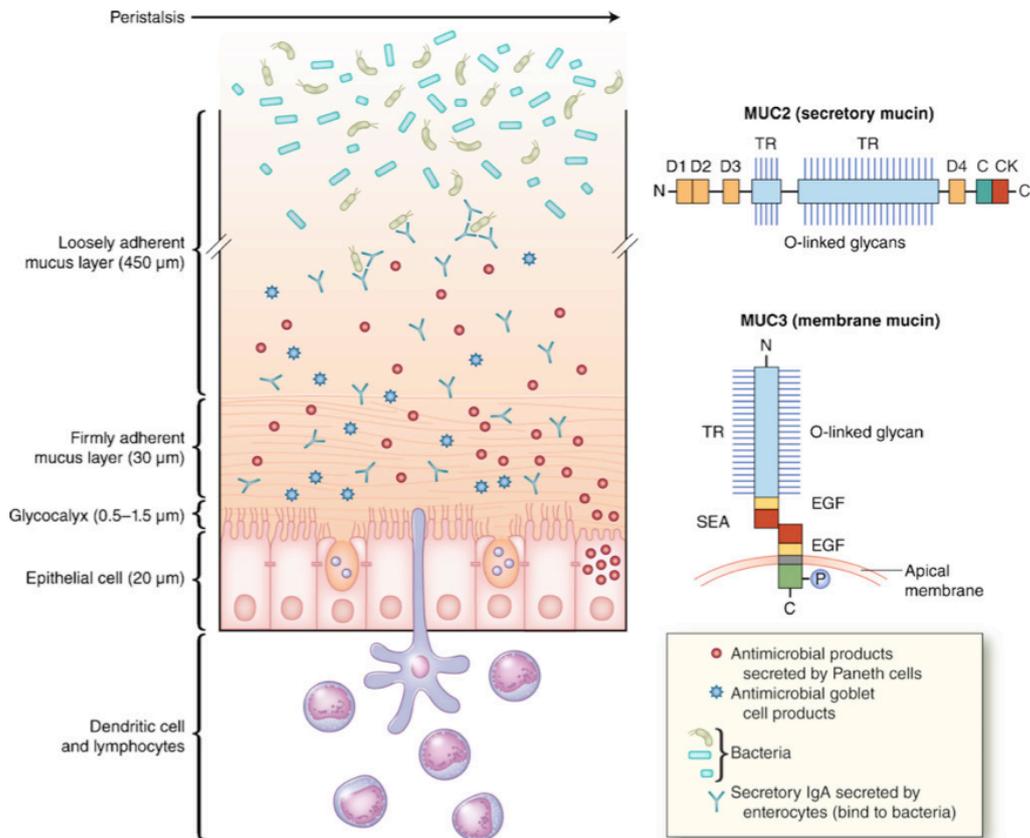


New gut signals and the microbiome

Some notes
Simon Mills 2014



Microbiome

Within the GI tract, the microbiota have a mutually beneficial relationship with their host that maintains normal mucosal immune function, epithelial barrier integrity, motility, and nutrient absorption. Disruption of this relationship alters GI function and disease susceptibility.

One universal activity of the intestinal microbiota is the break-down and fermentation of polysaccharides into short chain fatty acids (SCFAs). The predominant SCFAs produced by bacterial fermentation in the colon are acetate (C2), propionate (C3) and butyrate (C4), although the absolute and relative abundances of these vary depending on factors such as the host's diet and prevailing microbiota composition. SCFAs are rapidly taken up by the colonic mucosa, contributing up to 10% of daily calorific needs for the host, and act as important stimuli for the absorption of sodium and water by the colon.

Intestinal epithelial cells derive as much as 60–70% of their energy needs from butyrate so this SCFA in particular may have important indirect effects on intestinal barrier integrity and function by fuelling epithelial cell proliferation/repair and mucin production.

Butyrate may also reduce gut mucosal inflammation by inhibiting the activity of the transcription factor NF- κ B, therefore mediating the effects of pro-inflammatory cytokines, and inducing apoptosis and inhibition of proliferation in tumour cells. The predominant butyrate-producing bacteria in the human colon belong to the *Lachnospiraceae* and *Ruminococcaceae* families of the Firmicutes phylum, which are often depleted in dysbiosis. Acetate and propionate play a role in reducing intestinal inflammation via a G protein-coupled receptor (GPR43) expressed on neutrophils.

Propionate, a SCFA shown to induce vasodilation *ex vivo*, produces an acute hypotensive response *in vitro*. Its effects appear to be mediated by Olfr78, an olfactory G protein-coupled receptor expressed in the renal juxtaglomerular apparatus.

Pluznick JL, Protzko RJ, Gevorgyan H, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci USA*. 2013; 110(11): 4410-5.

The microbiota influences host gene expression, including genes that regulate nutrient absorption, mucosal barrier enhancement, xenobiotic metabolism, and angiogenesis. Similarly, the production and composition of mucin and the development of 5-hydroxytryptamine-secreting enterochromaffin cells is influenced by the microbiota. Germ-free rodents have an enlarged caecum, reflecting a gross disturbance in GI motility; its prompt reversal to normal size on bacterial colonization identifies the microbiota as a determinant of GI motility. The abnormal motility of germ-free animals probably reflects a combination of the lack of a mature enteroendocrine system, changes in neurotransmission, and immaturity of the mucosal immune system.

A number of studies have shown that the gut flora is especially efficient in harvesting energy from the diet in obese subjects, ultimately leading to a greater distribution of fatty acids to tissues, including the liver.

Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; 482(7384): 179-85.

Germ-free mice produce higher levels of adrenocorticotrophin hormone and corticosterone levels in response to stress, when compared to mice bearing a conventional microbiota. This effect was correlated with reduced levels of brain-derived neurotrophic factor (BDNF).

Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 2004; 558: 263-275

Antibiotic induced perturbation of the microbiota in mice produced a response profile in the gut wall reminiscent of changes seen in some patients with irritable bowel syndrome (IBS): subclinical inflammation or immune activation and visceral hyperalgesia (linked with raised levels of substance P). These changes were reversed when the mice were gavaged with *Lactobacillus*.

Verdu EF, Bercik P, Verma-Gandhu M, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006; 55:182-190

The results of one study indicate that reducing the indigenous microbiota (by antibiotics) blocks the stressor-induced increase in circulating IL-6 and iNOS mRNA in the spleen.

Bailey MT, Dowd SE, Galley JD et al. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation *Brain, Behavior, and Immunity* 2011; 25: 397-407

Abdominal pain is common in the general population and, in patients with irritable bowel syndrome, is attributed to visceral hypersensitivity. Researchers found that oral administration of specific *Lactobacillus* strains induced the expression of mu-opioid and cannabinoid receptors in intestinal epithelial cells, and mediated analgesic functions in the gut-similar to the effects of morphine.

Rousseaux C, Thuru X, Gelot A, et al. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med*. 2007; 13(1): 35-7.

Lactobacillus reuteri ingestion in rats stimulates of myenteric intrinsic sensory neurons and augments enteric nervous system inhibitory tone on intestinal circular muscle. This involved an inhibition of calcium-dependent potassium channels.

Kunze WA, Mao YK, Wang B, et al. *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J Cell Mol Med*. 2009; 13(8B): 2261-70.

Feeding *Lactobacillus rhamnosus* to healthy adult mice over a period of 4 weeks, induced anxiolytic and antidepressant-like effects, and alterations in the cognitive and emotional abilities towards aversive stimuli. In addition, this *Lactobacillus* treatment prevented an exaggerated HPA axis response after a stressful stimulus, and induced vagus-dependent changes in various GABA receptors in the prefrontal cortex, amygdala, locus coeruleus, and hippocampus.

Bravo JA, Forsythe P, Chew MV, et al. Ingestion of lactobacillus strain regulates emotional behavior and central gaba receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA* 2011; 108: 16050-16055.

Gut-brain axis and stress

Stress induces modifications of motility, secretion, visceral sensitivity, intestinal permeability, and local inflammatory responses in the gastrointestinal (GI) tract. Stress is involved in the initiation and relapse of experimental colitis.

Mast cells of the intestinal mucosa serve as end effectors of the brain-gut axis and release several mediators, cytokines, and chemokines that can profoundly affect GI physiology under stress conditions by inducing intestinal hyperpermeability and activation of mucosal immune function.

Stress decreases vagal efferent activity and increases sympathetic outflow and adrenomedullary activity, leading to increased norepinephrine and epinephrine levels, inhibiting immune cell functions and promoting intestinal inflammation.

Vagal activity has a counteractive anti-inflammatory role. Pro-inflammatory cytokines (eg IL-1, IL-6, and TNF α) released from the intestinal mucosa activate vagal responses that in turn activate the HPA axis, decrease production of proinflammatory cytokines, attenuate the systemic inflammatory response to endotoxin and intestinal inflammation, and modulate immune activity in the spleen.

Heart rate variability, a non-invasive electrocardiographic technique commonly used to assess autonomic imbalances, confirms the link between sympathetic and vagal imbalances and human GI problems.

Bonaz BL, Bernstein CN. Brain-Gut Interactions in Inflammatory Bowel Disease. *Gastroenterology* 2013;144: 36–49

Gut-brain axis and the microbiome

There is a functional communication between the gastro- intestinal (GI) and central nervous system (CNS). This communication is bidirectional and it involves anatomical connections like the vagus nerve and humoral components including the immune system and the hypothalamus-pituitary-adrenal (HPA) axis. In addition, there is increasing evidence suggesting another key player in this interaction: the intestinal microbiota. This had led to the proposal of what is now recognized as the microbiota–gut–brain axis. Moreover, the current evidence suggests that dysregulation of this axis could be an underlying cause of a variety of functional bowel disorders including irritable bowel syndrome (IBS).

The brain can influence microbiota composition indirectly through modifications in gastrointestinal motility, secretion, and intestinal permeability, or directly, via cytokines released into the gut lumen from enterochromaffin cells, neurons and immune cells of lamina propria. Intestinal motility is considered one of the most important control systems of the intestinal microbiota, by controlling its removal. On the other hand, enteric microbiota also play a role in the development and maintenance of both sensory and motor gut functions, through communication with the brain that occurs indirectly through interaction with epithelial-cell and receptor-mediated signalling and directly through stimulation of neuronal cells in the lamina propria, when intestinal permeability is increased.

Enterochromaffin cells

Integral to all the communications above are enterochromaffin cells, which serve as bidirectional transducers that modulate interrelationship between the gastrointestinal lumen and the nervous system. Enterochromaffin cells are innervated by sensory vagus nerve fibres. Enterochromaffin or Kulchitsky cells, are a type of endocrine cell occurring in the epithelia lining the digestive and the respiratory tracts. Together with other enteroendocrine

cells such as cholecystokinin- and secretin-producing cells in the small intestine, they sense the luminal content of the gut and control the function of the stomach, the gall bladder, the pancreas, and the intestine. Enterochromaffin cells are the body's most abundant endocrine cell type and contain about 90% of the body's store of serotonin (5-HT). In response to chemical, mechanical or pathological stimuli in the lumen and for example to the hormone gastrin, 5-HT activates both secretory and peristaltic reflexes, and activates vagal afferents (via 5-HT₃ receptors) that are important for example in the generation of nausea. Gut microbiota influence on enterochromaffin cells suggest a role in the regulation of visceral pain.

Evidence supports the concept that diarrhoea- and constipation-predominant irritable bowel syndrome depends on release of serotonin by enterochromaffin cells. Recent findings indicate that olfactory receptors of the enterochromaffin cell might prove to be a relatively accessible target for treatment of IBS, of conditions such as diarrhoea with vomiting caused by anticancer therapy, and of other gastrointestinal disorders.

Phenols, aldehydes and ketones have been shown as ligands for olfactory receptors on enterochromaffin cells. Among those so far characterised are thymol and geraniol for OR1G1 and eugenol and isoeugenol for OR73

Braun T, Volland P, Kunz L et al. Enterochromaffin Cells of the Human Gut: Sensors for Spices and Odorants. *Gastroenterology* 2007;132:1890-1901

Gut wall integrity and mucus production

The major function of intestinal goblet cells and their main secretory products, mucins, is the formation of several mucus layers which serve as the front line innate host defence mechanism. In health, commensal bacteria are trapped in a loosely adherent layer, formed by a secreted mucin glycoprotein (MUC2) to be eliminated by peristaltic movement. They should fail to reach a firmly-adherent mucus layer formed from epithelial membrane-bound mucins (MUC1, MUC3, MUC17) and other components.

A defective mucosal barrier, abnormal commensal bacteria, and defective host innate and adaptive immune response can result in intestinal inflammation and injury. Defective mucus layers, from lack of or mutated MUC2 mucin, or goblet cell depletion resulting from chronic excessive secretion of mucins, lead to increased bacterial adhesion to the surface epithelium and increased intestinal permeability.

MUC2 mucin production is mediated by activation of signalling pathways targeting the transcription factors that bind to specific sites on the MUC2 promoter. There is evidence that it is a protective response in inflammatory events. Activation of a transcription factor, nuclear factor NF- κ B, is a common event during inflammation in the gastrointestinal tract and MUC2 mucin has been shown to have NF- κ B binding sites in the promoter. Inflammatory cytokines, tumor necrosis factor alpha (TNF- α), interleukins (IL-4 and IL-13), a neuropeptide hormone, vasoactive intestinal peptide (VIP), and also prostaglandin, PGE2 have all been shown to upregulate MUC2 transcription. Other upregulatory bioactive factors include microbes and their products, toxins and growth factors.

Kim YS, Ho SB Intestinal Goblet Cells and Mucins in Health and Disease: Recent Insights and Progress *Curr Gastroenterol Rep* 2010; 12: 319-330

Gut wall integrity and IgA

Intestinal epithelial cells recognize and interact with commensal bacteria with moderate mucosal immunity, including the production of secretory immunoglobulin A.

Goto Y, Kiyono H. Epithelial barrier: an interface for the cross-communication between gut flora and immune system. *Immunol Rev.* 2012; 245(1): 147-63.

The intestinal mucosa contains the largest population of antibody-secreting plasma cells in the body, and in humans several grams of secretory immunoglobulin A (SIgA) are released into the intestine each day. In the gut lumen, SIgA serves as a first-line barrier that protects the epithelium from pathogens and toxins

Pabst O. New concepts in the generation and functions of IgA. *Nat Rev Immunol.* 2012; 12(12): 821-32.

Experiments have shown that SIgA normally cooperates with innate defence factors (like mucosal induction of regulatory T cells) to protect the epithelium and reinforce its barrier function. In the absence of SIgA, commensal gut bacteria overstimulate innate epithelial immunity at the expense of expression of genes that regulate fat and carbohydrate metabolism, resulting in possible lipid malabsorption. This shows that the intestinal epithelial barrier balances defence and nutrition, and that SIgA is essential to keep the balance between these two functions.

Brandtzaeg P. Gate-keeper function of the intestinal epithelium. *Benef Microbes.* 2013; 4(1): 67-82.

Bile

Thus far, bile has been considered an antibacterial, which was mainly attributed to direct and indirect antimicrobial actions of BAs. However, some intestinal bacteria such as *Escherichia coli* and *Listeria* as well as some protozoa (eg, *Giardia*) are BA tolerant and their growth may even be favoured in the presence of BAs, which conversely can suppress other symbiotic commensals. Bacterial overgrowth owing to reduced intestinal BAs is a long known complication of cholestasis and end-stage liver disease.

Trauner M, Halilbasic E. Nuclear receptors as new perspective for the management of liver diseases. *Gastroenterology* 2011;140:1120–1125.

Gut microbiota is also able to alter BA metabolism and signalling through deconjugation and dehydroxylation, resulting in formation of secondary, more hydrophobic BAs with altered ligand properties for BA receptors. Impaired enzymatic activity in IBD-related dysbiosis may affect luminal BA composition and inflammatory modulating potential.

Apart from their detergent properties in lipid digestion, bile acids (BAs) have more recently been recognized to have additional hormonal actions that control a range of metabolic and immune functions throughout the body via dedicated BA nuclear receptors such as farnesoid X receptor (FXR) and a G-protein-coupled receptor TGR5 (GPBAR-1). In the gut FXR maintains epithelial barrier integrity by induction of multiple genes involved in intestinal mucosal defence against inflammation and microbes that help to manage gut microbiota.

Porez G, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. *J Lipid Res* 2012; 53(9): 1723-37.

Cholesterol is converted to bile acids in the liver, which are then secreted into the intestine and either removed from the body or recycled back to the liver. The global effect of the gut flora so far noted is suppression of both BA synthesis in the liver (through FXR-dependent FGF15 induction) and BA import from the ileum. The FXR receptor not only affects cholesterol metabolism but is also involved in the body's sugar and fat metabolism.

On the other hand, the presence of gut microbiota leads to a more hydrophobic BA pool that is potentially more toxic, particularly in the liver. The concept of 'toxic bile' in liver disease has received a substantial amount of experimental exploration.

Poupon R. Ursodeoxycholic acid and bile-acid mimetics as therapeutic agents for cholestatic liver diseases: an overview of their mechanisms of action. *Clin Res Hepatol Gastroenterol* 2012; 36(Suppl. 1): S3-12.

Bile may indeed become more harmful essentially in cases of abnormal bile composition and/or when bile flow is reduced, allowing more prolonged contact between bile components and liver cell membranes. As the gut microbiota appears to be a major determinant of BA pool hydrophobicity, it may strongly impact bile toxicity and thus contribute to acute and chronic liver diseases especially in the context of BA overload. It has also been reported that the pathophysiology of primary biliary cirrhosis and primary sclerosing cholangitis involves abnormal immune responses to gut microbial antigens.

Henao-Mejia J, Elinav E, Thaiss CA, Flavell RA. The intestinal microbiota in chronic liver disease. *Adv Immunol* 2013; 117: 73-97.

BAs are primarily conjugated with glycine in humans. However taurine conjugation allows a more efficient formation of micelles and appears to be a natural response to a high fat diet. This may turn into less healthy outcomes: saturated milk fat (a common component of processed foods and confectionery) has been shown in mice to favour the formation of taurine-conjugated BAs. These were shown however also to generate organic sulphur, promote the growth of bacteria such as *Bilophila wadsworthia*, and express the sulphite reductase A gene that allows them to generate H₂S. These metabolites can act as gut mucosal barrier breakers, initiating intestinal inflammation. These insights may provide an explanation for observations dating back to the 1950s, suggesting a role for milk fat and milk products in the pathogenesis of Crohn's disease

Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in IL10/mice. *Nature* 2012;487:104–108

IBD

Epidemiologic, clinical, and experimental data have uncovered an important association between inflammatory bowel diseases (IBD) and several environmental factors such as diet, antibiotic usage, or microbial exposure during life. These environmental factors might exert their main influences via modulation of the gut's microbiota and it is currently believed that IBD reflects a microbiota-driven disease in a genetically susceptible host. Overall, there is now increasing agreement that IBD's microbiota is disturbed (commonly described as dysbiosis), certain "pathobionts" exist, and is characterized by low diversity of species and a high density of mucosal surface colonization

(*Nat Rev Gastroenterol Hepatol* 2012;9:599–608).

Diet

Many of the postulated benefits of consuming a diet that is high in fibre may be mediated, at least in part, via short-chain fatty acid (SCFA) production by intestinal microbes and it is known that changes in diet can affect SCFA profiles. For example, obese individuals fed low-carbohydrate diets showed severe depletion in faecal butyrate levels, probably as a result of reduced abundance of butyrate-producing gram-positive *Firmicutes* species (the most diverse range of dominant microbes in the gut) following the change in diet. Similarly, European children have lower amounts of SCFAs in their faeces than high-fibre consuming rural African children.

Dietary interventions designed to promote SCFA production in the colon may therefore have some efficacy in the treatment of dysbioses. In particular, resistant starch has often been reported to promote butyrate formation, which might then reduce symptoms via its putative anti-inflammatory effects. Non-starch polysaccharide dietary components such as cellulose also appear to increase butyrate production in vivo, although their recalcitrant nature and lower fermentability means that the effects may be less dramatic than those seen with resistant starch. However, these substrates may also act to generate faecal bulk, which might decrease transit time and therefore reduce exposure of the intestinal lumen to harmful or toxic compounds.

It appears, however, that the response of the intestinal microbiota to dietary interventions is influenced by the prevailing composition of the microbiota and so responses are specific to the individual.

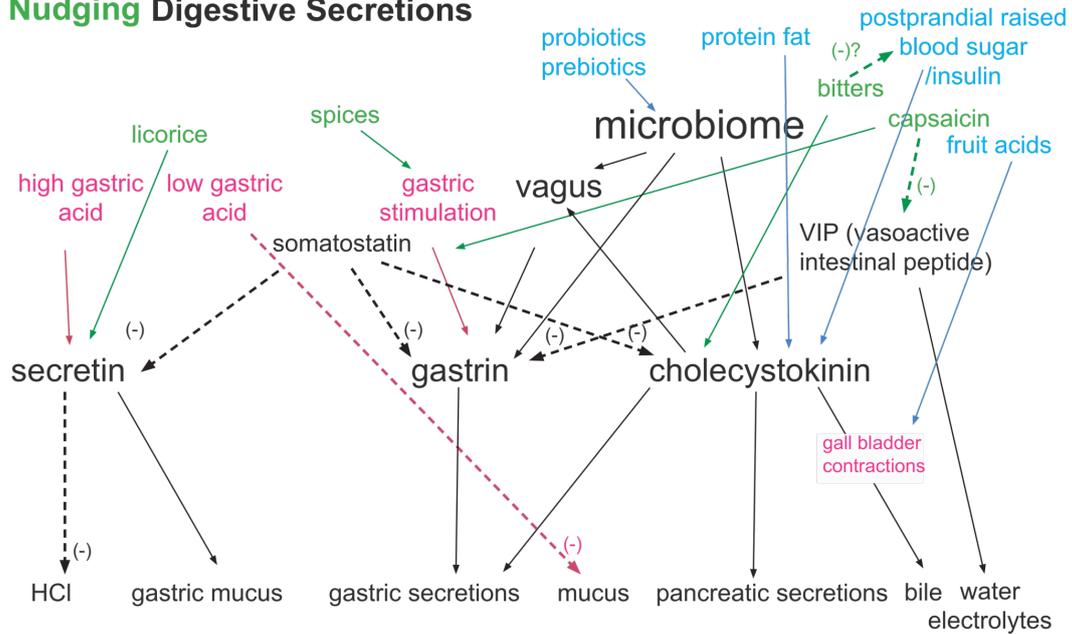
Walker AW, Lawley TD Therapeutic modulation of intestinal dysbiosis. *Pharmacological Research* 2013; 69: 75–86

High intake of total fat, polyunsaturated fatty acids, omega-6 fatty acids, and meats have been identified as risk factors for developing both Crohn's disease and ulcerative colitis, whereas high intake of fruits and dietary fibers may reduce Crohn's disease incidence and high vegetable intake lowers risk for ulcerative colitis.

Albenberg LG, Lewis JD, Wu GD. Food and the gut microbiota in inflammatory bowel diseases: a critical connection. *Curr Opin Gastroenterol* 2012;28:314–320.

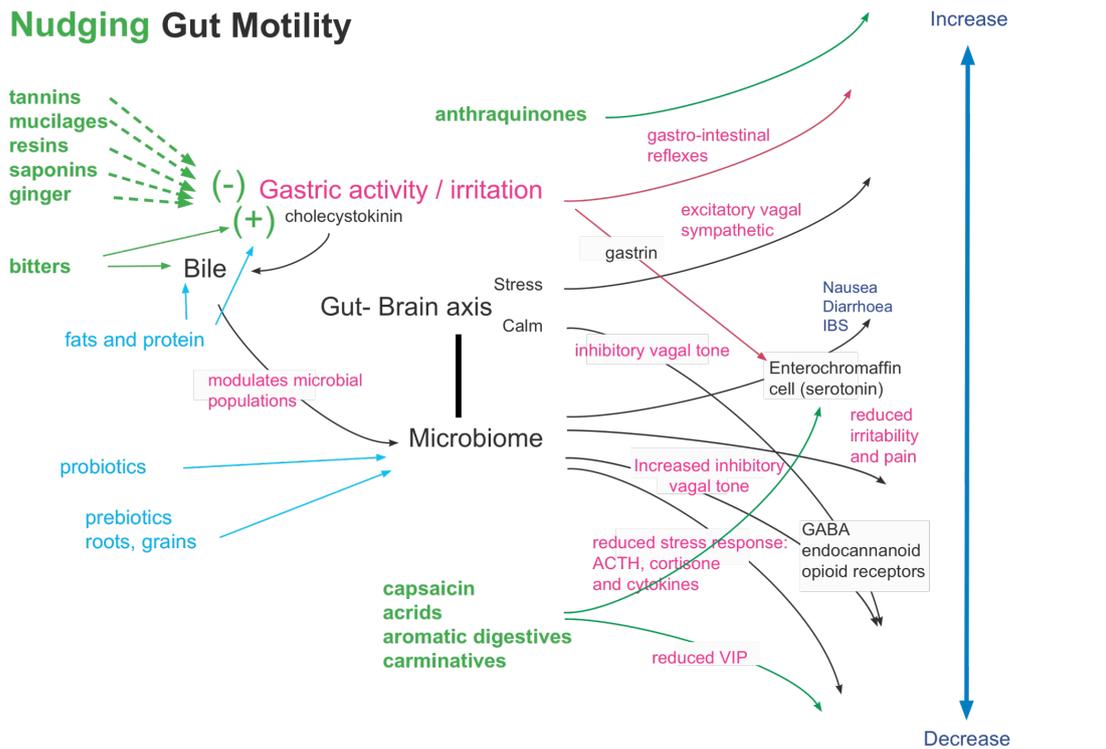
Recent studies have demonstrated how certain dietary components such as cruciferous vegetables interact with intestinal aryl hydrocarbon xenobiotic receptors and thereby regulate intestinal immune processes and the microbiota
 Tilg H. Diet and intestinal immunity. *N Engl J Med* 2012; 366: 181–183.

Nudging Digestive Secretions

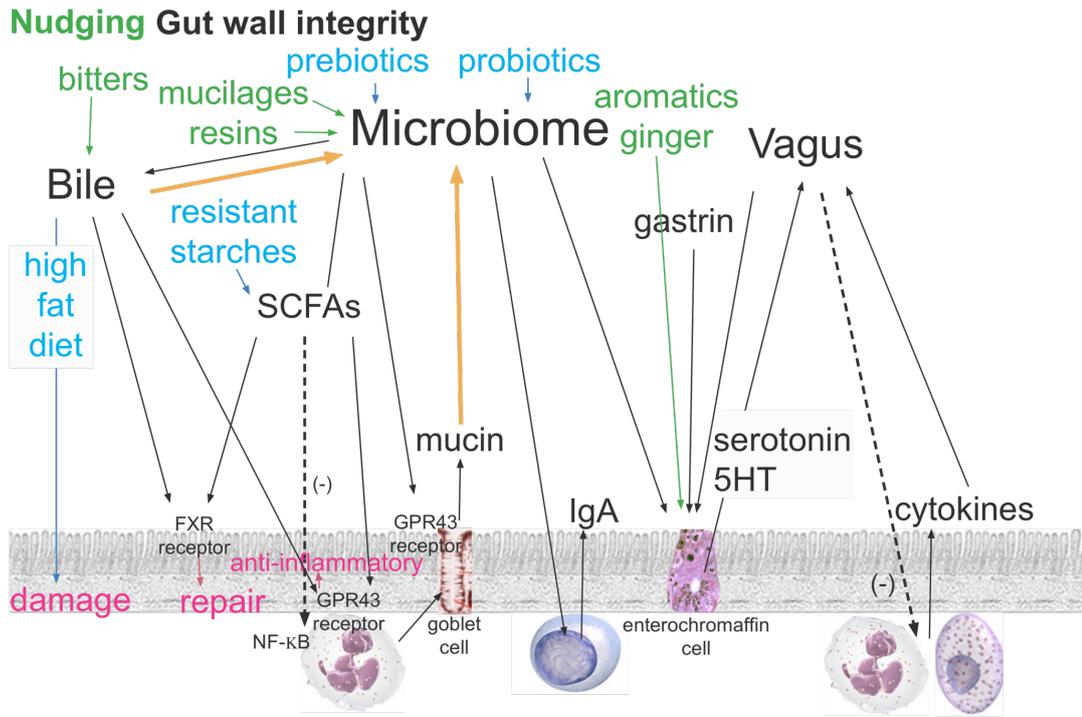


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Nudging Gut Motility



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