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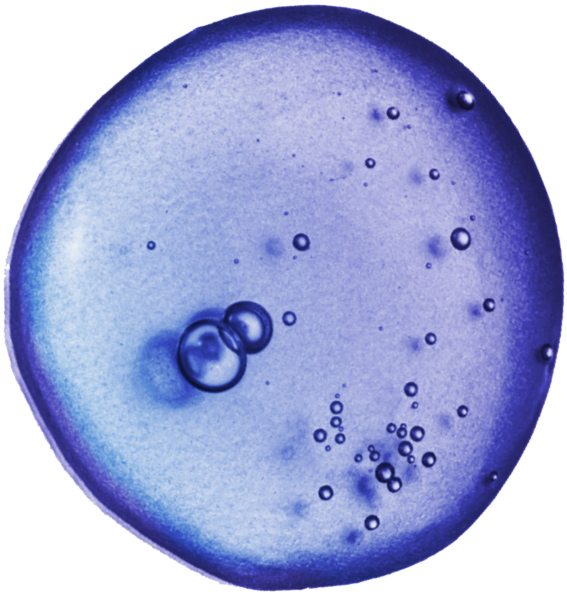
Microbiota: Their Clinical Relevance

HOW MICROBIAL ECOSYSTEMS AFFECT PHYSIOLOGY, IMMUNOLOGY, AND GASTROINTESTINAL FUNCTION

by Joseph Katzinger, ND

Introduction to the Microbiota

It's difficult to overstate the fundamental importance of gut and oral microbiota to human health and disease or the number of direct and indirect ways it influences human physiology. The multitude of bacteria, archaea, viruses, and fungi — collectively referred to as microbiota — are complex. Microbiota impact every organ and system of the body and contribute to a broad range of functions. These include the digestion of food; education of immune cells; endocrine and neurological signaling; regulation of human metabolism; and much more. A growing body of evidence implicates imbalances in microbial composition and activity in cardiovascular disease, diabetes, and gastrointestinal and neurological diseases, among others. It's difficult to point to another single influence on health and biology with as many intricate connections to human physiology and well-being as gut microbiota.



Considerable advances in the analysis of the gut microbiome (a term often used interchangeably with microbiota but which strictly refers to the collection of all microbial genes), as well as advances in metabolomics (analysis of microbial metabolites), have deepened our understanding of the tremendous functional capacity of our microbiota and the diversity of bioactive compounds that have local and systemic effects.[1] A recent estimate suggests the number of bacterial cells in the human body is approximately equal to the number of human cells, yet the microbiome includes at least 3.3 million genes — more than 150 times the human genome.[2,3]

This significance is only starting to be appreciated, with a recent paper indicating "a considerable part of the environmental influence on human health and disease risk may be mediated or modified by microbial communities." Indeed, the environment has a substantially greater role in shaping the human gut microbiome than human genetics. This was recently indicated by a cohort analysis that found no significant association between genetic ancestry and the microbiome but found over 20% of inter-person variability was associated with non-genetic factors such as diet, drugs, and anthropometric measurements. These findings raise the possibility that many environmental factors in health and disease may at least partly mediate their effects via changes to microbiota.

[1] Lynch, S. V., Ng, S. C., Shanahan, F., et al. (2019). Translating the gut microbiome: ready for the clinic?. Nature reviews. Gastroenterology & hepatology, 16(11), 656–661.
[2] Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. PLoS biology, 14(8), e1002533.
[3] Belizário, J. E., & Napolitano, M. (2015). Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. Frontiers in microbiology, 6, 1050.
[4] Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. Nature reviews. Microbiology, 19(1), 55–71.
[5] Rothschild, D., Weissbrod, O., Barkan, E., et al. (2018). Environment dominates over host genetics in shaping human gut microbiota. Nature, 555(7695), 210–215.

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Although this overview primarily focuses on bacterial populations (which have received the bulk of research efforts), it's important to note that the intestinal environment is also comprised of viral communities, including bacteria-infecting phage families (~90%) and eukaryotic viruses (10%); archaea; and fungi (Figure 1). For example, 0.03% of the fecal microbiome is comprised of fungi, and although fungi are estimated to be 3,300-fold less abundant than bacteria (microbiome studies suggest only 267 different fungal species), there is a high degree of variability between individuals, and fungal mycotoxins have been implicated in inflammatory conditions and disruption of the gut barrier.[6,7]

This overview provides a review of the diverse and unique roles the microbiota plays in human health and disease. It also reviews recent research related to the analysis of the metabolome and microbiome, with relevant clinical interventions and condition-specific protocols.

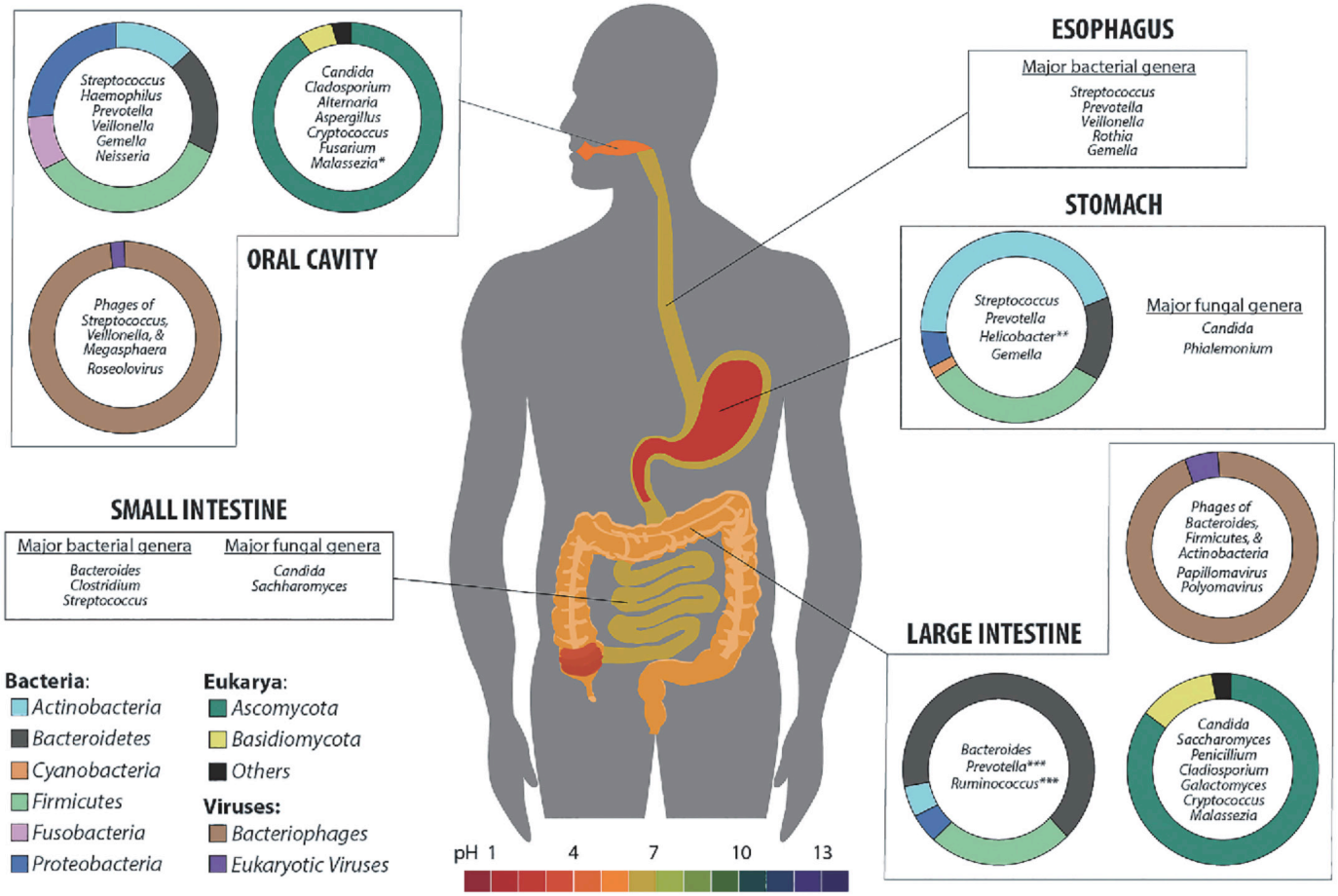

Figure 1: Microbiome composition of bacteria, eukarya, and viruses among the physiological niches of the human gastrointestinal tract. From Hillman, E. T., Lu, H., et al. (2017). Microbial Ecology along the Gastrointestinal Tract. Microbes and Environments, 32(4), 300–313.

[6] Hillman, E. T., Lu, H., Yao, T., et al. (2017). Microbial Ecology along the Gastrointestinal Tract. Microbes and environments, 32(4), 300–313.

[7] Wang, Z. K., Yang, Y. S., Stefka, A. T., et al. (2014). Review article: fungal microbiota and digestive diseases. Alimentary pharmacology & therapeutics, 39(8), 751–766.

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Physiological Function

Digestion of plant polysaccharides. Approximately 17 carbohydrate-active enzymes are formed in the human body, while the microbiota provides around 89. The gut microbiota actively digests dietary fiber, which the human body is unable to digest. This includes liberating short-chain fatty acids (SCFA) from indigestible dietary fibers	Synthesis of group B vitamins, K vitamins, a number of coenzymes (for example, flavin mononucleotide)
Participation in the metabolism of proteins, lipids, and fatty acids	Participation in the regulation of intestinal peristalsis
Energy supplies intestinal epithelial cells and regulates their proliferative activity	Influence on bone metabolism and pathogenesis of osteoporosis
Modulation of goblet cell functions and mucin secretion	Influence on the processes associated with the synthesis of neurotransmitters, myelination of neurons in the prefrontal cortex, and the development of the amygdala and hippocampus
Intestinal microorganisms affect the number of Paneth cells and hence the integrity of the epithelial barrier	Inhibition of the growth of pathogenic microorganisms is due to the activation of phagocytosis, the synthesis of antibacterial peptides, or the synthesis of bacteriocins that inhibit the growth of competitors
Stimulation of local and systemic immunity due to activation of the synthesis of IgA, interferons, and activation of immune cells	Influence the effectiveness of several drugs, in particular: antibiotics, proton pump inhibitors, metformin, vitamin D, and laxatives. It has been shown that the use of these drugs disrupts both the composition of the microbiota and its functional activity

Box 1: A partial list of functions of the microbiota. Adapted from Senchukova M. A. (2023). Microbiota of the gastrointestinal tract: Friend or foe? *World Journal of Gastroenterology*, 29(1), 19–42.

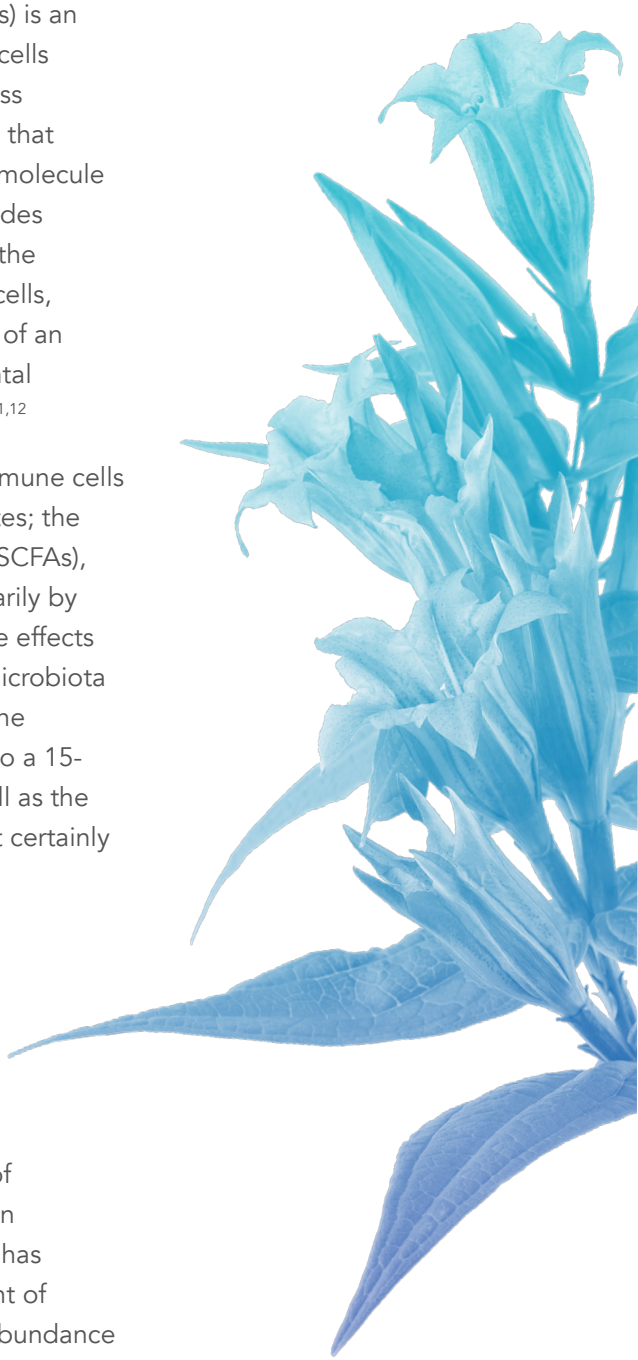
Immunological

It is now clear that microbial exposure during the postnatal and early infant period of life primes the immune system, playing critical roles in immune maturation and metabolism as well as influencing disease susceptibility and microbial tolerance, i.e., "learning" to differentiate harmful organisms from those that are beneficial, or at least spectators. At birth, epithelial cells express innate immune receptors. In response to microbial exposure, these receptor-mediated signals appear necessary for several key steps in development, such as the maintenance of the epithelial barrier, a relationship that continues into adulthood.[8] Both symbiotic and pathogenic bacteria activate host pattern recognition receptors (PRR), including toll-like receptors (TLRs), with symbiotes promoting tolerance and pathogens stimulating inflammation.[9]

[8] Fulde, M., & Hornef, M. W. (2014). Maturation of the enteric mucosal innate immune system during the postnatal period. Immunological reviews, 260(1), 21–34.

[9] Wang, S., Charbonnier, L. M., Noval Rivas, et al. (2015). MyD88 Adaptor-Dependent Microbial Sensing by Regulatory T Cells Promotes Mucosal Tolerance and Enforces Commensalism. Immunity, 43(2), 289–303.

Precisely how tolerance is promoted (to both self and foreign antigens) is an active area of research. However, it is evident that T regulatory (Treg) cells play a critical role in orchestrating this balance. To illustrate this process with an example, Bacteroides fragilis is a common human commensal that modulates the function of Treg cells through the immunomodulatory molecule polysaccharide A (PSA). PSA sits on the surface of B. fragilis and provides a unique signature for this bacteria that TLRs recognize. PSA induces the production of the anti-inflammatory signal IL-10 from migratory Treg cells, promoting tolerance and suppressing inflammation. This suppression of an inflammatory immune response has been demonstrated in experimental models of colitis, in which the administration of PSA provides a cure.[11,12]



Much of the coordination and interaction between microbiota and immune cells is mediated via signaling compounds, particularly microbial metabolites; the most well-studied of these are categorized as short-chain fatty acids (SCFAs), bile acids, and polyamines.[13] SCFAs, for example, are produced primarily by bacterial fermentation of dietary fiber in the colon. They have multiple effects both locally and systemically and are the primary mediators of host-microbiota cross-talk, with perhaps the strongest link between diet, health, and the microbiota. Higher dietary fiber intake, for instance, has been linked to a 15-30% reduction in all-cause and cardiovascular-related mortality, as well as the incidence of type 2 diabetes and colorectal cancer, and SCFAs almost certainly play a significant role in this risk reduction.[14]

At least three SCFA-sensing G-protein-coupled receptors (GPCRs or GPRs) are found not only on various immune cells but also on epithelial cells, pancreatic cells, adipocytes, and enteroendocrine cells that are central to regulating metabolism.[15] Immune cell modulation by SCFAs generally promotes tolerance and reduced inflammation. For example, models of colitis, arthritis, and asthma all demonstrate non-resolving inflammation with a deficiency of one of the GPCR subtypes (GPR43), as well as increased cardiac fibrosis.[16,17] In humans, lower expression GPR43 (as well as GPR41) on immune cells has been associated with a greater degree of arterial stiffness independent of conventional risk factors, with arterial stiffness also linked to a lower abundance of Lactobacillus spp. and a higher abundance of several species from the genus Clostridioides. This adds to a growing body of evidence suggesting that hypertension in humans may be causally associated with gut microbiota.[19]

[11] Perez-Lopez, A., Behnsen, J., Nuccio, S. P., et al. (2016). Mucosal immunity to pathogenic intestinal bacteria. Nature reviews. Immunology, 16(3), 135–148.
[12] Round, J. L., & Mazmanian, S. K. (2010). Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America, 107(27), 12204–12209.
[13] Yoon, J. H., Do, J. S., Velankanni, P., et al. (2023). Gut Microbial Metabolites on Host Immune Responses in Health and Disease. Immune network, 23(1), e6.
[14] Reynolds, A., Mann, J., Cummings, J., et al. (2019). Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet (London, England), 393(10170), 434–445.
[15] van der Hee, B., & Wells, J. M. (2021). Microbial Regulation of Host Physiology by Short-chain Fatty Acids. Trends in microbiology, 29(8), 700–712.
[16] Maslowski, K. M., Vieira, A. T., Ng, A., et al. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature, 461(7268), 1282–1286.
[17] Kaye, D. M., Shihata, W. A., Jama, H. A., et al. (2020). Deficiency of Prebiotic Fiber and Insufficient Signaling Through Gut Metabolite-Sensing Receptors Leads to Cardiovascular Disease. Circulation, 141(17), 1393–1403.
[18] Dinakis, E., Nakai, M., Gill, P. A., et al. (2021). The Gut Microbiota and Their Metabolites in Human Arterial Stiffness. Heart, lung & circulation, 30(11), 1716–1725.
[19] Muralitharan, R. R., Jama, H. A., et al. (2020). Microbial Peer Pressure: The Role of the Gut Microbiota in Hypertension and Its Complications. Hypertension (Dallas, Tex. : 1979), 76(6), 1674–1687.

While typically promoting tolerance, SCFAs can also enhance the antimicrobial activity of immune cells. For example, butyrate has been shown to improve resistance to enteropathogens and enhance the differentiation of macrophages by promoting gene expression of antimicrobial peptides (Figure 2). It's important to note that butyrate activates the antimicrobial activity of macrophages differently than occurs through other pathways. For example, stimulation with the pro-inflammatory membrane component of gram-negative bacteria, lipopolysaccharide (LPS), does enhance macrophage activity. But the subsequent inflammation is a stark contrast to the activation that occurs with butyrate. SCFAs have been found to increase immunity to extracellular and intracellular bacteria, viruses, parasites, and fungal infections.[13]

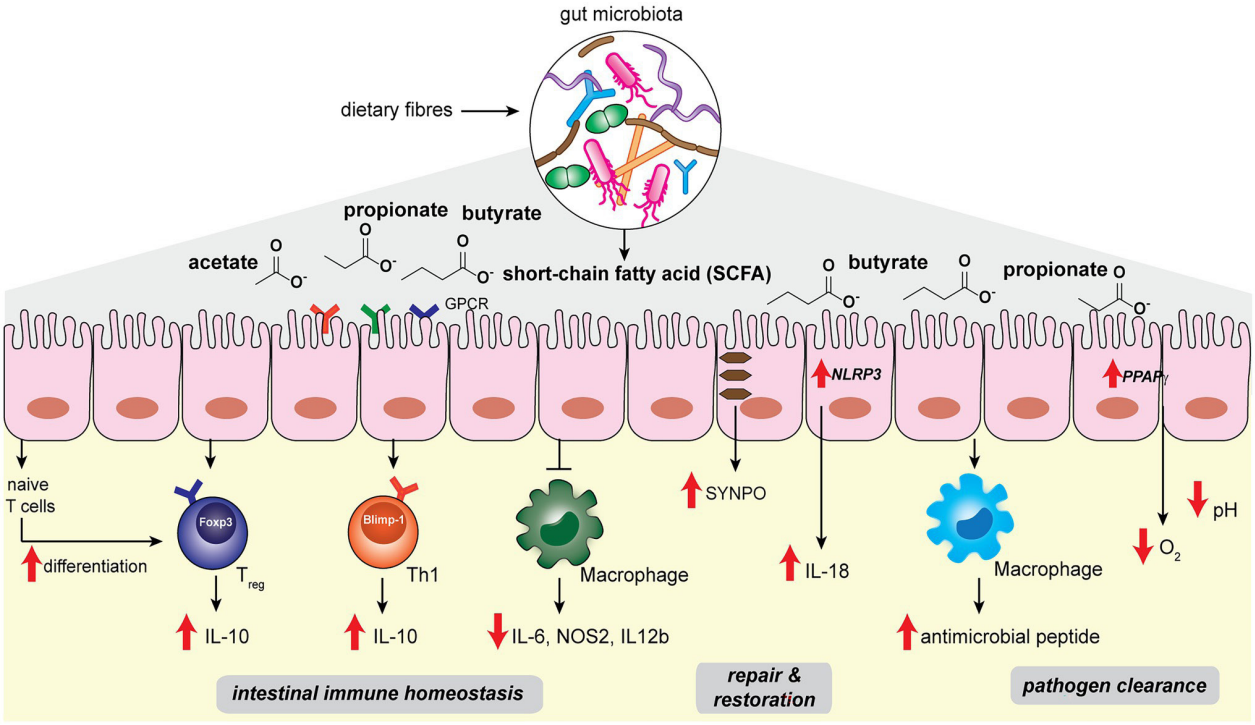


Figure 2: Gut microbiota-derived short-chain fatty acids such as acetate, propionate, and butyrate regulate host intestinal immune homeostasis; conduct epithelial repair and restoration; and facilitate pathogen clearance in the host gut niche. From Li, C., Liang, Y., & Qiao, Y. (2022). Messengers From the Gut: Gut Microbiota-Derived Metabolites on Host Regulation. Frontiers in microbiology, 13, 863407.

In addition to SCFAs, bile acids and polyamines are two other groups of bacterial metabolites recognized to influence immune function. The 5-15% of conjugated primary bile acids that are not reabsorbed in the ileum are deconjugated and biotransformed into secondary bile acids (such as deoxycholic acid (DCA) and lithocholic acid (LCA)) in the colon by microbiota. These secondary bile acids are essential signaling molecules that affect energy expenditure, metabolism, and glucose and lipid homeostasis, as well as having direct immune effects. For example, secondary bile acids bind to the Takeda G protein-coupled receptor (TGR5) and the G protein-coupled bile acid receptor 1 (GPBAR1) receptor, promoting a more tolerant expression of macrophages. This binding by bile acids may also inhibit inflammatory cytokine release after LPS exposure.[21] Bile acid derivatives also promote the differentiation of Treg cells, an effect that ameliorates susceptibility to colitis in experimental models.[22]

[20] Schulthess, J., Pandey, S., Capitani, M., et al. (2019). The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. Immunity, 50(2), 432–445.e7.
[21] Wang, J., Zhu, N., Su, X., et al. (2023). Gut-Microbiota-Derived Metabolites Maintain Gut and Systemic Immune Homeostasis. Cells, 12(5), 793.
[22] Song, X., Sun, X., Oh, S. F., et al. (2020). Microbial bile acid metabolites modulate gut RORγ+ regulatory T cell homeostasis. Nature, 577(7790), 410–415.

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Polyamines such as putrescine, spermidine, and spermine are important signaling molecules present in all eukaryotes, with gut microbiota as the primary source of production. For example, the amino acid arginine is metabolized to putrescine by *Bifidobacterium* sp, though most polyamine metabolism is more complex.[23]

Reductions in polyamine levels — particularly levels of spermidine — appear to occur with aging. Indeed, a higher spermidine intake has been associated with greater longevity in both animal models and humans. A prospective community-based cohort with over 800 participants found that, after adjustment for possible confounding variables, two-thirds of participants who consumed the most spermidine had a 24% lower all-cause mortality risk than the one-third of participants who consumed the least spermidine. In the study, this finding corresponded to an additional 5.7 years of life.

Spermidine appears to enhance tight junctions and restore the integrity of the gut mucosal barrier by inducing autophagy and modulating microbial populations.[24] In experimental models, polyamines have been shown to have an anti-obesity effect, promote T-helper cell differentiation, and limit intestinal inflammation.[13]

Gastrointestinal

Microbiota are crucial for proper intestinal epithelial cell and tight junction formation, which are essential for partitioning our internal and external environments, i.e., confining the microbiota to the intestinal lumen. This is no small task given that the gut has the largest surface area in the body, estimated to be 344 ft^2, approximately ½ the size of a badminton court.[25] This barrier is composed of three layers: a physical layer (comprised of epithelial cells, glycocalyx, and a mucous layer); a chemical barrier (comprised of gastric acid, digestive enzymes, mucopolysaccharides, glycoproteins, glycolipids, and other compounds); and an immunological barrier (including lymphoid follicles and Peyer's patches).[26]

Each of these layers has significant complexity. For example, the epithelial portion of the physical layer comprises the following cell types: columnar, goblet, enteroendocrine, Tufts, Paneth, and microfold (M), each with its own functions. For example, goblet cells produce mucus and deliver small antigens to dendritic cells in the lamina propria (the immune layer beneath the epithelium), helping to induce tolerance to both food and commensal microbiota. Paneth cells are primarily responsible for regulating the density of microbiota by generating antimicrobial peptides and for regulating stem cell homeostasis. This latter function is particularly important to the integrity of the epithelial barrier, as stem cells must continuously regenerate epithelial cells in response to injury and apoptosis.

[23] Kitada, Y., Muramatsu, K., Toju, H., et al. (2018). Bioactive polyamine production by a novel hybrid system comprising multiple indigenous gut bacterial strategies. Science advances, 4(6), eaat0062.
[24] Ma, L., Ni, Y., Wang, Z., et al. (2020). Spermidine improves gut barrier integrity and gut microbiota function in diet-induced obese mice. Gut microbes, 12(1), 1–19.
[25] Helander, H. F., & Fändriks, L. (2014). Surface area of the digestive tract - revisited. Scandinavian journal of gastroenterology, 49(6), 681–689.
[26] Senchukova M. A. (2023). Microbiota of the gastrointestinal tract: Friend or foe?. World journal of gastroenterology, 29(1), 19–42.

Epithelial cells also form intercellular junctions comprising three junctions: tight junctions (TJs), adherens junctions (AJs), and desmosomes (Figure 3).[27] These junctions have the difficult task of allowing penetration of essential ions, nutrients, and water while restricting entry to microbes and toxins. Integrity of these junctions has warranted considerable attention, as dysfunction of TJ complexes has been associated with numerous diseases, including inflammatory and metabolic disorders such as inflammatory bowel disease (IBD), non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), and obesity, while many phytochemicals have been found to increase TJ function.[28] For example, quercetin has been shown to improve TJ integrity in Caco-2 cells by modulating the activity of several TJ proteins, including claudin-1 and -4, ZO-2, and occludin.[29] Similarly, berberine has been shown to improve TJ integrity in Caco-2 cells at least in part by suppressing NF-κB signaling, a key modulator of intestinal inflammation, as well as inhibiting the inflammatory effects of LPS.[30,31]

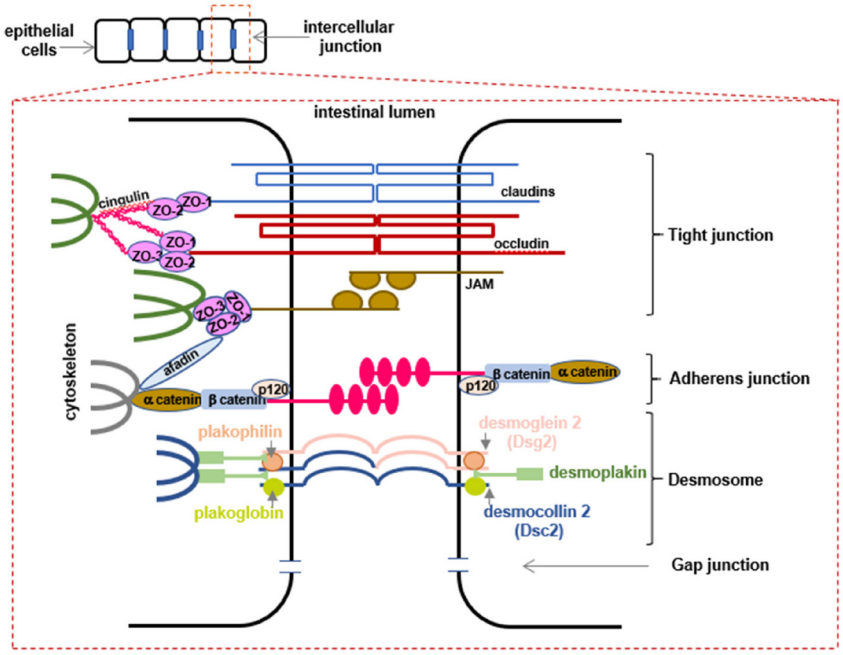

Figure 3: A representative schematic of the intercellular junctions in the intestinal epithelium. From Gieryńska, M., Szulc-Dąbrowska, L., Struzik, J., et al. (2022). Integrity of the Intestinal Barrier: The Involvement of Epithelial Cells and Microbiota-A Mutual Relationship. Animals : an open access journal from MDPI, 12(2), 145.
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[27] Gieryńska, M., Szulc-Dąbrowska, L., Struzik, J., et al. (2022). Integrity of the Intestinal Barrier: The Involvement of Epithelial Cells and Microbiota-A Mutual Relationship. Animals : an open access journal from MDPI, 12(2), 145.
[28] Lee, B., Moon, K. M., et al. (2018). Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. Journal of immunology research, 2018, 2645465.
[29] Suzuki, T., & Hara, H. (2009). Quercetin enhances intestinal barrier function through the assembly of zonula [corrected] occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. The Journal of nutrition, 139(5), 965–974.
[30] Gu, L., Li, N., Gong, J, et al. . (2011). Berberine ameliorates intestinal epithelial tight-junction damage and down-regulates myosin light chain kinase pathways in a mouse model of endotoxinemia. The Journal of infectious diseases, 203(11), 1602–1612.
[31] Izadparast, F., Riahi-Zajani, B., Yarmohammadi, F., et al. (2022). Protective effect of berberine against LPS-induced injury in the intestine: a review. Cell cycle (Georgetown, Tex.), 21(22), 2365–2378.