with all neurodegenerative disorders
  - decreases melatonin which protects brain through antioxidant and inflammatory-regulation activity
  - Increases quinolinic acid and decreases kynurenine

Cytokines in the brain

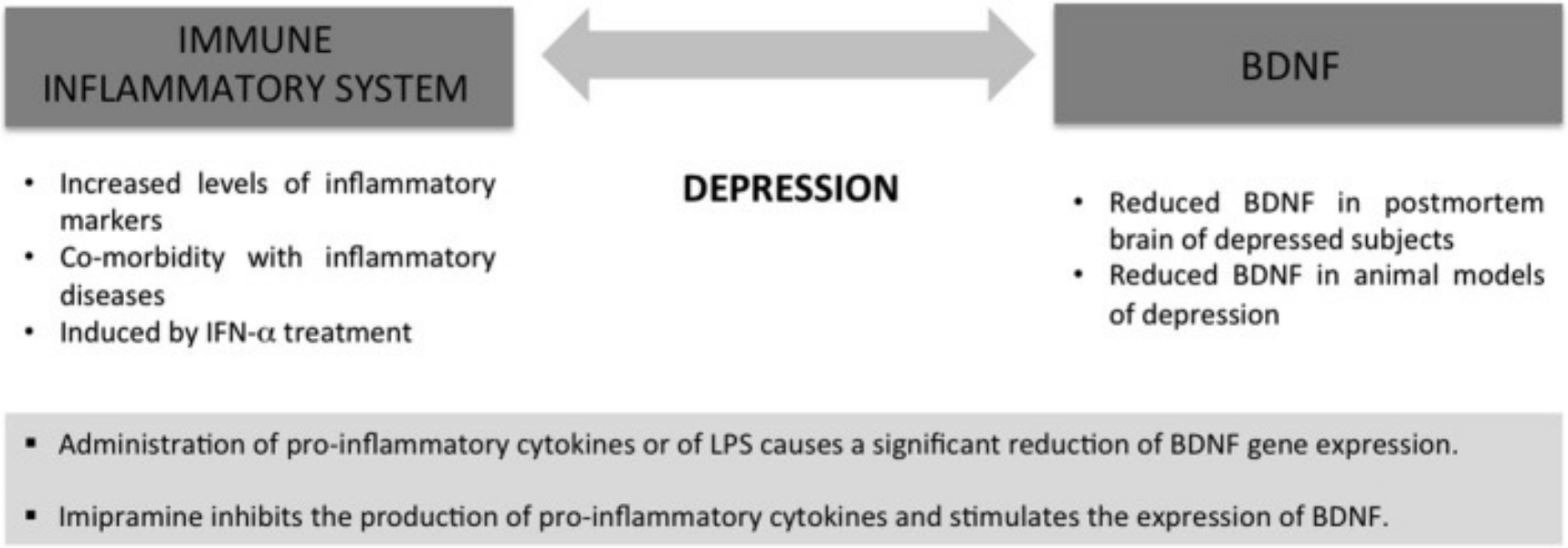
- Immune Effects
  - Stimulate microglial activation which in turn stimulates more cytokines – feed forward loop
  - Microglial cells destroy adjacent neurons and/or affect neurotransmitter function
  - Stimulate autoantibody production to neurons and receptors
  - Stimulate MAO breakdown of Serotonin/Dopamine
- Neuroendocrine
  - Disrupts H-P-T-G-A axis due to pituitary inflammation and sympathetic affects on adrenals, increasing immune dysfunction
- Behavior
  - Cytokines affect sleep, mood, appetite, addiction

Cytokines and the Brain: Implications for Clinical Psychiatry. Am J Psychiatry. 2000

The Inflammatory Hypothesis of Depression. Focus 2012

Immune system to brain signaling: neuropsychopharmacological implications. Pharmacol Ther 2011

Inflammation stimulates neurodegeneration and inhibits neuroplasticity



Picture from: Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Front Cell Neurosci*. 2014

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Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders.

J Neurol Sci. 2007 Jun

- Stress **production of free radicals** worsens the mitochondrial function, creates micropores in membrane and induces apoptosis.
- Senescence is accompanied by a higher degree of reactive oxygen species production, and by **diminished functions of the endoplasmic reticulum.**
- In the event of a dysfunction of the endoplasmic reticulum, unfolded proteins aggregate in it, forming potentially toxic deposits which tend to be resistant to degradation.

- Tryptophan is metabolized via several pathways, the main one being the kynurenine pathway. A central compound of the pathway is kynurenine (KYN), which can be metabolized in two separate ways: one branch furnishing kynurenic acid, and the other 3-hydroxykynurenine and quinolinic acid.
- **Quinolinic acid is a specific agonist at the N-methyl-d-aspartate receptors, and a potent neurotoxin with an additional and marked free radical-producing property.**
- These changes may disturb normal brain function and can add to the pathomechanisms of many brain diseases.

Increases in Kynurenate

- Causes:
  - Leaky gut/Dysbiosis/Infection
  - **Stress, inflammation and decreased glutathione**
  - **Insufficient B6**, magnesium, zinc
  - Pollutant exposure and liver overload
  - Low conversion of Tryptophan to Serotonin
  - Methylation deficits, elevated homocysteine
  - Increased Tryptophan intake
  - Overweight
  - Oral Contraceptives
- Effects:
  - Anxiety
  - Depression
  - **Autism – C. Diff infection**
  - Neurodegeneration
  - Parkinson's
  - Alzheimer's

Treatment for tryptophan metabolism problems

- Look at bigger neurotransmitter picture
- Decrease Inflammation (gut)
- **Increase Glutathione Status**
- Methyl Renew to replenish B6 and Methyl-nutrients
- Calorie Restriction
- Exercise
- Lower BMI
- Melatonin and 5 HTP supplementation

Never supplement with Tryptophan or 5-HTP unless this pathway is balanced:

Increase Glutathione and Methylation

Improve Leaky Gut and lower LPS and inflammation

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Inhibitory effect of dehydroepiandrosterone (DHEA) on brain monoamine oxidase activity: in vivo and in vitro studies.

Life Sci. 2009 Oct 21;85(17-18):652-6.

- DHEA significantly reduced (-24%) total MAO activity at 120mg/kg dose. No significant difference was observed when MAO A and MAO B activities were independently analyzed.
- An inhibitory effect of DHEA on MAO activity may be involved in the **antidepressant and neuroprotective effects of the steroid**. Since MAO inhibition reduces neurodegeneration in clinical trials for Parkinson's disease, our results suggest that DHEA may be useful to treat depression and to prevent neuronal death in this disorder.

DHEA Uses

- Depression (stimulates serotonin, dopamine)
- Fatigue
- Stress/Adrenal Support
- Brain Injury (reduces inflammation)
- Autoimmune (Lupus, Psoriasis)
- Infections (slows viral replication)
- Longevity
- Menopause/Hot Flashes
- Suggest using in combination with DIM

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Effect of dehydroepiandrosterone on the immune response and gut microbiota in dextran sulfate sodium-induced colitis mice

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ARTICLE INFO

Keywords:
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Gut microbiota

ABSTRACT

Dehydroepiandrosterone (DHEA) possess anti-inflammatory, anti-oxidant and immune-regulating function in animals and humans, but there is not enough information about the mechanisms underlying its beneficial effects. The present study investigated the effect and mechanism of DHEA in dextran sulfate sodium (DSS)-induced colitis mice. The findings showed that DHEA relieved the decreasing of body weight, the increasing of disease activity index, the enhancing of spleen weight, the shortening of colon length and the rising of myeloperoxidase activity; meanwhile, histopathological analysis showed that DHEA maintained a relatively intact structure of colon in DSS-induced colitis mice. DHEA decreased the malondialdehyde content, superoxide dismutase activity and inducible nitric oxide synthase protein level; meanwhile, DHEA also inhibited the secretion of tumor necrosis factor-α, interleukin-1β and interleukin-6 in DSS-induced colitis mice. Importantly, our results showed that DHEA blocked the activation of nuclear factor-kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways; and it inhibited the Nod-like receptor protein 3 inflammasome activation in DSS-induced colitis mice. Furthermore, DHEA markedly promoted the intestinal barrier function by up-regulation zonula occludens-1 expression level. The 16S rDNA gene sequencing demonstrated that DHEA decreased the *Pseudomonas* abundance in DSS-induced colitis mice. In conclusion, our data demonstrated that DHEA reduces oxidative damage through regulating antioxidant enzyme activity; inhibits pro-inflammatory cytokines production by blocking the activation of p38 MAPK and NF-κB signal pathway; protects colon barrier integrity *via* increasing tight junction protein expression and modulating gut microbiota taxa; all that finally alleviates DSS-induced experimental colitis in mice.

1. Introduction

Ulcerative colitis (UC) is a main form of the inflammatory bowel diseases (IBD) and characterizes by body weight loss, abdominal pain, diarrhea and rectal bleeding (Conrad et al., 2014). UC mainly occurs in the colonic mucosa and lesions mostly develop from the distal end of colon, and it causes millions of patients in the worldwide (Cosnes et al.

the inhibitors of NLRP3 may be used as the potential therapeutic drug for IBD treatment (Perera et al., 2017). In addition, the disturbance of intestinal microbiota and epithelial barrier damage were along with UC (Rubio et al., 2018). It well known that a good tight junction (TJ) of intestines is essential to maintain intestinal function and prevents the pathogenic microorganism's invasion, thereby exerting a barrier function and safeguarding the body health. Although many studies reported

frontiers in Cell and Developmental Biology

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PMID: 32117978

Mitochondria: An Integrative Hub Coordinating Circadian Rhythms, Metabolism, the Microbiome, and Immunity

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Abstract

Go to:

There is currently some understanding of the mechanisms that underpin the interactions between circadian rhythmicity and immunity, metabolism and immune response, and circadian rhythmicity and metabolism. In addition, a wealth of studies have led to the conclusion that the commensal microbiota (mainly bacteria) within the intestine contributes to host homeostasis by regulating circadian rhythmicity, metabolism, and the immune system. Experimental studies on how these four biological domains interact with each other have mainly focused on any two of those domains at a time and only occasionally on three. However, a systematic analysis of how these four domains concurrently interact with each other seems to be missing. We have analyzed current evidence that signposts a role for mitochondria as a key hub that supports and integrates activity across all four domains, circadian clocks, metabolic pathways, the intestinal microbiota, and the immune system, coordinating their integration and crosstalk. This work will hopefully provide a new perspective for both hypothesis-building and more systematic experimental approaches.

Keywords: mitochondria, circadian rhythmicity, metabolism, intestinal microbiota, immune system

Circadian Rhythmicity

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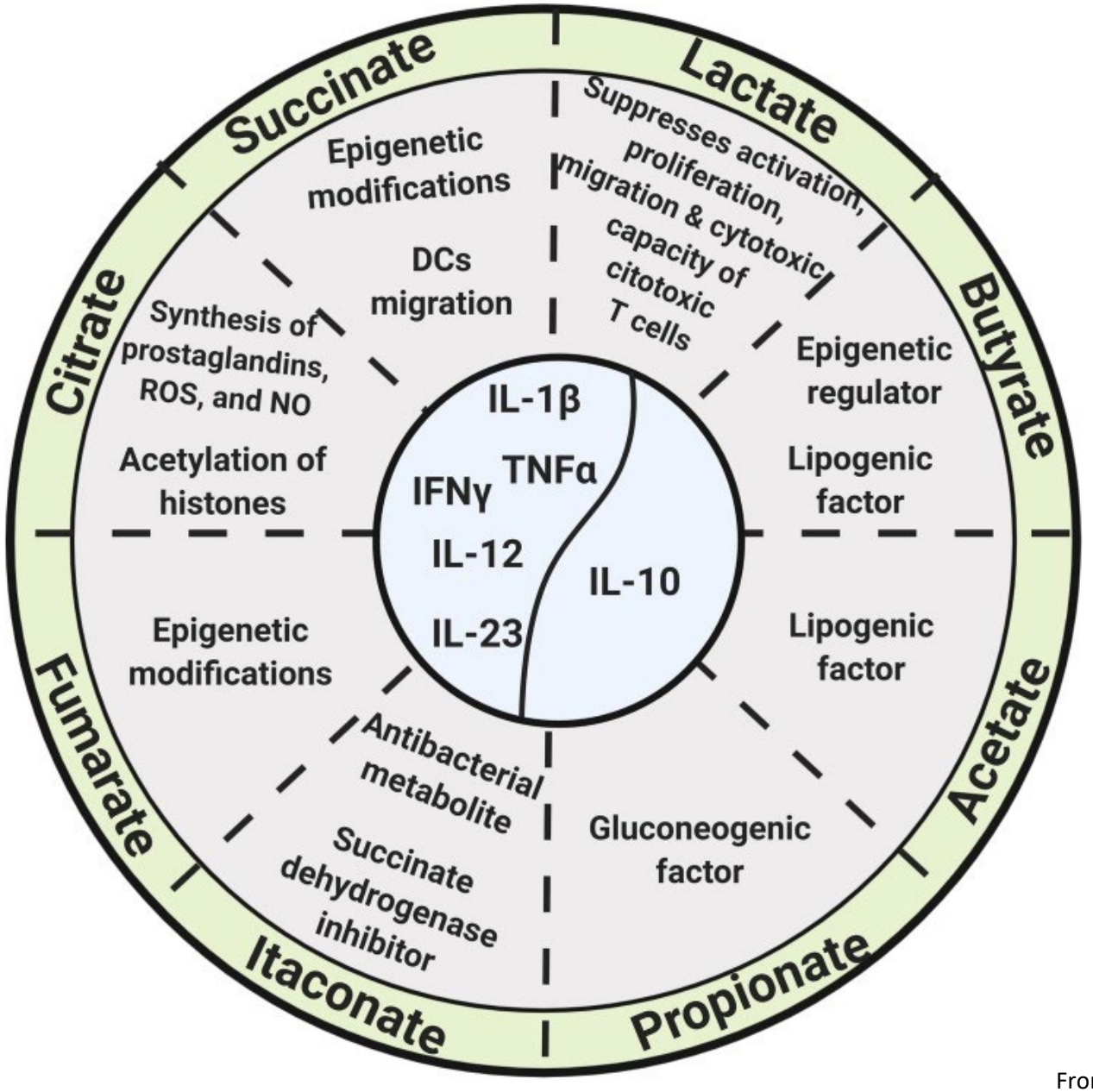
Circadian rhythms were first observed in 1729 by Jean-Jacques d'Ortous de Mairan, who noticed that the leaves of the Mimosa plant moved with a periodicity of 24 h, even in the absence of light, thus suggesting the presence of an internal clock. It is now recognized that circadian rhythmicity integrates a mechanism for the timely coordination of cellular and broader physiological functions (Roenneberg and Merrow, 2005).



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- Succinate is produced from succinyl CoA by succinyl-CoA synthetase and accumulates in the cytoplasm of monocyte/macrophages and dendritic cells (DCs) upon LPS stimulation (Pistollato et al., 2010; Williams and O'Neill, 2018). Succinate accumulation, along with the induction of the glycolytic enzyme hexokinase-1, increases the activity of the respiratory chain complex II, promotes the production of mROS, stabilizes HIF-1α, regulates the transcription of pro-IL-1β, and activates the NOD-like receptor protein 3 (NLRP3) inflammasome, increasing the production of IL-1β (Chandel et al., 2000; Moon et al., 2015; Garaude et al., 2016; Mills et al., 2016, 2017; Figure 2).
- LPS stimulation leads to the succinylation of several enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), and the glutamate carrier-1 (Tannahill et al., 2013).
- A specific receptor for extracellular succinate (SUCNR1/GPR91) is present in hepatic, renal, retinal, and immune cells, and its ligation leads to the secretion of various hormones, growth factors, and cytokines (Ariza et al., 2012) and regulates DCs migration into lymph nodes, as well as DCs antigen presentation (Figure 2). The SUCNR1/GPR91 receptor can synergize with TLR3 and TLR7, increasing the production of pro-inflammatory cytokines (Rubic et al., 2008).

Front Cell Dev Biol. 2020; 8: 51.

The Metabolome

- The metabolome is the repertoire of small biomolecules present in cells, tissues, and body fluids, and its composition is at the core of the health status of individuals. The development of new “metabolomic platforms” has revealed that a number of metabolites present in several biological samples, such as serum and urine, vary in concentration following a circadian rhythmicity (Martínez-Lozano et al., 2014; de Raad et al., 2016). Among them are glycolysis-related metabolites, such as glucose, glucose-6-phosphate, bisphosphoglycerate, and lactate; tricarboxylic acid (TCA) cycle-related molecules, such as acetate, acetyl CoA, citrate, isocitrate, and malonate; amino acids and their derivatives; lipid metabolites; nucleotides; antioxidants; and coenzymes such as NAD, FAD, and coenzyme A (Krishnaiah et al., 2017).
- Interestingly, the daily variation in the bacterial composition within the intestine implies a daily variation in the concentration of some bacteria-derived metabolites, and the hundreds of microbiota-derived metabolites that have been identified are regarded as components of the human metabolome (Belizário et al., 2018). Thus, linking eukaryotic- and bacterial-derived metabolites with the other three biological domains is discussed here.
- In attempting to convey the view that mitochondria support and integrate the communication between the four mentioned biological domains, the specific roles of mitochondria are discussed in the next sections.

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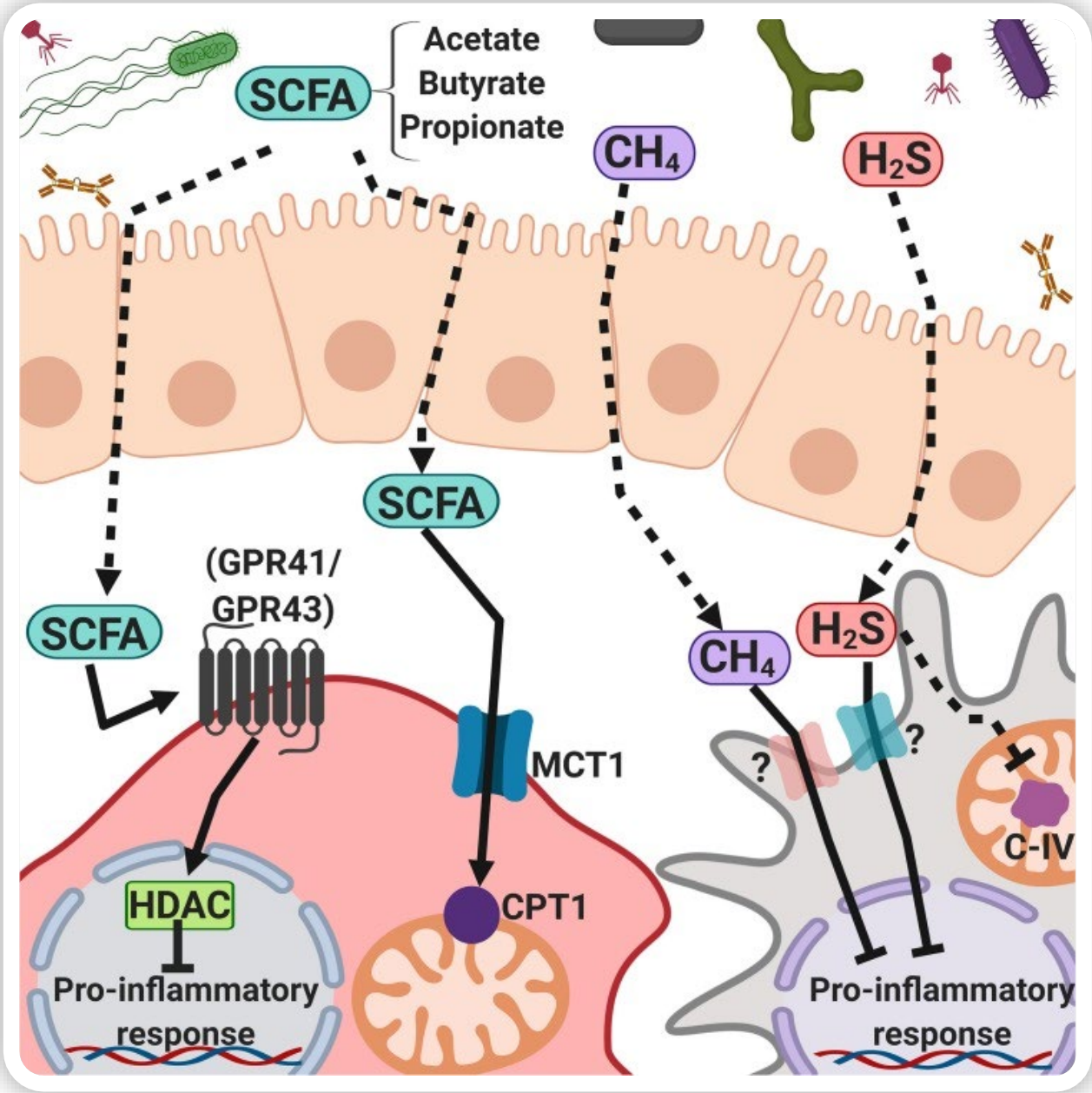

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A higher proportion of SCFA-producing bacteria within the intestinal microbiota is associated with a reduction in the risk of developing obesity, insulin resistance, and type 2 diabetes, since these compounds, particularly butyrate, increase cellular respiration and fatty acid oxidation (Belizário et al., 2018). Acetate, butyrate, and propionate are the most abundant SCFAs and represent 90–95% of the total SCFAs present in the colon.

SCFA

Mitochondria as target of microbial influence

Mitochondrial metabolic stress induces mitochondrial dysfunction, which may lead to the disruption of the intestinal epithelial barrier, allowing *E. coli*, and perhaps other bacteria, to cross the epithelium (Nazli et al., 2004; Wang et al., 2015). Microbial products, such as butyrate and urolithin A, enhance mitochondrial functions (Ryu et al., 2016), and others, such as betaine, methionine, and homocysteine, activate signaling pathways that regulate mitochondrial dynamics in the intestinal epithelium (Lin and Wang, 2017).

E. coli-secreted colanic acid is endocytosed and is capable of inducing Drp1-dependent mitochondrial fission (Han et al., 2017), and *Pseudomonas aeruginosa* secretes N-(3-oxo-dodecanoyl)-L-homoserine lactone (3OC12), a molecule thought to subvert immune defenses. In several cell types, such as in bronchial epithelial cells, 3OC12 is hydrolyzed by the enzyme lactonase paraoxonase 2 (PON2) present in mitochondria, yielding 3OC12 acid, which accumulates within mitochondria, causing mitochondrial and cytosolic acidification, increase in intracellular Ca^{2+} concentration and activation of stress signaling kinases (Horke et al., 2015).

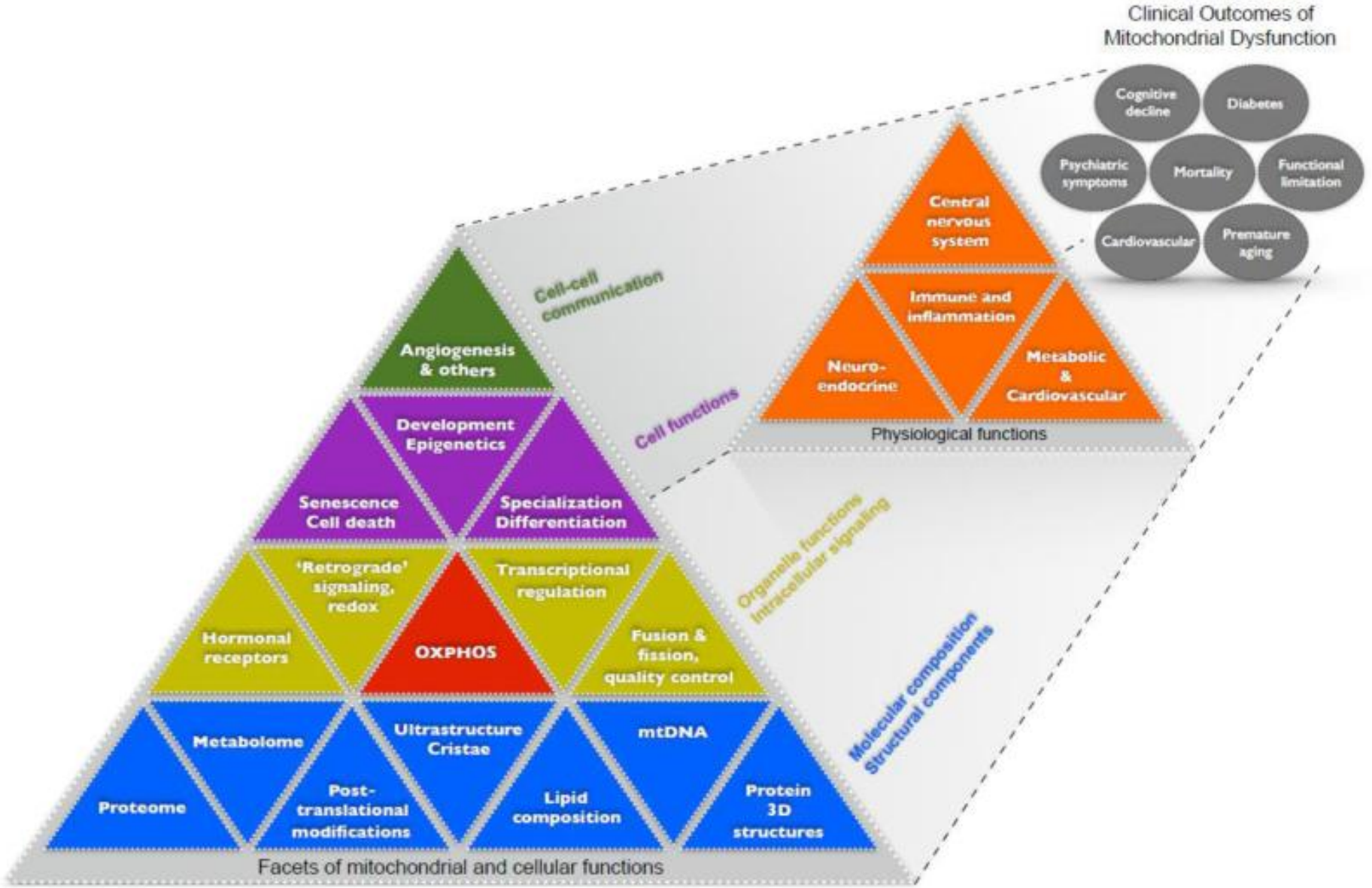
Polymorphisms in the mitochondrial genes of the ND5, CYTB, and D-loop regions have been associated with variations in the composition of the intestinal microbiota (Ma et al., 2014); mutations in the ATP8 gene increase the relative abundance of Bacteroidales, Deferribacteraceae, Desulfovibrionaceae, and Helicobacteraceae, suggesting that mitochondria play a role in defining the microbiome (Hirose et al., 2017).

It has also been suggested that mitochondria from intestinal cells are highly responsive to microbiotic signaling, with implications in inflammatory processes and colorectal cancer (Andersson et al., 1998; Jackson and Theiss, 2019).

The rise of mitochondria in medicine.

Mitochondrion. 2016 Sep;30:105-16.

- Once considered exclusively the cell's powerhouse, mitochondria are now recognized to perform multiple essential functions beyond energy production, impacting most areas of cell biology and medicine. Since the emergence of molecular biology and the discovery of pathogenic mitochondrial DNA defects in the 1980's, research advances have revealed a **number of common human diseases** which share an underlying pathogenesis involving mitochondrial dysfunction. Mitochondria undergo function-defining dynamic shape changes, communicate with each other, regulate gene expression within the nucleus, modulate synaptic transmission within the brain, release molecules that contribute to oncogenic transformation and trigger inflammatory responses systemically, and influence the regulation of complex physiological systems. Novel mitopathogenic mechanisms are thus being uncovered across a number of medical disciplines including genetics, oncology, neurology, immunology, and critical care medicine. Increasing knowledge of the bioenergetic aspects of human disease has provided new opportunities for diagnosis, therapy, prevention, and in connecting various domains of medicine.



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- Mutations in mitochondrial DNA are usually single nucleotide substitutions, single base insertions, or deletions.
- Because each cell contains thousands of mitochondria, nearly all organisms house low levels of mitochondrial variants, conferring some degree of heteroplasmy. Although a single mutational event might be rare in its generation, repeated mitotic segregation and clonal expansion can enable it to dominate the mitochondrial DNA pool over time. When this occurs, it is known as reaching threshold, and it usually results in physiological consequences.

Gut bacteria signaling to mitochondria in intestinal inflammation and cancer.

Gut Microbes. 2019 Mar 26:1-20.

The gastrointestinal microbiome plays a pivotal role in physiological homeostasis of the intestine as well as in the pathophysiology of diseases including inflammatory bowel diseases (IBD) and colorectal cancer (CRC). Emerging evidence suggests that gut microbiota signal to the mitochondria of mucosal cells, including epithelial cells and immune cells. Gut microbiota signaling to mitochondria has been shown to alter mitochondrial metabolism, activate immune cells, induce inflammasome signaling, and alter epithelial barrier function. Both dysbiosis of the gut microbiota and mitochondrial dysfunction are associated with chronic intestinal inflammation and CRC. This review discusses mitochondrial metabolism of gut mucosal cells, mitochondrial dysfunction, and known gut microbiota-mediated mitochondrial alterations during IBD and CRC.

Essential Mitochondrial Support Agents

- CoQ10
- Quercetin
- Lipoic Acid
- Vitamin C, E, A, D
- N-Acetyl-Cysteine/Glutathione/Redox
- Ribose
- Adaptogens (Eleuthero, Rhodiola, Schisandra, Ashwagandha)
- Melatonin
- B-Vitamins (NAD)

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The Gut Microbiome and Its Role in Obesity

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Abstract

Go to:

The human body is host to a vast number of microbes, including bacterial, fungal and protozoal microoganisms, which together constitute our microbiota. Evidence is emerging that the intestinal microbiome is intrinsically linked with overall health, including obesity risk. Obesity and obesity-related metabolic disorders are characterized by specific alterations in the composition and function of the human gut microbiome. Mechanistic studies have indicated that the gastrointestinal microbiota can influence both sides of the energy balance equation; namely, as a factor influencing energy utilization from the diet and as a factor that influences host genes that regulate energy expenditure and storage. Moreover, its composition is not fixed and can be influenced by several dietary components. This fact raises the attractive possibility that manipulating the gut microbiota could facilitate weight loss or prevent obesity in humans. Emerging as possible strategies for obesity prevention and/or treatment are targeting the microbiota, in order to restore or modulate its composition through the consumption of live bacteria (probiotics), nondigestible or limited digestible food constituents such as oligosaccharides (prebiotics), or both (synbiotics), or even fecal transplants.

The human gastrointestinal tract is colonized by large numbers of microorganisms, including bacteria, archaea, viruses, fungi and protozoa, collectively known as the gut microbiota. The human gut microbiota (see Table 1) consists of up to 100 trillion microbes and possesses at least 100 times more genes (the microbiome) than are present in the entire human genome.[1] These microbes serve a number of important functions including: producing additional energy otherwise inaccessible to the host by breaking down soluble fiber; producing vitamins such as biotin, folate and vitamin K; metabolizing xenobiotics such as the inactivation of heterocyclic amines formed in meat during cooking; preventing colonization by

Microbiota and metabolism

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GI360™; stool

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Doctor: Sample Doctor
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3755 Illinois Ave.
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Age: 44
Sex: Male

Sample Collection	Date/Time
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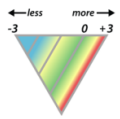
Date Reported	09/02/2020
Specimens Collected	3

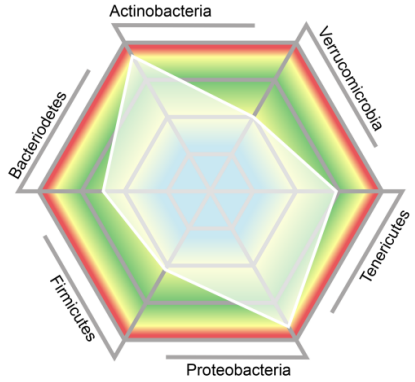
Microbiome Abundance and Diversity Summary

The abundance and diversity of gastrointestinal bacteria provide an indication of gastrointestinal health, and gut microbial imbalances can contribute to dysbiosis and other chronic disease states. The GI360™ Microbiome Profile is a gut microbiota DNA analysis tool that identifies and characterizes more than 45 targeted analytes across six Phyla using PCR and compares the patient results to a characterized normobiotic reference population. The web chart illustrates the degree to which an individual's microbiome profile deviates from normobiosis.

LEGEND



The web image shows the relative diversity and balance among bacteria belonging to the six primary Phyla. The white shaded area represents the patient's results compared to a normobiotic reference population. The center of the web represents less abundance while the outer edges represent more than normobiotic.



Dysbiosis Index

The Dysbiosis Index the (DI) is calculated strictly from the results of the Microbiome Profile, with scores from 1 to 5. A DI score above 2 indicates dysbiosis; a microbiota profile that differs from the defined normobiotic reference population. The higher the DI above 2, the more the sample deviates from the normobiotic profile. The dysbiosis test and DI does not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

DI Score

4

Key Findings

Actinomycetales, Very High	↑	*Blastocystis* spp., Observed	Occult Blood, Detected
Escherichia spp., Very High	↑	*Dientamoeba fragilis*, Observed	
Clostridia Class, Very Low	↓	WBC, Abnormal	
Faecalibacterium prausnitzii, Very Low	↓	Consistency, Abnormal	
Lachnospiraceae, Very Low	↓	*Candida albicans*, Cultured	
Lactobacillus ruminis & Pediococcus acidilactici, Very High	↑	*Candida lusitaniae*, Cultured	
Phascolarctobacterium spp., Very High	↑	*Rhodotorula mucilaginosa*, Cultured	
Veillonella spp., Very Low	↓	Lactoferrin, Very High	
Actinobacteria, Low	↓	Lysozyme, Very High	
Bifidobacterium spp., Low	↓	pH, Very Low	

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Microbiome Bacterial Abundance;Multiplex PCR

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LEGEND

-3	-2	-1	0	+1	+2	+3
Very Low		Low	Within Reference Interval	High	Very High	

Results are graphed as deviations from a normobiotic population. Normobiosis or a normobiotic state characterizes a composition of the microbiota profile in which microorganisms with potential health benefits predominate in abundance and diversity over potentially harmful ones.

Actinobacteria	Result	Reference Interval
Actinobacteria	-1	0
Actinomycetales	+2	0
Bifidobacterium spp.	-1	0

Bacteriodetes	Result	Reference Interval
Alistipes spp.	0	0
Alistipes onderdonkii	0	0
Bacteroides fragilis	0	0
Bacteroides spp. & *Prevotella* spp.	0	0
Bacteroides spp.	+1	0
Bacteroides pectinophilus	-1	0
Bacteroides stercoris	0	0
Bacteroides zoogleoformans	-1	0
Parabacteroides johnsonii	0	0
Parabacteroides spp.	0	0

Firmicutes	Result	Reference Interval
Firmicutes	0	0
Bacilli Class	0	0
Catenibacterium mitsuokai	0	0

Notes:
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Methodology: Multiplex PCR

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Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Clostridia Class	-2		0
Clostridium methylpentosum	-1		0
Clostridium L2-50	0		0
Coprobacillus cateniformis	0		0
Dialister invisus	0		0
Dialister invisus & Megasphaera micronuciformis	0		0
Dorea spp.	0		0
Eubacterium biforme	0		0
Eubacterium hallii	0		0
Eubacterium rectale	0		0
Eubacterium siraeum	0		0
Faecalibacterium prausnitzii	-2		0
Lachnospiraceae	-2		0
Lactobacillus ruminis & Pediococcus acidilactici	+2		0
Lactobacillus spp.	0		0
Phascolarctobacterium spp.	+3		0
Ruminococcus albus & R. bromii	0		0
Ruminococcus gnavus	+1		0
Streptococcus agalactiae & Eubacterium rectale	-1		0
Streptococcus salivarius ssp. *thermophilus & S. sanguinis*	0		0

Notes:
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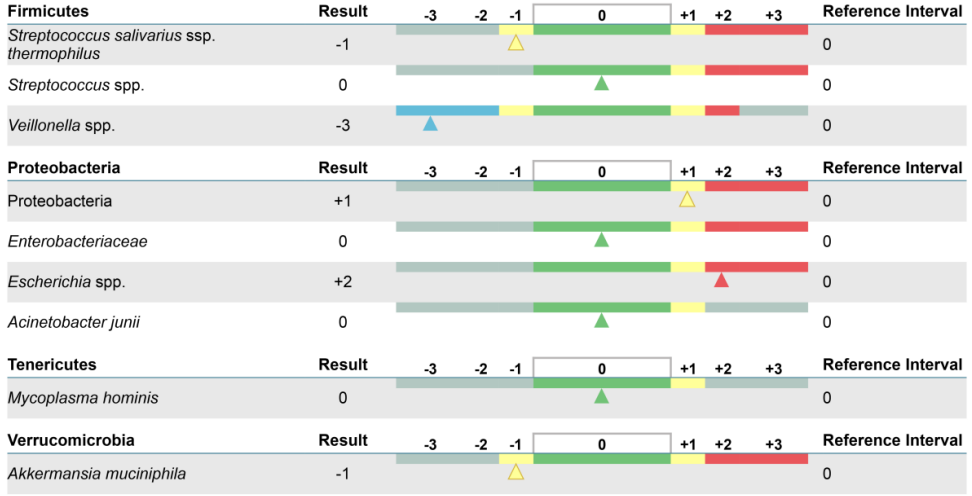
Firmicutes	Result	Reference Interval
Streptococcus salivarius ssp. *thermophilus*	-1	0
Streptococcus spp.	0	0
Veillonella spp.	-3	0

Proteobacteria	Result	Reference Interval
Proteobacteria	+1	0
Enterobacteriaceae	0	0
Escherichia spp.	+2	0
Acinetobacter junii	0	0

Tenericutes	Result	Reference Interval
Mycoplasma hominis	0	0

Verrucomicrobia	Result	Reference Interval
Akkermansia muciniphila	-1	0



Microbiome Abundance Information:

- The GI360™ Microbiome Profile is a focused gut microbiota DNA analysis tool that identifies more than 45 targeted analytes across six phyla using a CE-marked multiplex PCR system. Patient results are compared to a highly defined normobiotic reference population (n > 1,100). The white shadowed web plot within the hexagonal diagram illustrates the degree to which an individual's microbiome profile deviates from normobiosis. The center of the diagram represents less bacterial abundance while the outer edges represent greater than normobiosis. Deviation from a hexagon-shaped plot indicates variant diversity of the microbial community. Key findings for patient's microbiome profile are summarized in the table below the diagram, and detailed results for all of the analytes are presented on the next 3 pages of the report. Detailed results for the specific bacteria are reported as -3 to +3 standard deviations, as compared to the normobiotic reference population.

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Methodology: Multiplex PCR

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GI Pathogens;Multiplex PCR

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Viruses	Result
Adenovirus F40/41	Negative
Norovirus GI/GII	Negative
Rotavirus A	Negative

Pathogenic Bacteria	Result
Campylobacter (*C. jejuni*, *C. coli* and *C. lari*)	Negative
Clostridioides difficile (Toxin A/B)	Negative
Escherichia coli O157	Negative
Enterotoxigenic *Escherichia coli* (ETEC) lt/st	Negative
Salmonella spp.	Negative
Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2	Negative
Shigella (*S. boydii*, *S. sonnei*, *S. flexneri* & *S. dysenteriae*)	Negative
Vibrio cholerae	Negative

Parasites	Result
Cryptosporidium (*C. parvum* and *C. hominis*)	Negative
Entamoeba histolytica	Negative
Giardia duodenalis (AKA *intestinalis* & *lamblia*)	Negative

Notes:
Methodology: Multiplex PCR

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Parasitology; Microscopy

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Protozoa	Result
Balantidium coli	Not Detected
Blastocystis spp.	Few
Chilomastix mesnili	Not Detected
Dientamoeba fragilis	Rare
Endolimax nana	Not Detected
Entamoeba coli	Not Detected
Entamoeba hartmanni	Not Detected
Entamoeba histolytica/Entamoeba dispar	Not Detected
Entamoeba polecki	Not Detected
Enteromonas hominis	Not Detected
Giardia duodenalis	Not Detected
Iodamoeba bütschlii	Not Detected
Isospora belli	Not Detected
Pentatrichomonas hominis	Not Detected
Retortamonas intestinalis	Not Detected

Cestodes - Tapeworms	Result
Diphyllobothrium latum	Not Detected
Dipylidium caninum	Not Detected
Hymenolepis diminuta	Not Detected
Hymenolepis nana	Not Detected
Taenia	Not Detected

Trematodes - Flukes	Result
Clonorchis sinensis	Not Detected
Fasciola hepatica/Fasciolopsis buski	Not Detected
Heterophyes heterophyes	Not Detected
Paragonimus westermani	Not Detected

Nematodes - Roundworms	Result
Ascaris lumbricoides	Not Detected

Notes:
Methodology: Microscopy

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Parasitology; Microscopy

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Nematodes - Roundworms	Result
Capillaria hepatica	Not Detected
Capillaria philippinensis	Not Detected
Enterobius vermicularis	Not Detected
Hookworm	Not Detected
Strongyloides stercoralis	Not Detected
Trichuris trichiura	Not Detected

Other Markers	Result	Reference Interval
Yeast	Not Detected	Not Detected – Rare
RBC	Rare	Not Detected – Rare
WBC	Many	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Not Detected	Not Detected – Few
Charcot-Leyden Crystals	Not Detected	Not Detected
Pollen	Not Detected	Not Detected

Macroscopic Appearance	Result	Reference Interval
Color	Brown	Brown
Consistency	Loose	Soft
Mucus	Negative	Negative

Parasitology Information:

- This test is not designed to detect *Cyclospora cayetanensis* or *Microsproridia* spp.
- Intestinal parasites are abnormal inhabitants of the gastrointestinal tract that have the potential to cause damage to their host. The presence of any parasite within the intestine generally confirms that the patient has acquired the organism through fecal-oral contamination. Damage to the host includes parasitic burden, migration, blockage and pressure. Immunologic inflammation, hypersensitivity reactions and cytotoxicity also play a large role in the morbidity of these diseases. The infective dose often relates to severity of the disease and repeat encounters can be additive.
- There are two main classes of intestinal parasites, they include protozoa and helminths. The protozoa typically have two stages; the trophozoite stage that is the metabolically active, invasive stage and the cyst stage, which is the vegetative inactive form resistant to unfavorable environmental conditions outside the human host. Helminths are large, multicellular organisms. Like protozoa, helminths can be either free-living or parasitic in nature. In their adult form, helminths cannot multiply in humans.

Notes:
Methodology: Microscopy, Macroscopic Observation

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Microbiology

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Pathogenic Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Aeromonas	NG		No Growth
Edwardsiella tarda	NG		No Growth
Plesiomonas shigelloides	NG		No Growth
Salmonella	NG		No Growth
Shigella spp.	NG		No Growth
Vibrio cholerae	NG		No Growth
Vibrio	NG		No Growth
Yersinia	NG		No Growth

Imbalance Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Klebsiella pneumoniae	1+		No Growth

Yeast	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Candida albicans	2+		0+ – 1+
Candida lusitaniae	2+		0+ – 1+
Rhodotorula mucilaginosa	1+		0+ – 1+

Microbiology Information:

- **Pathogenic bacteria** consist of known pathogenic bacteria that can cause disease in the GI tract. They are present due to the consumption of contaminated food or water, exposure to animals, fish, or amphibians known to harbor the organism. These organisms can be detected by either Multiplex PCR or microbiology culture.
- **Imbalanced bacteria** are usually neither pathogenic nor beneficial to the host GI tract. Imbalances can occur when there are insufficient levels of beneficial bacteria and increased levels of commensal bacteria. Certain commensal bacteria are reported as dysbiotic at higher levels.
- **Yeast** may normally be present in small quantities on the skin, in the mouth and intestine. While small quantities of yeast may be normal, yeast observed in higher quantities is considered abnormal.

Notes:
NG = No Growth
Methodology: Culture and identification by MALDI-TOF and conventional biochemicals

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Stool Chemistries

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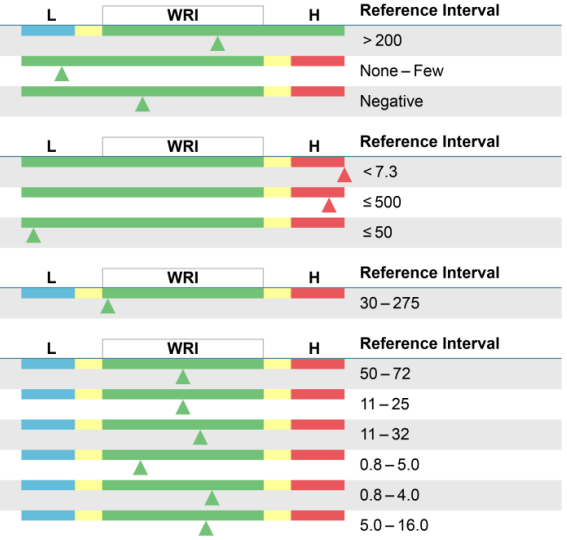
Digestion / Absorption	Result	Unit	Reference Interval
Elastase	343	µg/mL	> 200
Fat Stain	None		None – Few
Carbohydrates†	Negative		Negative

Inflammation	Result	Unit	Reference Interval
Lactoferrin	124	µg/mL	< 7.3
Lysozyme*	814	ng/mL	≤ 500
Calprotectin	12	µg/g	≤ 50

Immunology	Result	Unit	Reference Interval
Secretory IgA*	38.6	mg/dL	30 – 275

Short Chain Fatty Acids	Result	Unit	Reference Interval
% Acetate‡	60		50 – 72
% Propionate‡	17		11 – 25
% Butyrate‡	21		11 – 32
% Valerate‡	1.7		0.8 – 5.0
Butyrate‡	2.4	mg/mL	0.8 – 4.0
Total SCFA's‡	11	mg/mL	5.0 – 16.0

Intestinal Health Markers	Result	Unit	Reference Interval
pH	5.4		5.8 – 7.0
β-glucuronidase*	547	U/L	100 – 1200
Occult Blood	Positive		Negative



Chemistry Information:

- **Elastase** findings can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency. Correlations between low levels and chronic pancreatitis and cancer have been reported.

Notes:
RI= Reference Interval, L (blue)= Low (below RI), WRI (green)= Within RI (optimal), WRI (yellow)= Within RI (not optimal), H (red)= High (above RI)
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†This test has been modified from the manufacturer's instructions and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements.
‡This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use.
Methodology: Elisa, Microscopy, Colormetric, Gas Chromotography, ph Electrode, Guaiac

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Stool Chemistries

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Chemistry Information:

- **Fat Stain:** Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea.
- **Carbohydrates:** The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.
- **Lactoferrin** and **Calprotectin** are reliable markers for differentiating organic inflammation (IBD) from function symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse.
- **Lysozyme** is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients.
- **Secretory IgA** (sIgA) is secreted by mucosal tissue and represents the first line of defense of the GI mucosa and is central to the normal function of the GI tract as an immune barrier. Elevated levels of sIgA have been associated with an upregulated immune response.
- **Short chain fatty acids (SCFAs):** SCFAs are the end product of the bacterial fermentation process of dietary fiber by beneficial flora in the gut and play an important role in the health of the GI as well as protecting against intestinal dysbiosis. Lactobacilli and bifidobacteria produce large amounts of short chain fatty acids, which decrease the pH of the intestines and therefore make the environment unsuitable for pathogens, including bacteria and yeast. Studies have shown that SCFAs have numerous implications in maintaining gut physiology. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation. Levels of **Butyrate** and **Total SCFA** in mg/mL are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.
- **pH:** Fecal pH is largely dependent on the fermentation of fiber by the beneficial flora of the gut.
- **Occult blood:** A positive occult blood indicates the presence of free hemoglobin found in the stool, which is released when red blood cells are lysed.
- **β-glucuronidase** is an enzyme that breaks the tight bond between glucuronic acid and toxins in the intestines. The binding of toxins in the gut is protective by way of blocking their absorption and facilitating excretion.

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Yeast Susceptibilities

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Candida albicans

Natural Agents	Low Susceptibility / High Susceptibility
Berberine*	
Caprylic Acid*	
Uva Ursi*	
Plant Tannins*	
Oregano*	
Undecylenic Acid*	
Grapefruit Seed Extract*	

Non-Absorbed Antifungals	Low Susceptibility / High Susceptibility
Nystatin	

Azole Antifungals	Resistant	S-DD	Susceptible
Fluconazole			●
Itraconazole			●
Ketoconazole			●

Susceptibility Information:

- **Natural antifungal** agents may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative activity is reported for each natural agent based upon the diameter of the zone of inhibition or no growth zone surrounding the disk. Data based on over 5000 individual observations were used to relate the zone size to the activity level of the agent. A scale of relative activity is defined for the natural agents tested.
- **Non-absorbed antifungals** may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed using standardized commercially prepared disks impregnated with Nystatin. Relative activity is reported based upon the diameter of the zone of inhibition or no growth zone surrounding the disk.
- **Susceptible** results imply that an infection due to the fungus may be appropriately treated when the recommended dosage of the tested antifungal agent is used. **Susceptible - Dose Dependent (S-DD)** results imply that an infection due to the fungus may be treated when the highest recommended dosage of the tested antifungal agent is used. **Resistant** results imply that the fungus will not be inhibited by normal dosage levels of the tested antifungal agent.

Notes:
*This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U. S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use. The results are not intended to be used as a sole means for clinical diagnosis or patient management decisions.

Analyzed by DOCTOR'S DATA, INC. • 3755 Illinois Avenue, St. Charles, IL 60174-2420 USA • LAB DIR: Erlo Roth, MD • CLIA ID: 14D0646470

Yeast Susceptibilities

Order: SAMPLE REPORT
Client #: 12345
Doctor: Sample Doctor
Doctor's Data, Inc.
3755 Illinois Ave.
St. Charles, IL 60174

Patient: Sample Patient
Age: 44
Sex: Male

Sample Collection	Date/Time
Date Collected	08/31/2020
Date Received	09/01/2020
Date Reported	09/02/2020
Specimens Collected	3

Candida lusitaniae

Natural Agents	Low Susceptibility / High Susceptibility
Berberine*	
Caprylic Acid*	
Uva Ursi*	
Plant Tannins*	
Oregano*	
Undecylenic Acid*	
Grapefruit Seed Extract*	

Non-Absorbed Antifungals	Low Susceptibility / High Susceptibility
Nystatin	

Azole Antifungals	Resistant	S-DD	Susceptible
Fluconazole			●
Itraconazole			●
Ketoconazole			●

Susceptibility Information:

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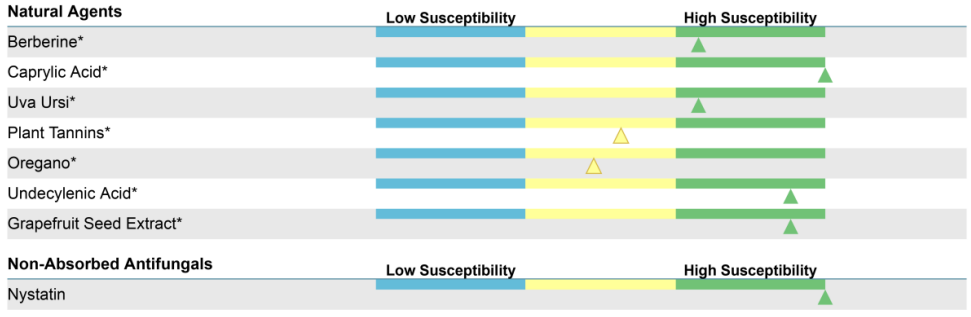
Rhodotorula mucilaginosa



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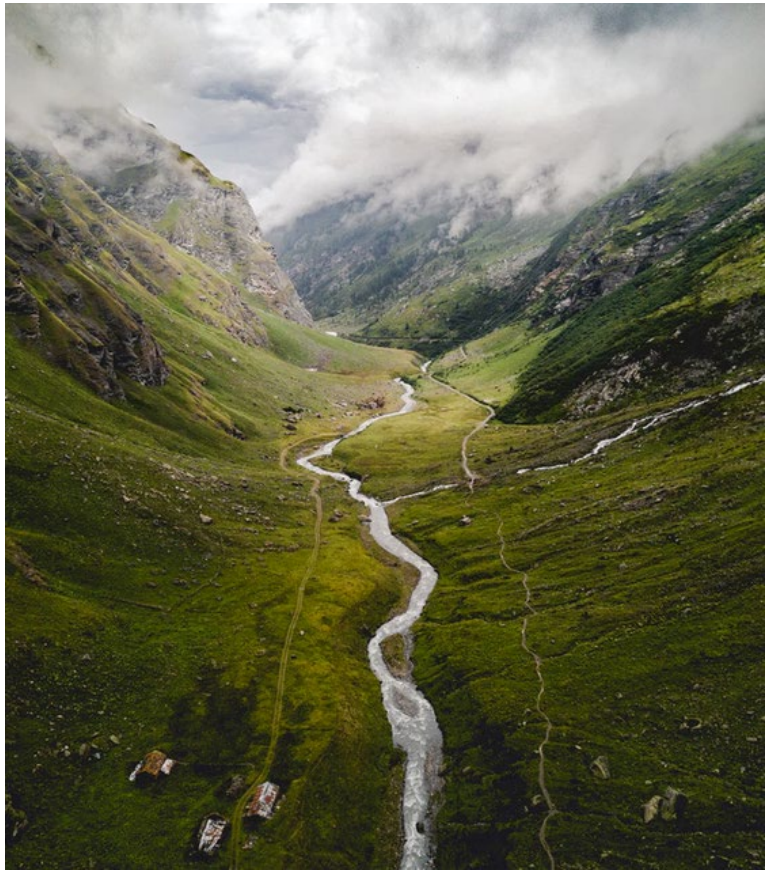
SCIENCE+INSIGHT

Melatonin and Gut Health



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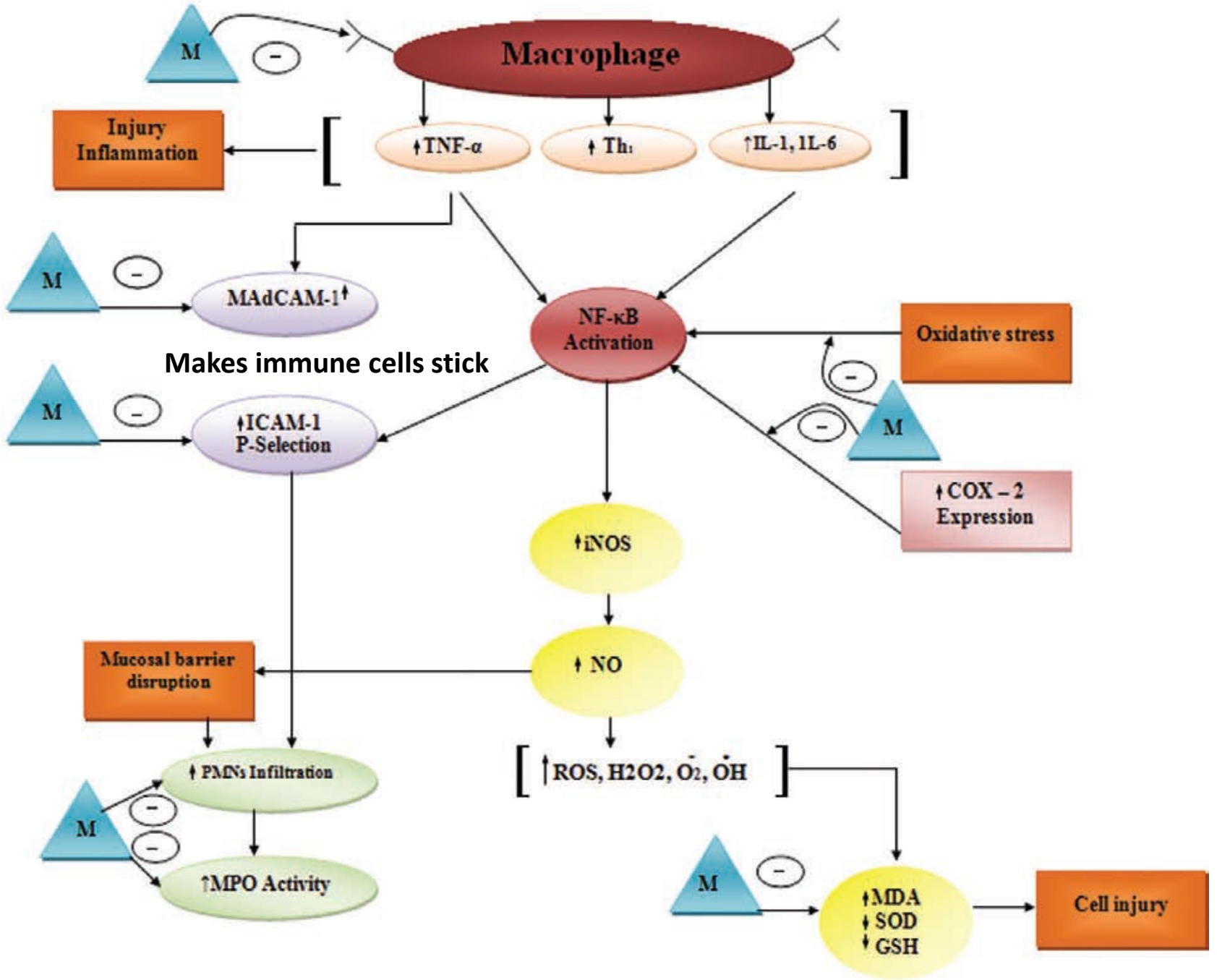
SCIENCE + INSIGHT

Melatonin, a promising supplement in inflammatory bowel disease: a comprehensive review of evidences.

Curr Pharm Des. 2011 Dec;17(38):4372-8.

- Inflammation and oxidative process are associated with inflammatory bowel disease (IBD). Regarding anti-inflammatory and antioxidant potentials, **melatonin has been found beneficial in several experimental and clinical studies including inflammatory bowel disease (IBD).**

- **The majority of these studies indicate that melatonin has a positive impact on IBD with no or negligible side effects.** Such results have been mostly explained through free radical scavenging and diminishing inflammation.



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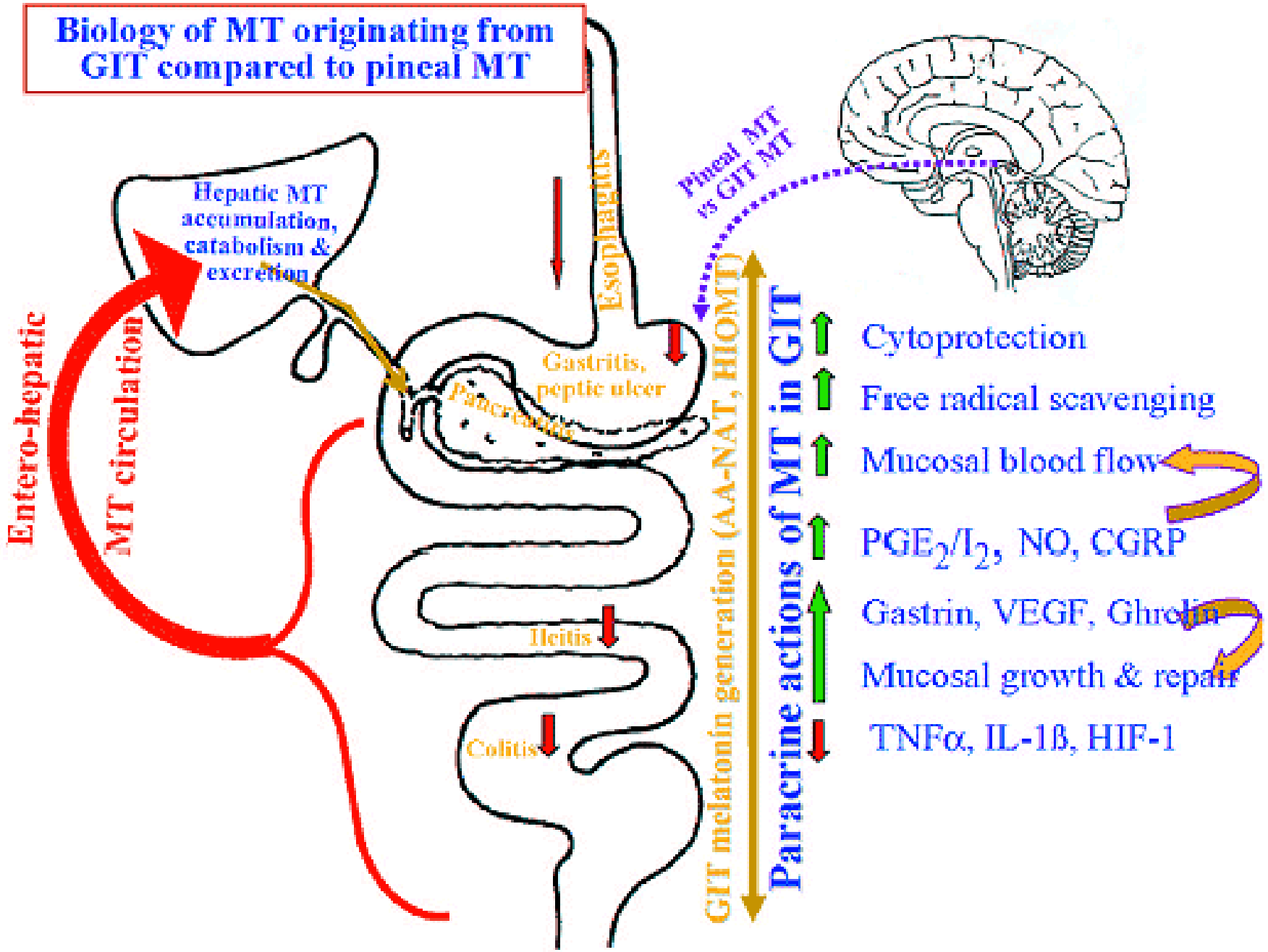
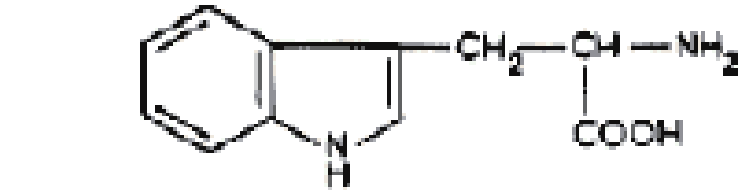
Enteric melatonin

- The extent of melatonin secreted from GI ENS is **400 times higher than that of pineal gland** in the CNS suggesting an important role of melatonin in the GI tract. Melatonin is known to be beneficial in GI disorders (esophagitis, gastritis, peptic ulcer, and pancreatitis), regulation of fecal water content, IBS, and IBD due to its potent anti-oxidant, immunoregulating, anti-inflammatory and enteroprotective (antioxidant) properties,

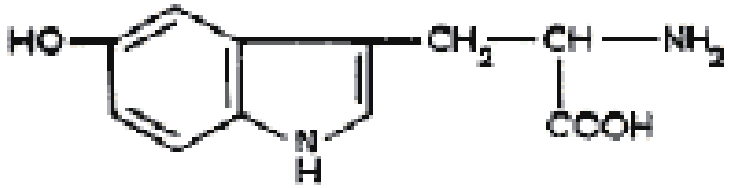
Biology of MT originating from GIT compared to pineal MT





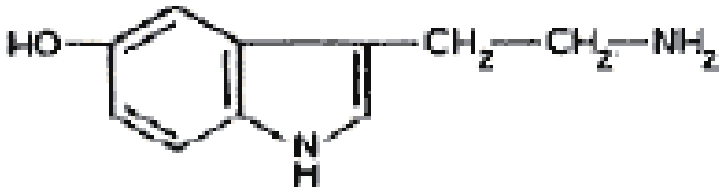
L-Tryptophan

Tryptophan 5-hydroxylase



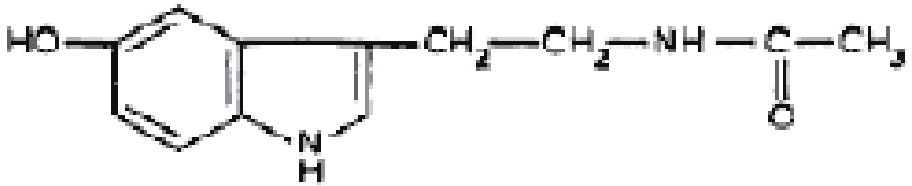
5-hydroxytryptophan

Aromatic L-amino acid decarboxylase



Serotonin

Serotonin N-acetyltransferase (NAT)



N-acetylserotonin

Hydroxyindol--O-methyl transferase (HIOMT)

Melatonin

Melatonin and Methylation

- **Methylation problems (genetic or acquired) leads to lack of melatonin**

Champier J et al, **Folate depletion changes gene expression of fatty acid metabolism, DNA synthesis, and circadian cycle in male mice.** Nutr Res. 2012

Folate deficiency alters the secretion of melatonin, a hormone involved in circadian rhythm entrainment, and causes hyperhomocysteinemia because of disruption of homocysteine metabolism. Adverse effects of homocysteine include the generation of free radicals, activation of proliferation or apoptosis, and alteration of gene expression. **This study shows that, in the mouse liver, dietary folate depletion leads to major changes in expression of several genes involved in fatty acid metabolism, DNA synthesis, and expression of circadian genes.**

IBD induces methylation deficit

Oussalah A, **Meta-analysis: hyperhomocysteinaemia in inflammatory bowel diseases.** Aliment Pharmacol Ther. 2011

- Twenty-eight studies evaluated the plasma homocysteine level and/or hyperhomocysteinaemia risk in IBD patients. Five studies assessed the association of hyperhomocysteinaemia with thrombosis. **The mean plasma homocysteine level was significantly higher in IBD patients when compared with controls (weighted mean difference (WMD)=3.75** µmol/L. The mean plasma homocysteine level did not differ between ulcerative colitis (UC) and Crohn's disease (CD). **The risk of hyperhomocysteinaemia [>12] was significantly higher in IBD patients when compared with controls [odds ratio (OR)=4.65].** Plasma folate level was inversely correlated with IBD risk associated with MTHFR C677T polymorphism (P=0.006).

- CONCLUSIONS: **The risk of hyperhomocysteinaemia is significantly higher in IBD patients when compared with controls.** The risk assessment of hyperhomocysteinaemia-related thrombosis in IBD requires further investigation. Deficient folate status is associated with a higher impact of MTHFR C677T polymorphism on IBD risk.
- **Take Home: IBD causes acquired methylation deficit regardless of MTHFR gene status. If MTHFR +, folate deficiency may trigger IBD. MTHFR SNP's do not seem to be sufficient for IBD development.**

Bondarenko LA. **Role of methionine in nocturnal melatonin peak in the pineal gland.** Bull Exp Biol Med. 2004

- Methionine dose-dependently stimulated O-methylation of hydroxyindoles in the pineal gland and contributed to the nocturnal melatonin peak in adult male Wistar rats. Methionine is involved in the maintenance of diurnal rhythms by regulating biochemical transformations of indoles in pinealocytes.

- **Take home: Methylation improves melatonin synthesis**

Melatonin and LG

Sun X et al., **Melatonin reduces bacterial translocation by preventing damage to the intestinal mucosa in an experimental severe acute pancreatitis rat model.**

Exp Ther Med. 2013 Dec;6(6):1343-1349.

The level of *E. coli* DNA in the melatonin (MT) group was significantly lower than in rats in the pancreatitis (SAP) group. No *E. coli* DNA was detected in the control group. **Villus height and crypt depth in the ileum were significantly higher in the MT and control groups compared to the SAP group, and were significantly higher in the MT group than in the SAP group.** These results suggested that melatonin prevented gut barrier dysfunction and reduced bacterial translocation, resulting in reduced pancreatic-associated infections and decreased early mortality rates.

Sommansson A et al, **Melatonin inhibits alcohol-induced increases in duodenal mucosal permeability in rats in vivo.** Am J Physiol Gastrointest Liver Physiol. 2013 Jul

We recently found that melatonin decreases basal duodenal mucosal permeability, suggesting a mucosal protective mode of action of this agent. Perfusing the duodenal segment with ethanol, red wine, or HCl induced concentration-dependent increases in paracellular permeability. Luminal ethanol and wine increased, whereas **HCl transiently decreased duodenal motility.**

Administration of melatonin significantly reduced ethanol- and wine-induced increases in permeability…

These results suggest that melatonin may serve important gastrointestinal barrier functions.

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SCIENCE+INSIGHT

Celinski K **Effects of melatonin and tryptophan on healing of gastric and duodenal ulcers with Helicobacter pylori infection in humans.** J Physiol Pharmacol. 2011

- Melatonin (MT) and its precursor L-tryptophan (TRP) are implicated in the protection of gastric mucosa against aspirin-induced lesions and in the acceleration of healing of idiopathic gastro-duodenal ulcers.
- Treatment with omeprazole 20 mg twice daily combined with placebo (group A), MT administered in a dose of 5 mg twice daily (group B) or TRP applied in a dose of 250 mg twice daily (group C). **At day 21, all ulcers were healed in patients of groups B and C but only 3 out of 7 in group A of gastric ulcers and 3 out of 7 in duodenal ulcers.**
- **Take home – melatonin protects mucosa by direct antioxidant protection as well as increasing leptin**

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Pancreatic Insufficiency

- **Signs and Symptoms:**
  - Bloating after meals – a sense that food just “sits” in stomach
  - Abdominal discomfort - especially 15 minutes or more after eating
  - Flatulence
  - **Glucose intolerance (exocrine and endocrine function mirror each other)**
  - Decreased absorption of nutrients (fats, proteins, carbohydrates)
  - Edema (hypoalbuminemia)
  - Bleeding tendency (vitamin K deficiency)
  - Weight loss
  - Failure to thrive in children
  - Azotorrhea
  - Steatorrhea; pale, foul-smelling, or bulky stool

Pancreatic insufficiency (exocrine)

Causes:

- Impaired Production
  - Pancreatic Cancer
  - Insulin resistance
  - Smoking
  - Crohn's
  - IBD
  - Dysbiosis/Infection (giardia, h. pylori, SIBO)
  - Food allergy/sensitivity
  - Gastritis/enteritis
- Impaired Secretion
  - Hepatobiliary or pancreatic obstruction
  - Sympathetic overactivation causing sphincter malfunction
  - Small intestine obstruction
  - Lymphoma

Pancreatic insufficiency (exocrine)

- Potential Laboratory/Diagnostic Tests
  - Serum albumin low
  - Serum amylase elevated
  - Pancreatic elastase - stool
  - Chymotrypsin – stool or serum
  - Plain film abdominal X-ray – pancreatic calcification
  - Ultrasound – will exclude gallstone or a dilated biliary system
  - Endoscopic retrograde cholangiopancreatography

DIGESTION /ABSORPTION

	Within	Outside	Reference Range
Elastase	> 500		> 200 µg/mL
Fat Stain	Few		None - Mod
Muscle fibers	None		None - Rare
Vegetable fibers	Rare		None - Few
Carbohydrates	Neg		Neg

Elastase findings can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency. Correlations between low levels and chronic pancreatitis and cancer have been reported. **Fat Stain:** Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea. **Muscle fibers** in the stool are an indicator of incomplete digestion. Bloating, flatulence, feelings of "fullness" may be associated with increase in muscle fibers. **Vegetable fibers** in the stool may be indicative of inadequate chewing, or eating "on the run". **Carbohydrates:** The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.

Pancreatic insufficiency treatment

- Lifestyle – as with all digestive issues, stress must be addressed (RR) and exercise program initiated, breathing etc.
- Supplements
  - Pancreatin
  - Chymotrypsin
  - HCL
  - Ox bile
  - Bromelain
  - Papain
  - Glutamine
  - NAC


Food is medicine

You are what you eat (farm raised fish):

Kortner TM et al, **Dietary soyasaponin supplementation to pea protein concentrate reveals nutrigenomic interactions underlying enteropathy in Atlantic salmon (Salmo salar)**

BMC Vet Res. 2012

- Use of plant ingredients in aquaculture feeds is impeded by high contents of antinutritional factors such as saponins, which may cause various pharmacological and biological effects. In this study, transcriptome changes were analyzed using a 21 k oligonucleotide microarray and qPCR in the distal intestine of Atlantic salmon fed diets based on five plant protein sources combined with soybean saponins.

- **RESULTS:** Diets with corn gluten, sunflower, rapeseed or horsebean produced minor effects while the combination of saponins with pea protein concentrate caused enteritis and major transcriptome changes. **Acute inflammation was characterised by up-regulation of cytokines, NFkB and TNF alpha related genes and regulators of T-cell function, while the IFN-axis was suppressed. Marked down-regulation of xenobiotic metabolism was also observed, possibly increasing vulnerability of the intestinal tissue.** A hallmark of metabolic changes was dramatic down-regulation of lipid, bile and steroid metabolism. Impairment of digestion was further suggested by expression changes of nutrient transporters and regulators of water balance (e.g. aquaporin, guanylin). Furthermore, augmented synthesis of polyamines needed for cellular proliferation (up-regulation of arginase and ornithine decarboxylase) and increased mucus production (down-regulation of glycan turnover and goblet cell hyperplasia) was elevated.

Inspiration

If you are not inspired to live healthy, in every way possible, then how can you BE an inspiration to those you are trying to serve?

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Dr. Lundell's Educational Opportunities

- **Functional Medicine Series**
  - **6, 12 to 15 hour classes**
  - **Complete all 6, take exam, submit case study and receive Certification**
    1. **Blood Chemistry, Inflammation, Methylation**
    2. **Autoimmune Triad (Epigenetics, Leaky Gut, Environmental)**
    3. **Functional Gastroenterology**
    4. **Functional Endocrinology: Adrenal, Thyroid (Testing and Treatment)**
    5. **Stress Hormones, Sex Hormones from Womb to Tomb.**
    6. **Case Studies, Practical Application**
- **www.drbrandonlundell.com**
- **hhc@drbrandonlundell.com (email us for a $500 off code!)**

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Functional Medicine – Nutritional Pathology Certification

- Pathology has been defined as that branch of medicine which treats the essential nature and primary causative factors of disease.

- Nutritional Pathology therefore, is the study of the cellular basis for ill health - the true causes being primarily nutritional aberrations, environmental inputs and lifestyle factors. Lifestyle and nutritional means to restore proper function must be employed to establish and maintain essential health throughout the lifespan.

- **6-Module Course with Lab Manual, FM Resource Kit, FM coaching**

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Nutritional Pathology Certification

- **MODULE 1 (Nutritional Pathology Introduction and Essential Principles)**

  Interpretation of Blood Chemistry, Case Studies, Infections, FM lab tests, Clinical Pearls, Insulin Resistance, Inflammation, Toxins, Methylation, SNP testing, Completing the FM Therapy Approach. Bonus: B12 Deficiency Epidemic, Inflammation from womb to tomb. Anti-inflammatory diet. Methylation Made Simple. You will walk away with information on how to immediately implement NP into your practice and begin helping people right away.

- **MODULE 2 (Immunology, Autoimmune Triad)**

  Introduction and Overview of the Autoimmune Triad, Immunology 101, Symptoms, Labs, Inflammation Drives Autoimmune, Holistic Approach to Autoimmune, TH1/TH2 System, Clinical Pearls, Advanced Labs for Root Causes, Case Study – Genetics and Methylation, Epigenetics, Natural Support, Kidney Support, Environmental Pollutants, Zonulin, Gut and Autoimmune: The New Frontier, Dysbiosis Protocols, Infections, Case Study Putting it all Together.

- **MODULE 3 (Functional Gastroenterology from A-Z)**

  Infections, C. Diff, H. Pylori etc.. IBS/IBD mechanisms, Gut-Brain Research, Dysbiosis/SIBO, Effective Strategies for Leaky Gut, Neuroendocrine Control of GE, Gastritis, Ulcers, Gallbladder, Low HCL, Pancreatic Insufficiency, Food Testing, Stress Effects on Gut, Vagal Nerve Stimulation, Which Diet and Probiotics Work. ***Bonus****: Leaky gut protocols, Food Sensitivity Testing – do's and don'ts.*

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Nutritional Pathology Certification

- **MODULE 4 (Endocrinology I)**

Introduction and Principles to Endocrinology, Neuro-endocrine Basics, Receptors, Parathyroid, Osteoporosis, EPO, H-P-A Axis, Adrenals in Depth, Adrenal Testing and Treatment, Neurotransmitters, Mental Health, Thyroid physiology, Why the Thyroid Epidemic, Thyroid Clinical Pearls, Thyroid Treatments that work, Case Study.

- **MODULE 5 (Endocrinology II, Detoxification)**

Male Hormones, Prostate, Low T, Female Hormones, Estrogen, Metabolism, PCOS, Endometriosis, Fertility Support, Developmental Origins of Adult Diseases, Preconception, Prenatal Support, , Why Are Our Kids' Brains and Bodies So Messed Up?, Neuro-Endocrine Disruptors, Toxins in Air, Food, Water, Invisible Enemy – Detecting Hidden Sources of Toxins with Labs, Living a Toxin-Less Life, Supporting Detox – all phases.

- **MODULE 6 (Cardio-Vascular, Metabolism/Energy Regulation, Case Studies)**

Cardiovascular support, Arrythmias, ATP regulation, Ribose, Congestive Heart Failure, Hypertension, Chronic Fatigue Syndrome/Fibromyalgia, *Neuroendocrinology, Metabolic Syndrome, Insulin Resistance, Metabolism, Effects of Chronic Stress: Autoimmune and Insulin Resistance, Clinical Pearls and Case Studies. Lyme Disease Case Study, Hyperbaric Oxygen Therapy, Immune Testing*

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Nutritional Pathology Certification

- Each Module is $475 and each can be taken by itself. However, it is recommended that if you are completing the entire course, or are relatively new to Functional Medicine, that you take each course sequentially.

- If you purchase the **Entire Course** all at once, you will also receive the Functional Medicine Resource Kit (see Table of Contents) as a bonus. **[$599 discount] – all for $2950.**

- Final Exam will be given after all modules have been completed (open book). Upon passing final exam, you will be given a certificate of completion in **Nutritional Pathology and Functional Medicine.**

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www.drbrandonlundell.com

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Questions

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Thank You!

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