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Chapter 14

# The gut microbiome: its role in brain health

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## Chapter outline



## Introduction

The demand for solutions to digestive health issues is accelerating, especially since both the scientific literature and the popular press currently dedicate significant resources to promoting awareness of what has come to be known as "gut health." In a quest to find solutions to their own health issues, consumers have embraced a new terminology that includes such terms as *microbiome*, *SIBO (small intestine bacterial overgrowth), PPIs (protein pump*

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*inhibitors), FMT (fecal microbial transplant), FODMAPS (fermentable oligo-, di-, monosaccharides and polyols), MCAS (mast cell activation syndrome)*, and others. As with any innovation, there can be initial confusion, especially when the science has yet to catch up with interventions that are already being implemented; in this context, *gut health* is no exception.

This chapter describes the history of microbiome research, positioning this relatively new field within the context of the interrelationship between the gastrointestinal (GI) tract and the brain, known as the *Gut-Brain Axis*. In so doing, it explores known and putative mechanisms to explain the bidirectional interactions clearly at play, highlighting clinical examples where the microbiome, the host intestinal epithelium, and its underlying immune system impact human health and behavior. Although the vagus nerve (VN) is an integral part of the bidirectional communication network linking the gut and the brain, it is not the only means by which the gut communicates with the brain. Several other pathways directly link the brain and the gut microbiota with the clinical significance of this becoming increasingly apparent [1,2].

Links between neuropsychiatric disorders such as depression, generalized anxiety disorder, and immune dysfunction have opened up new therapeutic options for addressing such conditions [3]. Similarly, neurodegenerative and inflammation-related brain conditions such as Parkinson disease (PD), Alzheimer disease (AD), schizophrenia, and autistic spectrum disorder are being considered within the context of the gut-brain axis, similarly paving the way for new therapeutic interventions [4].

As interventions, live probiotic supplements and to a lesser extent prebiotics that support the growth of the probiotic microbes are popular choices of both clinicians and consumers across a range of conditions [5], even though the evidence for benefit in brain health remains equivocal and many unanswered questions remain [6].

## The human gut microbiome—a new frontier in medicine

Until recently, the major determinant of the unique microbial signature of an individual was believed to have been encoded within the host's genome [7]. However, it appears that the genetics of the host plays a minor part, the major determinant being the environment. Specifically, the microbiome is not significantly associated with genetic ancestry or with individual SNPs, and that previously reported associations are not replicated across different studies [8]. This is clinically very relevant because it suggests that interventions to alter the environment of the microbiome may be effectively conducted across diverse genetic backgrounds.

### The history of microbiome research

Contemporary readers may unwittingly think that the term "microbiome" appeared relatively recently in the scientific literature, especially since misattribution of the term to Nobel Laureate microbiologist Joshua Lederberg in 2001 has been frequently requoted. In fact, the term "microbiome" first appeared in 1962 when it was adopted in conjunction with the incorporation of germ-free (GF) animals into common laboratory practice [9]. By 1988, Whipps et al. had defined the microbiome in a manner that is largely in keeping with its modern usage in microbiology: "A characteristic microbial community occupying a reasonably well-defined habitat which has distinct physicochemical properties. The term this way not only refers to the microorganisms involved but also encompasses their theatre of activity." [10] Defined in this manner, the microbiome may be considered a key component of the gut ecosystem, where the "theatre" also includes the host epithelium and its underlying immune network.

Three decades later, our growing understanding of the gut microbiome in human physiology has led to a dramatic shift in the way we perceive the thousand or more microbial taxonomic units that inhabit the gut lumen. Where the intestinal lumen was once considered to be a temporary repository for the wastes produced after food digestion and nutrient absorption, the gut microbiome is now classified by some as an organ in its own right [11].

### The human microbiome project—harnessing modern lab technology

Following closely behind the completion of the Human Genome Project (HGP) in 2003, the Human Microbiome Project (HMP), which began in 2007, was seen to be a logical, conceptual, and experimental extension [12]. The HGP leveraged advances in high-throughput DNA sequencing technologies to extend the HGP research to the human microbiome. One primary objective of the HMP is to determine a reference genome for a human microbiome. When such a reference genome has been determined, it is considered possible to better characterize dysbiosis and microbiome-related conditions associated with either health or disease [13].

With microbiome research no longer limited to culture-based techniques, the understanding of its role in human physiology has significantly advanced with the conception of 16s rRNA techniques [14] in 1977, and more recently Next-Generation Sequencing (NGS) technologies in 2005 [15]. Such techniques can explain at least in part, the acceleration in the research being conducted in this field [16].

With the benefit of further post-2007 research, it has been subsequently considered that the characterization of a

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"healthy" reference genome may no longer be a practical definition; an alternative hypothesis is that of a healthy "functional core" which comprises the metabolic and other molecular functions performed by the microbiome in a particular habitat but which is not necessarily provided by the same microorganisms in different people [17].

The Metagenomics of the Human Intestinal Tract (MetaHIT) project was founded in 2008 and aimed to sequence the microbial genomes of fecal samples derived from both diseased (inflammatory bowel disease and obesity) and healthy individuals with a view to better understand the microbial links to disease [16].

## Modern links between microbiome and human behavior

An evolving awareness of the diverse roles of the microbiome and its symbiotic relationships with its human host has resulted in a radical shift from thinking of microbes solely in terms of pathogenicity to considering their essentiality in normal human physiology [15]. The impact of the resident gut microbes is now being extensively researched in the context of a dysfunctional nervous system where there is considerable overlap in the mechanistic underpinnings of conditions described using the terms neuroinflammatory [18], neuropsychiatric [4], and neurodegenerative diseases [19–21]. Mental health issues such as depression and anxiety are the focus of intense research into the links between the gut microbiome and immune imbalances associated with uncontrolled inflammation [3,4,20], as is the impact of psychosocial and physical stress signaling systems linking the gastrointestinal (GI) tract and central nervous system (CNS) [22].

## What microorganisms constitute the gut microbiome?

While the "gut microbiome" refers to the collective genome of all the microorganisms inhabiting the human GI tract, the organisms themselves are described collectively as the "microbiota," a dense and diverse population that includes bacteria, bacteriophages, viruses, fungi, protozoa, and archaea, with bacteria dominating [23].

The microbiota are not evenly distributed throughout the GI tract, largely due to differences in host physiology at its different functional levels. The colon, where luminal contents reside for extended periods of time, exhibits the most dense microbial population. Estimates of the number of bacteria inhabiting the healthy human GI tract are $10^{13}$–$10^{14}$ viable bacteria per gram of luminal content and collectively contain at least 100-fold as many genes as does the entire human genome [24]. The diverse bacterial population of the human intestine is estimated to represent 50 different phyla, 1000 different species [23], and 7000 subspecies [25].

In this way, suggests Gill et al. [24], "humans are superorganisms whose metabolism represents an amalgamation of microbial and human attributes."

Of the 50 or so phyla identified in the human GI tract, four phyla dominate the densely populated colon: Firmicutes (64%), Bacteroidetes (23%), Proteobacteria (8%), and Actinobacteria (3%) [23].

## Dysbiosis

Even though it is accepted that a balanced gut microbiota is important for health, a so-called "normal" human microbiome has not yet been defined [26]. The colon is dominated by obligate anaerobes which is a likely consequence of the severe oxygen limitation characteristic of this region of the GI tract. Such obligate anaerobes ferment those diet-derived carbohydrates that have escaped earlier digestion, metabolizing them into short-chain fatty acids (SCFAs) including butyrate, a major signaling molecule in host physiology. Oxygen deprivation with dominance of obligate bacteria in the colon ensures production of metabolites that maintain gut homeostasis [27,28].

Dysbiosis occurs when this pattern is disrupted by an expansion of facultative anaerobes, typically belonging to the Phylum Proteobacteria. Such disruption may be the result of antibiotic or nonantibiotic pharmaceutical treatment [29], which can eliminate epithelial hypoxia to drive the shift of bacterial communities from obligate to facultative anaerobes; this is considered the hallmark of dysbiosis [26].

Of clinical relevance is the observation that chronic psychosocial stress in an animal model resulted in an expansion of Proteobacteria [30]. In a human population, dysbiosis together with increased Proteobacteria were associated with the onset of cardiovascular events and are found in atherosclerotic plaques [31,32], suggesting that elevated Proteobacteria may represent a "microbial signature" of disease [33].

## Microbiome analysis

In keeping with a trend toward the development of clinical strategies for personalized nutrition, identity of the microbial population via a stool sample is available to both clinicians and consumers from some laboratories. The reports typically categorize the microorganisms into one of the four dominant phyla, together with identification at genus and species level [34]. Although this form of testing is still in its infancy and its value uncertain, there are those who regard the information derived from microbiome research as the key to better understanding human health and nutrition [35].

## Linking the gut microbiome to the brain

The term "gut-brain axis" was first mentioned in 1980 in describing the existence of a mechanism relating plasma

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cholecystokinin levels to cerebrospinal fluid as a "gut to brain axis" [36]. In the ensuing four decades, 1297 related publications are indexed in PubMed, where 22 are clinical trials and 601 are reviews (accessed 6th October 2019).

By 2014, it was suggested that the growing body of information connecting human neurological function to the host's luminal microbiome required a reevaluation of many concepts in health and disease; in short, a paradigm shift would be necessary. The observed bidirectional signaling between the gut and the brain was seen to involve multiple mechanisms. These findings have resulted in speculation that alterations in the gut microbiome may play a pathophysiological role in human brain diseases, including autism spectrum disorder, anxiety, depression, and visceral and chronic pain [37] as well as the neurodegenerative and other conditions mentioned earlier.

Experimental work continues to show that brain development is abnormal when the gut microbiome is absent [38,39]. Furthermore, maternal and early-life nutrition play a key role in establishing the microbiome and take place at the same time as the occurrence of neurodevelopmental plasticity, suggesting a complex dialogue between the GI tract and the brain. Several lines of evidence show that the gut microbiota interacts with diet, drugs, and stress, both prenatally and postnatally [40].

Reference to this growing body of literature indicates that human health can be both positively and negatively impacted by the microbiota, not just in the GI tract but in distant organs and systems.

## The GI tract and its microbiome as an ecosystem

No discussion of the microbiome would be complete without consideration of the ecosystem to which it belongs. As with land-based ecosystems, each component of the human gut ecosystem is responsive to the continuously changing conditions surrounding it, its survival dependent on its ability to adapt to its ever-changing environment [41]. Within the human GI tract, the intestinal epithelial cells (IEC), their underlying immune network, and the commensal microbiota are the major components of this ecosystem [41]. The gut is equipped with a multilayered biological system capable of maintaining homeostasis, while at the same time, engaging in cross-talk between the host and the microbial symbionts [42].

The host's first line of defense consists of enterocytes interspersed with specialized cells together with overlying mucous layers to restrict intrusion of potentially reactive antigens and undesirable luminal microorganisms. Its second line of defense is dependent on the underlying immune cells of the innate and acquired immune systems, an important role being to distinguish between beneficial and pathological antigens; in so doing, its exerts tolerogenic responses to the commensal microbes [42].

Investigation into the bidirectional nature of the host-microbe relationship indicates that the composition of the human microbiota is determined by an interaction of host genetics and diet with an environment that includes chemicals such as antibiotics [43].

It seems, however, that on average, the genetic differences between humans within a population are too small to outweigh diet as a determinant of microbiome composition [34]. Nevertheless, a preponderance of therapeutic inventions directed at restoring homeostasis to the gut ecosystem is focused on the administration of probiotic supplements without targeted interventions aimed at restoring the defensive functions of the specialized enterocyte [44].

### How the microbiota shift rapidly with dietary change

To illustrate the rapid and dynamic way in which gut ecology can shift, a 2014 study [45] showed that when humans were switched between a plant-based and an animal-based diet in a 4-day intervention, the composition of the microbiota changed significantly in accordance with the changes in macronutrient proportions. The participants on the carnivore-style diet increased their dietary fat approximately twofold, whereas those on the herbivore-style diet increased their plant carbohydrate approximately threefold.

Those participants consuming the plant-based diet increased their levels of the *Firmicutes* that metabolize polysaccharides; in so doing, the proportions of butyrate-producing *Roseburia, Eubacterium rectale*, and *Ruminococcus bromii* significantly increased, an advantage for the colonocytes for which butyrate is the preferred energy source.

By contrast, the animal-based diet, naturally higher in dietary fat, increased bile secretion, promoting the growth of bile-tolerant microbes such as *Bilophila wadsworthia, Alistipes*, and *Bacteroides* [45]. The abundance and activity of *B. wadsworthia* on the animal-based diet was considered to support a link between dietary fat, bile acids, and the outgrowth of microorganisms capable of triggering inflammatory bowel disease. This has clinical implications for those individuals trialing the many popular "branded" diets—Ketogenic, Paleolithic, Carnivore, low-FODMAP as examples; each of these is based on significant changes in the proportions of animal versus plant food, macronutrients, prebiotics, and other fibers [46].

The symbiotic relationship between the microbiota and the IECs forms an ecosystem where the host provides the

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microbes with a suitable physical environment, required nutrients, and prebiotics that can be metabolized to provide the host with beneficial signaling molecules. The microbes in turn, provide the host with essential nutrients, immune-modulating signals, and some antimicrobial activities. In this way, both commensal and exogenous probiotic bacteria interact with the host to maintain homeostasis [47]. In this regard, a Systems Biology model has been proposed that posits circular communication loops amid the brain, gut, and gut microbiome and that perturbation at any level can propagate dysregulation throughout the circuit [6].

As the science continues to unfold, it becomes clear that intricate signaling and cross-talk between the microbes and their human hosts is *joining the dots* in our understanding of why the presence of a diverse microbiota benefits not just digestive health but assists in driving the processes of health or disease in distant organs, including the nervous system [48].

### How germ-free animals help elucidate the mechanisms

To study host-microbial interactions in health and disease, GF rodents are being used increasingly as a relevant model [49]. For example, when GF animals are subsequently colonized with commensal microorganisms, complex signaling pathways have been shown to result in the expression of genes encoding tight-junction (TJ) proteins; as a result, the mucosal barrier can be enhanced [50]. Perhaps counter-intuitively, studies using GF animal models have demonstrated that mice lacking gut microbiota are resistant to diet-induced obesity, liver steatosis, and insulin resistance and that an etiologic mechanism associated with the effects of endotoxin can be advanced [51].

Studies using GF animals are equally valuable as a means of comparing the influence of the microbiota on the gut-brain axis [20]. For example, the investigation of anxiety-like and depressionlike behaviors in GF compared with normal animals indicates that responses to physical or psychosocial stresses are linked to the presence or absence of the microbiota [52,53]. Further research along these lines showed that the low-anxiety phenotype was accompanied by long-term changes in genes associated with plasticity in the hippocampus and amygdala of these animals [54]. What is further evolving in this line of evidence is that the impact on animal behavior is specific to the bacterial species employed to colonize the GF animals [20].

Compared with conventionally raised animals, when microorganisms are absent from the intestines of GF mice, the blood-brain barrier (BBB) is more permeable to macromolecules, mediated by reduced expression of key tight-junction proteins in the brain endothelial cells. When GF animals were either colonized or administered the SCFA fermentation metabolite, butyrate, the gut became less permeable and the barrier enhanced [55].

In a similar way, long-term antibiotic treatment of mice diminishes the gut microbiota and is sufficient to decrease neurogenesis in the hippocampus of adult mice, impairing their ability to recognize objects [56]. Interestingly, these phenotypes could be rescued with probiotics and voluntary exercise as the intervention. Neurogenesis can also be promoted by serotonin, with the gut bacteria shown to play a role in serotonergic pathways in both the gut and the brain [57].

These few examples illustrate the essential interconnectedness of the microbiota and neural function.

## Shifting the therapeutic emphasis from the probiotic toward the host

Development of a healthy gut mucosa is a *bidirectional event between the host and the gut microbiota*, creating an environment that allows the specific members to establish persistent colonization via utilization of host-derived dietary glycans [58].

Even so, it appears that current therapeutic strategies to restore gut homeostasis are mostly reliant on manipulation of the microbiota using antibiotics, probiotics, prebiotics, phage therapy, polyphenols, and fecal microbiota transplantation, rather than approaches that target the host epithelial cells and their underlying immune network [59,60].

Popular treatment options to restore the gut barrier and eliminate dysbiosis were investigated in a 2019 cross-sectional survey of complementary and integrative medicine practitioners. The most frequent clinical recommendations in descending order were: zinc, multistrain probiotics, vitamin D, glutamine, *Curcuma longa,* and the yeast, *Saccharomyces boulardii*. Other recommendations include advice to reduce alcohol, gluten, and dairy consumption. The authors indicate that these clinicians are prescribing in accordance with published literature, although they do not describe a rationale for such interventions [44].

With a different emphasis, a 2018 scientific review, entitled *Colonocyte Metabolism Shapes the Gut Microbiota*, [61] supports the claim that it is primarily the host colonocyte driving the microbiome—rather than the reverse, with this line of thinking supported recently by others [27,62].

Furthermore, these authors claim that because the human immune system already has mechanisms to balance the colonic microbiota, harnessing this host control mechanism for therapeutic means could provide an alternative to targeting the microbes themselves for remediation of dysbiosis.

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Where the popular current focus on addressing dysbiosis is on manipulating the microbiota with antimicrobials, pro- and prebiotics, it may be time to shift the emphasis closer to optimizing enterocyte and colonocyte metabolism as the primary drivers of dysbiosis in the colon.

In support of this hypothesis, evidence exists that the IECs engage endogenous mechanisms that include but are not limited to the synthesis of protective mucus by specialized Goblet Cells, the synthesis and release of secretory IgA by plasma cells, the production of selective antimicrobial peptides by Paneth cells, neurotransmitter synthesis by enterochromaffin cells, together with synthesis and release of a number of hormones by the enteroendocrine cells [63].

In addition, IECs contain sophisticated monitoring systems that include tolllike receptors and dendritic cells to detect possible threats to which healthy epithelial cells can respond [63].

In the context of the gut-brain axis, enhanced therapeutic strategies to restore homeostasis in the gut ecosystem may, in turn, lead to better clinical outcomes in conditions associated with CNS dysfunction.

## The intestinal epithelial cell

The single layer of IECs lining all anatomical hollow spaces share many common properties; even so, each is specialized to respond to its local environment. Although the IECs in this regard line the luminal surface from esophagus to anus, the types of cells differ regionally to accommodate the many different conditions within the human GI tract (Fig. 14.1).

Notably, comparison of the intestinal epithelium in the small intestine and that of the colon shows marked differences which are related to the requirements at each intestinal region. The small intestine is primarily involved in digestion and absorption and is contiguous with a relatively low microbial population, whereas a more dense microbial population occupies the colon, where fermentation of those carbohydrates that escape digestion within the small intestine is a key function [62].

IECs are continuously replaced every 4–5 days through a process of renewal and migration, initiated by maturation of the stem cells at the base of the villus crypt [64]. As the cells mature, they progress upwards along the villi, differentiating as they mature. The differentiated cells include

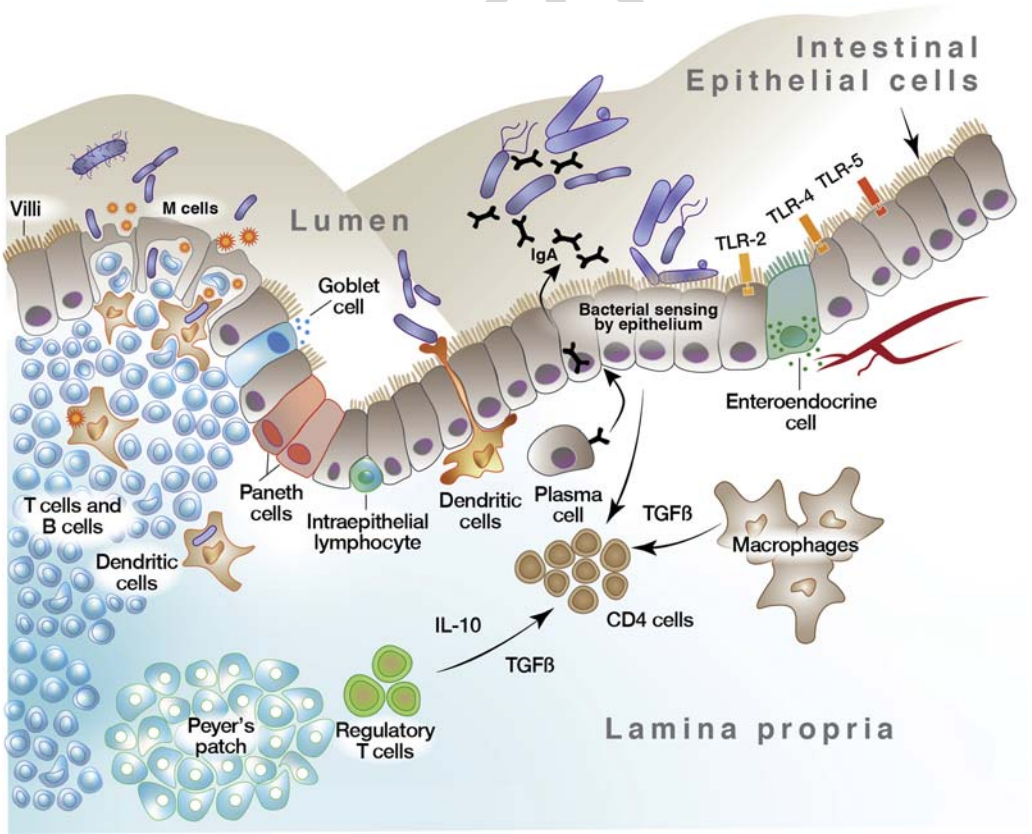


**FIGURE 14.1 The intestinal epithelium and its various specialized cell types.** The single layer of mucin-protected cells described as the "intestinal epithelium" is contiguous with the immune network of the Lamina propria on the systemic side and the microbiota on the luminal side. A complex array of differentiated cell types and intricate signaling pathways are integral to the ability of this epithelium to maintain homeostasis in a continuously hostile environment.

enterocytes as the most abundant of these cells; the major functions of the major specialized gut epithelial cells are briefly described below.

### Goblet cells

Goblet cells secrete mucins to protect and lubricate, making up around 10% of the total IECs and recognized as significant contributors to barrier maintenance [64]. In addition to the secretion of mucin as a primary component of innate immune defenses, goblet cells secrete antimicrobial proteins, chemokines, and cytokines demonstrating functions in innate immunity beyond barrier maintenance [65].

### Paneth cells

Of particular note are the small intestinal Paneth cells which protect the stem cells in the base of the crypt and which release a range of antimicrobial compounds [66]. The well-studied defensins are tolerogenic toward commensal microbes but are potent antimicrobials toward pathogens, making them useful therapeutic targets for addressing dysbiosis [66].

### Enteroendocrine cells

Enteroendocrine cells (EECs) are found scattered throughout the epithelium of the GI tract from the stomach to the rectum. EECs release gut hormones in response to meal-related stimuli and thereby exert actions ranging from the local control of gut motility and secretion to the regulation of insulin release and food intake. EECs are traditionally classified according to the principal hormone they produce. Some hormones, such as serotonin (5-HT), are produced along the whole length of the gut, playing important roles in motility [67].

Enterochromaffin cells are an EEC subtype (<1% of total intestinal epithelia) which produce >90% of the body's serotonin and have been suggested to affect a variety of physiological and pathophysiological states. As such, they play a significant role in GI motility and the migrating motor complex, as well as nausea and visceral hypersensitivity [68–70].

### IEC signaling molecules

In addition, IECs carry a number of surface molecules capable of detecting the presence of pathogens. These surface molecules are typically proteins and classified as Pattern Recognition Receptors (PRRs). The PRRs fall into two groups—the Pathogen-Associated Molecular Patterns (PAMPs) which detect the chemical signatures of microorganisms and the Damage-Associated Molecular Patterns (DAMPs) which detect antigens and other potential toxins. PAMPs and DAMPs both alert the IECs to potential threats, with resultant signaling mechanisms initiating the appropriate range of responses designed to protect the host [71].

In a complex interconnectedness, the IECs which are sandwiched between the gut lumen and the underlying immune cells operate as sensors to signals from both the host immune cells and the gut microbes, mediating the balance between secreting cytokines, chemokines, and hormones [62].

### Dendritic cells

As components of the intestinal epithelium, dendritic cells (DCs) are central to the initiation of primary immune responses. They are the only antigen-presenting cells capable of stimulating naive T cells, and hence they are pivotal in the generation of adaptive immunity [72]. DCs can promote the development of regulatory T cells (Treg) with inflammation-suppressive activity. The induction of tolerogenic DCs can be achieved by vitamin D receptor agonists, known to shape DC phenotype and function and as such are targets for pharmaceutical research, especially related to autoimmunity [73].

## The gut barrier

An appropriately dynamic gut barrier is critical to the maintenance and restoration of intestinal homeostasis. On the one hand, IECs must provide absorptive surfaces that allow the entry of water and nutrients; while on the other hand, they must be capable of excluding microbes and antigens, most of which are food-derived. Furthermore, the gut functions with the continuous challenge of responding to pathogens while remaining relatively unresponsive to commensal microflora, food proteins, and other antigens [74].

Collectively, healthy IECs provide an essential and selective intestinal barrier and in large part, determine gut homeostasis. The gut barrier is primarily composed of the continuous single-celled layer of IECs, a protective mucous overlay, and the underlying lamina propria (LP) [75]. The LP resides below the IECs and houses the majority of the cells involved in immune responses. Cells of the LP are conveniently located in close proximity to the surface epithelium and also to the surrounding vessels and nerve fibers, suggesting strong functional associations with both [76].

## The blood-brain barrier

The BBB, which is comprised of endothelial cells, astrocytes, pericytes, and adjacent neurons, regulates the passage of essential components into and out of the CNS, minimizing the influence of toxic compounds and

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pathogens [77]. This may include pathogens which have crossed the intestinal barrier and carried by the circulation to the CNS.

As with the epithelial cells of the GI tract, the TJ backbone in brain endothelial cells consists of transmembrane proteins (occludins, claudins, and junctional adhesion molecules [78]) which regulate the passage of molecules in and out of the CNS [79]. BBB dysfunction can contribute to neurological disorders in a passive way by vascular leakage of blood-borne molecules into the CNS and in an active way by guiding the migration of inflammatory cells into the CNS [80].

The source of the inflammatory cytokines carried systemically to the CNS may have been generated by perturbations at the gut-immune interface, providing a specific link between the gut ecosystem and the brain [81]. A number of CNS inflammation-related disorders, such as multiple sclerosis, human immunodeficiency, virus infection, or AD, have been linked to the production of proinflammatory cytokines, matrix metalloproteases, and reactive oxygen species, which adversely affect CNS barriers [81].

## The tight junction—effects of exogenous factors

An essential component of the intestinal barrier is the paracellular junction that connects the IECs to each other. The TJ is a complex mechanism that is somewhat analogous to a spring-loaded hinged gate with several types of latches that allow it to open and close. The environment-responsive molecules, *occludins, claudins,* and *JAM* proteins act as the latches, with *zonulin* acting as the spring. The role of the TJs is to regulate the paracellular permeability, allowing the "gate" to open and close as required. When this mechanism is perturbed, the gut barrier is compromised and intestinal permeability is increased, allowing entry of unwanted molecules and/or microbes.

The term, "leaky gut" has crept into popular vernacular and misrepresents the dynamic nature of the gut barrier; consequently, interventions to address the issue clinically are often inappropriate in that the layperson believes that the gut barrier is akin to there being holes in a pipe that must be "sealed." Gluten is widely considered within the lay community to be the primary dietary factor responsible for destabilizing the gut barrier. This notion, together with that promoted in a widely publicized book [82] claiming an association between wheat intake and adiposity, has led to the growing trend of gluten avoidance behavior in many countries, even when celiac disease is not present [83,84].

Both dietary factors and endogenous metabolic factors [85] provide the signals known to influence the components of the TJs. Gluten research confirms its role in destabilizing zonulin and thereby in opening the TJs; however, it is just one factor of many [86]. It is noteworthy that many of the additives and processing aids being used in producing gluten-free foods with the appearance, taste, and mouth-feel of the gluten-containing original have been shown to adversely affect gut barrier integrity [87]. Enzymes such as *transglutaminase* together with a number of commonly used emulsifiers have been identified as having destabilizing effects on the gut barrier and in some cases thought to contribute to a rise in autoimmune conditions [88].

## The tight junction—endogenous mechanisms

As described earlier, perturbation of the gut barrier is considered to be typically the result of reactions from microbes and food-derived molecules in the gut lumen; however, more recently it has become apparent that there are other factors which play a significant role in destabilizing the gut barrier as well as the BBB (Fig. 14.2).

### *Redox-inflammation*

Redox signaling has been shown to tightly modulate the inflammatory response, whereas tissue damage subsequent to unregulated inflammation generates more reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) [89]. The simultaneous presence of both perturbed redox and inflammation homeostasis acts as a feed-forward process to promote a dysfunctional barrier.

When redox-dependent signal transduction mechanism such as this occurs within the gut ecosystem, components of the TJ such as occludin adversely impact the gut barrier [90]. Oxidative stress has been suggested to act as an initiator and/or mediator of many human diseases and because the cerebral vasculature is particularly susceptible to oxidative stress, the maintenance of the BBB is a critical factor in maintaining CNS homeostasis [91].

### *Alkaline phosphatase*

Another biomolecule produced by the healthy host is intestinal alkaline phosphatase (IAP), an endogenous protein expressed by the intestinal epithelium, and believed to play a vital role in maintaining gut homeostasis and wellbeing [92]. Loss of IAP expression or function is associated with increased intestinal inflammation, dysbiosis, bacterial translocation, and subsequently systemic inflammation.

Its expression is known to be affected by prematurity, starvation, and inflammation. IAP's antiinflammatory effect is associated with inhibition of toxic microbial ligands such as bacterial lipopolysaccharide (LPS). Its ability to degrade LPS and other toxic ligands is essential in protecting the host from sepsis during acute inflammation and chronic inflammatory conditions such as inflammatory bowel disease [93].

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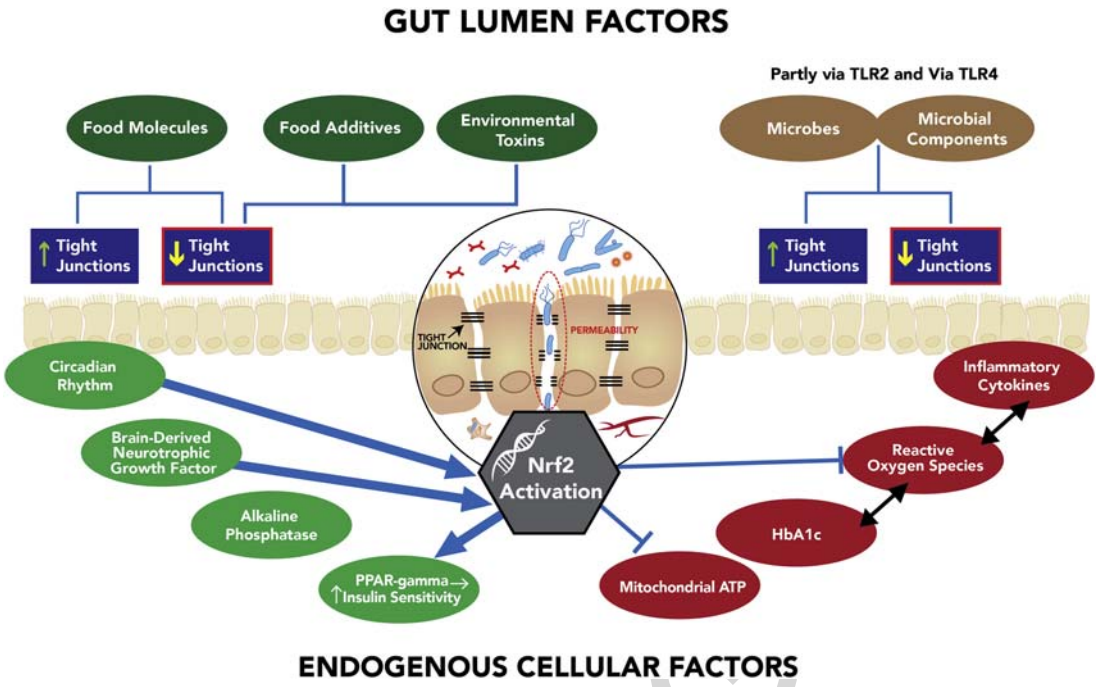


**FIGURE 14.2 Key factors contributing to gut barrier integrity.** Through its tight junctions, the gut barrier is impacted by numerous exogenous factors that include food, environmental toxins, and microbes and their metabolites. It is significantly impacted by endogenous factors associated with inflammation, oxidative stress, circadian rhythms, and poor metabolic control. Hyperglycemia is directly correlated with poor gut barrier function (*Copyright | Christine Houghton 2019*).

Of importance is the fact that this protective effect does not occur in GF animals [92]. However, oral supplementation with IAP in mice improves gut barrier function and prevents luminal proinflammatory factors from gaining access to the circulation. Mechanistically, IAP treatment preserved the localization of the ZO-1 and Occludin proteins of the TJ during inflammation, and as such, was also associated with improved epithelial barrier function [94].

## *Hyperglycemia*

Utilizing animal models of obesity and hyperglycemia, it has been shown that hyperglycemia drives intestinal barrier permeability, through GLUT2-dependent transcriptional reprogramming of IECs and alteration of TJ integrity [95]. Related findings included the observation that the genes most affected by hyperglycemia were those associated with TJ dynamics and also that glycosylated hemoglobin (HbA1c) levels as a measure of longer-term hyperglycemia correlated with microbial influx of both pathogens and commensal microbes.

In turn, hyperglycemia-mediated barrier disruption allowed the systemic influx of microbial products with associated enteric infection; reversal of these effects occurred when hyperglycemia was corrected. By this mechanism, a hyperglycemic environment favors immune dysfunction with diabetic patients especially susceptible to infections and with measurably impaired neutrophil function, humoral immunity, antioxidant defenses, urinary antibacterial activity, and GI and urinary motility [96].

## *Circadian rhythm*

Another factor that significantly impacts the gut ecosystem is circadian rhythm. The human biological clock is a remarkable built-in mechanism, which works according to the Earth's rotation and to variations in light, temperature, and environment to create a 24-h rhythm [97]. Originally identified in the brain, the circadian clock system has since been found in the GI tract. Whereas light controls the brain's central clock, meal timing has profound effects on the intestinal peripheral clock. This clock serves as a cue for rhythmic setting of absorption, epithelial renewal, and barrier functions. Related effects have been identified in Mast cells as key effector cells of allergic reactions and which have been shown to display diurnal variation [97].

Furthermore, not only are daylight and food intake involved in circadian control of gut homeostasis but such control is also responsive to microbial metabolites, adding to the library of intricate signaling mechanisms associated with gut homeostasis [98]. There is now a growing understanding that permeability at the BBB is also dynamically controlled by circadian rhythms and sleep. In addition, sleep promotes the clearance of metabolites along the BBB, with these mechanisms implicated in brain diseases including epilepsy [99].

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## How intestinal microbes communicate with the host

A number of host mechanisms bidirectionally connect the gut ecosystem and distant organs including the brain. These pathways communicate neurally via the VN and spinal cord, as well as via an endocrine link through the hypothalamic-pituitary-adrenal axis (HPA). However, there are three other pathways that involve neurotransmitters, cytokine signaling (immune) and microbial metabolites such as the SCFA (metabolic); degradation products of the amino acid, L-tryptophan also impact (Fig. 14.3).

In addition, the potential pathogenicity or tolerogenicity of the microbes of the gut lumen is detected by various monitoring systems within the gut epithelium and its associated immune cells within the LP [73,100]. A key property of the gut lumen microbes is that they continuously "converse" with the immune system via the IECs. Notably, gram-positive and gram-negative bacteria engage in different "conversations." IECs naturally recognize and interact with commensal bacteria and give instructions to the underlying mucosal immune cells to "initiate an immunological balance between active and quiescent conditions, to eventually establish intestinal homeostasis" [63].

Bacteria inhabiting the GI tract interact with human cells via one of the PRRs, namely the tolllike receptor (TLR). TLR2 plays an important role in enabling cells of the innate immune system to recognize conserved structural motifs on the surface of a wide array of bacteria [101], playing a major role in gram-positive bacterial recognition [102]. TLR2 is the recognition site for peptidoglycans and lipoteichoic acid (LTA) on the wall of gram-positive bacteria, whereas TLR4 recognizes endotoxin or lipopolysaccharide (LPS) on gram-negative bacteria. These receptors are the initiating step in cytokine synthesis and are widely expressed in cells throughout the body, including the neurons [103].

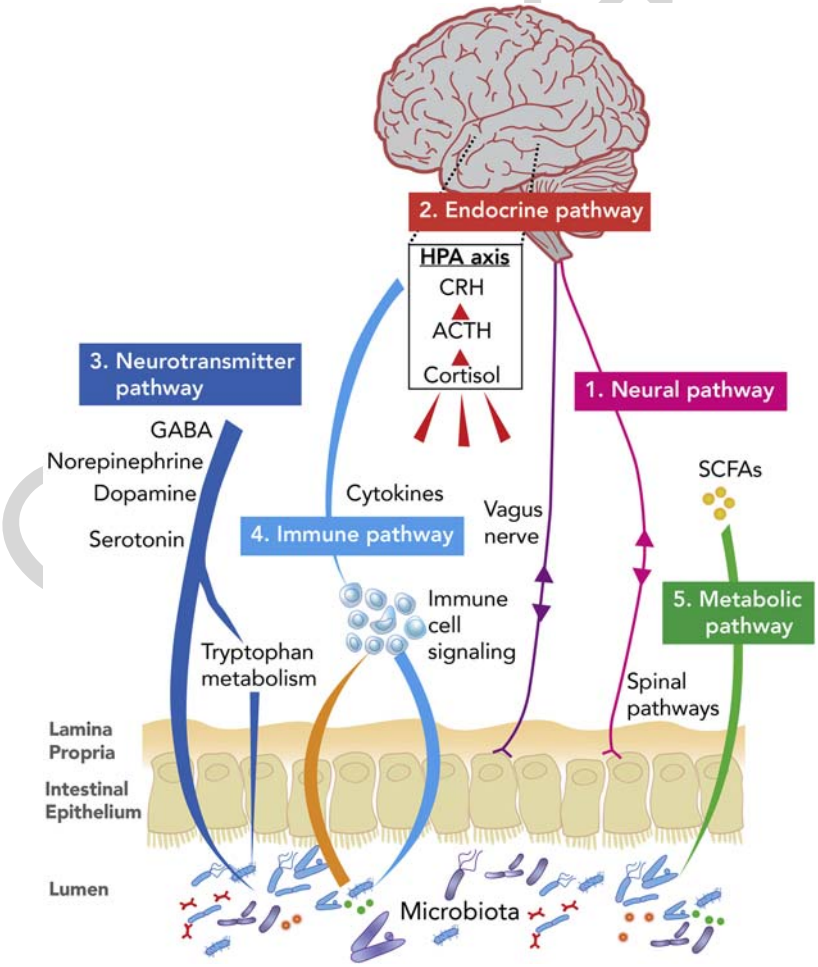


**FIGURE 14.3 The gut-brain axis.** The gut and the brain communicate bidirectionally via several pathways which include; **neural** via the vagus nerve, **endocrine** via the HPA axis, **immune** via cytokines, **metabolic** via microbially generated short-chain fatty acids and via **neurotransmitters**, some of which are synthesized by microbes.

At first thought to be associated only with inflammatory disease, TLR4 is now known to play a significant role in noninfectious and chronic disease. TLR4 is present on the surface of both hemopoietic and nonhemopoietic cells including endothelial cells, cardiac monocytes, and cells of the CNS [104], suggesting that this mechanism may impact other physiological systems as a predisposing process to initiating chronic disease.

In the context of the gut-brain axis, TLR4 is expressed on vagal afferent fibers such that these fibers can sense bacterial products to activate the brain [105]. LPS can also directly activate vagal afferent fibers [106].

## From dysfunctional gut ecology to chronic disease

As mentioned in the "How germ-free animals help elucidate the mechanisms section," animals lacking gut microbiota are resistant to diet-induced obesity, liver steatosis, and insulin resistance, supporting a concept that the gut microbiota contribute significantly to the development of impaired insulin sensitivity and nonalcoholic fatty liver disease (NAFLD) [51]. The multifactorial nature of this association may include the ability of the microbiota to generate toxins such as LPS.

Since insulin resistance leads to an inability of the system to appropriately regulate glucose, the blood biomarker HbA1c becomes elevated. As HbA1c was shown earlier to contribute to impairment of the gut barrier TJs, a feed-forward loop is generated, allowing influx of LPS and antigens. Fig. 14.4 graphically illustrates the process.

## LPS, neurobehavioral and neurodegenerative diseases

Although serum LPS is present in the bloodstream of all healthy individuals, it is found to be elevated in patients with conditions such as severe autism, liver cirrhosis, diabetes, aging, amyotrophic lateral sclerosis, AD, and chronic infection, especially sepsis [107]. It is similarly elevated in a dose-related manner in neurobehavioral conditions by depressing mood, increasing anxiety, and impairing long-term memory; in each case, acute systemic inflammatory markers are also elevated [108].

## Linking the VN to gut ecosystem communication

Of the mechanisms by which the gut and brain communicate, the neural mechanisms play a key role. The VN is considered to be at the interface of the gut-brain axis, communicating through the autonomic nervous system (Fig. 14.3). A perturbation of this axis is associated with the pathophysiology of neurodevelopmental, neurodegenerative, and neuropsychiatric disorders [109,110]. Likewise, abnormal vagal tone is observed in inflammatory and motility disorders of the bowel [111,112].

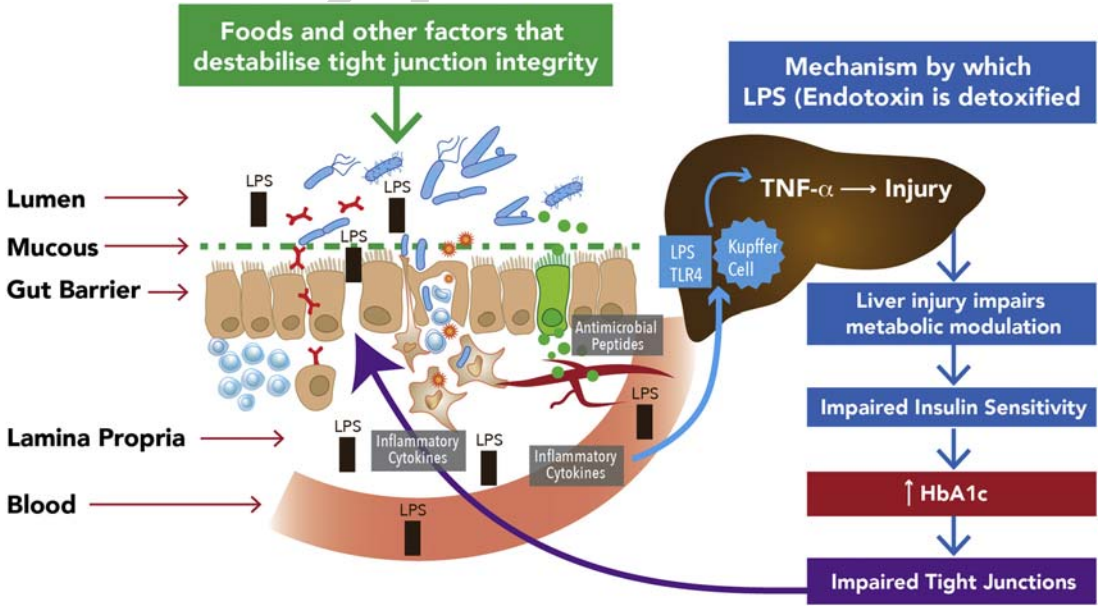


**FIGURE 14.4 The intricate bidirectional mechanisms between the gut ecosystem and systemic organs as the primary driver of cardiometabolic disease.** Gut barrier dysfunction and glucose dysregulation drive metabolic disease in a self-perpetuating loop. When the gut barrier is impaired, LPS has two primary effects; (1) entry via the paracellular spaces to the bloodstream and (2) initiation of the synthesis of inflammatory cytokines. LPS travels via the bloodstream to the liver where it attaches to TLR4 to initiate inflammation with subsequent hepatic damage. Such damage leads to insulin resistance and elevated HbA1c. In turn, HbA1c further impacts the gut barrier, further contributing to influx of LPS and antigens.

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Of the cranial nerves, the parasympathetic VN is a mixed nerve with antiinflammatory properties and is made up of 80% afferent and 20% efferent fibers. In this way, the VN can sense microbial metabolites and transfer this information via its afferent fibers to the CNS from which it can generate an appropriate response [2]. Vagal afferent fibers are distributed to all levels of the GI tract wall but do not cross the epithelial layer; they are therefore not in direct contact with the luminal microbiota and can only indirectly sense signals from the microbiota [2].

Both dysbiosis and stress are associated with irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), with stress stimulating the sympathetic nervous system while inhibiting the VN [113]. It is not yet known whether the dysbiosis is a cause or a consequence of an abnormal gut-brain response [114,115].

Stimulation of the VN which is an approved therapy for epilepsy and depression may also have potential to restore gut homeostasis [2].

## Other biochemical influences on neural function in the gut-brain axis

### Extending the roles of neurotransmitters

To support the classical roles of the neurotransmitters in the CNS, a significant role for epinephrine (E), norepinephrine (NE), dopamine (DA), and 5-HT in gut physiology has been identified. In this capacity, they are associated with major functions of the GI tract; motility, nutrient absorption, innate immune function, and the microbiome [116]. Where neurotransmitter levels are dysregulated in pathological conditions such as IBD and PD, modulators within the gut-brain axis appear to be potential therapeutic targets [117].

In the era of the microbiome, the classical role of selective serotonin reuptake inhibitors (SSRIs) as antidepressants is being challenged in that their effect in the brain may not be their only target [22]. Interestingly, in animals following a subdiaphragmatic vagotomy, SSRIs lose their ability to relieve depressionlike symptoms. This suggests that VN-dependent gut-brain signaling contributes to the effects of oral SSRIs, highlighting the therapeutic potential for vagal stimulation as an alternative treatment of mood disorders [118] but in also highlighting the clinical relevance of addressing gut ecology in patients presenting with depressive illness [119].

High comorbidities exist between psychiatric symptoms such as anxiety (via the HPA) and GI tract disorders such as IBS [120]. In the same way, there is demonstrable comorbidity between the neurodevelopmental and psychiatric impairment seen in autistic spectrum disorder and GI tract dysfunction [121].

### Synthesis of neurotransmitters and effects on the host

A detailed discussion of the pathways associated with neurotransmitter synthesis is beyond the scope of this review. However, not only does the host have the ability to synthesize its essential neurotransmitters but the bacteria also have this capability. Individual bacterial species have been identified for their ability to increase the levels of the neurotransmitters dopamine, noradrenaline, serotonin, gamma-amino butyric acid, acetylcholine, histamine, and others [122], complicating the clinician's ability to determine the primary disease drivers in individual case presentations.

### Serotonin—unraveling the complexities

The role of the microbiota in influencing neurotransmitter levels adds a new level of complexity to our understanding of disease-generating mechanisms. Serotonin has been the focus of extensive gut-brain axis research aiming to unravel distinct mechanisms at the level of the gut and the brain separately, as well as those associated with their interrelationship.

Approximately 95% of the body's serotonin is produced by the EECs of the gut [123] where the synthetic pathways are identical to those in the brain and wherein the amino acid L-tryptophan is the substrate for synthesis via the rate-limiting enzyme, *tryptophan hydroxylase* [117].

Following immune activation or the stress response, L-tryptophan can be metabolized to produce either kynurenine or quinolinic acid, neither of which can cross the BBB if produced outside the brain, whereas L-tryptophan can cross [123]. Quinolinic acid is neurotoxic when produced within the brain, whereas kynurenine is neuroprotective. It has recently been shown that the gut microbiota regulate which of the competing metabolic pathways will dominate metabolism of L-tryptophan and the consequent effects on cognitive and GI tract functions [124].

### Upstream factors in neural function

**Induction of cellular defense mechanisms.** In addition to the foregoing discussion of the role of the microbiota in brain function, this chapter would be incomplete without reference to the *upstream* biochemical factors that govern cellular function. Human cells rely on a range of endogenous mechanisms that govern their ability to defend themselves against various assaults and to maintain homeostasis. The transcription factor Nrf2 is significant in that its activation, typically by an environmental, exogenous, or dietary stressor, leads to the induction of several hundred genes associated with core processes related to cellular defense [125].

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Nrf2 is activated by a variety of factors which include exercise and diet-derived bioactives [126]. Numerous phytochemicals can activate Nrf2 but many are of such low bioavailability that it is doubtful that they can individually reach sufficient intracellular micromolar concentration to impact gene expression, although in combination the effect may be different [127].

Nrf2 is sometimes described as "a multi-organ protector," "the master redox switch," and "a guardian of health span and gatekeeper of species longevity." These terms are indicative of its significance in cellular homeostasis. The crucifer-derived phytochemical sulforaphane (SFN) is the most potent naturally occurring Nrf2 activator with an absolute bioavailability of around 80% [128]. Its diversity of application in humans is illustrated in a number of clinical trials across a diverse array of conditions [129].

### The role of BH4 in neurotransmitter synthesis

A lesser-known property of Nrf2 is that it is associated with synthesis of dopamine, adrenaline, noradrenaline, serotonin, and melatonin.

Just as serotonin is synthesized from L-tryptophan via *tryptophan hydroxylase*, dopamine is synthesized from the amino acid L-tyrosine via the enzyme *tyrosine hydroxylase*. Both enzymes require the cofactor tetrahydrobiopterin (BH4). The amino acid L-phenylalanine can be converted to L-tyrosine via another BH4-dependent enzyme, *phenylalanine hydroxylase*. These enzymes are readily inactivated by oxidation, suggesting that an oxidative cellular environment may impair the synthesis of these neurotransmitters [130].

BH4 is also the cofactor for nitric oxide synthases (NOS); however, inadequate BH4 leads to uncoupling of NOS and the production of highly oxidative radicals, thereby impairing the activity of the hydroxylases [131]. BH4 is synthesized by the rate-limiting enzyme *GTP cyclohydrolase 1*, an enzyme that is regulated by Nrf2, a key mediator of the antioxidant response. Activation of Nrf2 to induce expression of the gene coding for *GTP cyclohydrolase 1* may simultaneously restore redox status.

Dopamine is highly susceptible to oxidative assault and in PD, a progressive neurodegenerative disorder, there is selective loss of dopaminergic neurons in the substantia nigra. Quinone reductase, an enzyme encoded by the Nrf2 target gene, and NADPH:oxidoreductase (NQO1) effectively protect dopaminergic cells. SFN acts as a potent activator of Nrf2 and an NQO1 inducer protects neurons and nonneuronal cells [132].

### The role of BDNF in neurological function

Brain-derived neurotrophic factor (BDNF) is abundantly and widely expressed in the nervous system and is involved in regulating the growth of neurons, neurogenesis, and neuroplasticity [133]. Of relevance here is its role in regulating the epithelial TJ proteins to enhance barrier function, even in the presence of inflammatory cytokines [134]. In an animal study, antimicrobials altered the hippocampal expression of BDNF independent of inflammatory activity, changes in levels of gastrointestinal neurotransmitters and vagal or sympathetic integrity. The authors concluded that the dysregulated microbiota and associated intestinal dysbiosis might contribute to psychiatric disorders in patients with bowel disorders [135].

An additional property of BDNF is its ability to activate Nrf2 and its target genes. The significance of this was demonstrated in a model wherein depressed animals with low hippocampal BDNF levels exhibited decreased nuclear translocation of Nrf2, leading to a state of persistent oxidative stress. These results reveal a novel role for BDNF in controlling redox homeostasis because activating Nrf2 translocation restored redox homeostasis and reversed vulnerability to depression [136].

Because BDNF can modulate both the neuronal redox environment and epithelial barrier function, it would be clinically useful to know how to enhance it. Acute low-intensity exercise was shown, in an animal study, to activate hippocampal BDNF expression, supporting the role of appropriate exercise in human health [137].

## Nutrition-specific requirements of the host and its microbiota

### Nourishing the host cells

Human nutrition is an established science that aims to provide for the needs of humans in different environments and across different stages of the life cycle. However, the evolving discussion around the role of the microbiota in the GI ecosystem adds a new dimension to the science of human nutrition.

In aiming to restore homeostasis to the gut ecosystem, the host diet should optimize macro- and micronutrient intake for the IECs and their underlying immune network, ensuring that protein is of appropriate quality to supply L-glutamine for the enterocytes of the small intestine, especially since an inflamed small intestine has a heavy demand for this nonessential amino acid [138].

In line with the earlier discussion on the multiple roles of Nrf2 in core cellular processes, a diverse array of phytochemicals including SFN-yielding vegetables is recommended [139,140]. SFN's ability to readily cross the BBB may partly explain its neuroprotective effects in a range of neurological conditions [141].

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## Nourishing the microbiota

The symbiotic relationship that exists between the microbiota and the human host is evident when considering the nutrient requirements of each. Essentially, the host provides food for the microbes, which in turn consume that food to produce metabolites necessary for the health of the host; generated primarily in the colon, the SCFAs are the most important of these.

### *Dietary fat*

As discussed earlier, the macronutrient composition of the host diet has a major and rapid impact on the diversity and relative proportions of microbes inhabiting the GI tract. A diet high in fat, especially saturated fat, is associated with reduced microbial diversity, and in a 6-month randomized controlled trial of healthy young adults, an unfavorable shift to gram-negative microbial species with abnormal metabolic biomarkers was observed [142]. The known adverse effects of a high-fat diet on the host also adversely impact the microbiota.

### *Dietary protein*

High relative protein intake influences the microbiota, promoting the proliferation of bile-tolerant species, while decreasing the abundance of the saccharolytic microbes required to metabolize nondigestible carbohydrates that escape enzymatic digestion in the small intestine [45].

### *Dietary indigestible prebiotics*

A range of carbohydrate foods, insoluble fibers, resistant starches, and polyphenols are metabolized by the microbiota, each influencing microbial composition. The major SCFA metabolites acetate, butyrate, and propionate are the result of microbial fermentation of dietary fiber, with butyrate preferred by the colonocyte.

These metabolites act as signaling molecules that supply energy to the colonocytes in particular, improving the intestinal environment and directly affecting various host peripheral tissues [143]. The SCFAs act as signal transduction molecules via G-protein coupled receptors on target cells and also as epigenetic regulators of gene expression by the inhibition of histone deacetylase (HDAC) [143].

Because SCFAs cross the BBB, there is considerable interest in dietary interventions that favor production of SCFAs in order to target key inflammatory pathways. These pathways are dysregulated in cardiovascular disease, type II diabetes, and systemic inflammation and are consistently linked to an attenuated lifespan in schizophrenia [144].

The effects of SCFAs on brain health are a new area of research with little available human evidence. However, a 2018 animal study investigated the direct effect of SCFAs on behaviors associated with psychosocial stress. The intervention increased responsiveness to an acute stressor and exhibited behavioral test-specific antidepressant and anxiolytic effects [145].

## How does nature maintain the gut-microbiome-brain axis?

### The host cells

In addressing the IEC as a primary target, mechanisms to maintain homeostasis of these cells and their underlying cells are a key consideration. The range of exogenous and endogenous factors governing the integrity of the gut barrier is critical to this process.

As the science of redox homeostasis has evolved, it has become clear that the *Free Radical-Antioxidant* theory of the past was just too simplistic and that high doses of direct-acting antioxidant vitamins in particular can inhibit the cell's protective responses by masking nutrigenomic signals [146]. The role of Nrf2 in inducing the expression of hundreds of protective genes as a primary defensive mechanism would appear to better explain the endogenous mechanisms that have sustained human life for millennia, to a large extent as the response to diet and exercise [147].

Although the polyphenols per se exhibit very low bioavailability, the metabolites produced by microbial fermentation are independently bioactive [127]. A diet rich in a wide variety of plant foods exhibits significant activation of Nrf2 as a result of the additive effect of many such foods. The importance of plant foods in modulating redox-inflammation status was investigated in a clinical trial where young adults were asked to consume different quantities of plant food over 30 days. Biomarkers of inflammation, CRP, TNF-alpha, IL-6, and others, together with homocysteine were significantly lowered by a diet containing >660 g of (nonorganic) vegetables daily [139].

It is likely that the same or greater quantity of vegetables simultaneously upregulated Nrf2, although that was not measured. In an era in which it may not be possible to persuade patients to consume >660 g of vegetables daily, a high sulforaphane-yielding whole broccoli sprout supplement may be an appropriate prescription [126,129].

### The microbiota

An environment that supports the health of the IEC and immune network will most likely also support the microbiota, assuming that appropriate prebiotics are part of the regular dietary intake. The effect of the diet on the microbiota is influenced by seasonal variations, and this is

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apparent in traditional societies such as the Hadza hunter-gatherers in Tanzania, who exhibit an exceptionally diverse microbiome. Such seasonal effects are less likely to occur in those consuming a modern Western diet, which contributes to lower diversity [148].

A detailed discussion of the available prebiotics that will enhance proliferation of the butyrate-producers and other desirable microbes is beyond the scope of this chapter and readers are referred to a recent comprehensive review of the subject [46].

## Therapeutic interventions

### Probiotics as therapy

Although it is tempting to consider that probiotics might achieve the desired restoration of a dysfunctional gut ecosystem and its target axes, this would address only one-half of the bidirectional relationship between the host and its resident microbiota. Nevertheless, probiotic supplementation is a key intervention recommended by integrative and complementary medicine clinicians for a range of conditions.

When considering the rationale for probiotic supplementation, how does one reconcile such therapy in light of the following unanswered dilemmas? (1) Probiotics typically do not colonize the host, leaving no trace in a stool sample within a few weeks of cessation [149]; (2) Diet can rapidly and reproducibly influence the microbiota [45]; (3) A metagenomic analysis can identify species not available as supplements [150]; (4) Post antibiotic therapy, reconstitution of the microbiome has been shown to take 5 months longer with a probiotic than without [151].

Nevertheless, strong evidence supports the hypothesis that the efficacy of probiotics is both strain-specific and disease-specific for a number of diseases and many clinical trials have achieved successful outcomes in this manner [152]. It appears, however, that the potential for a particular strain to provide benefit is commonly conflated with the notion that any probiotic supplement claiming a high microbial count will benefit the host and reestablish the microbiome after antibiotic therapy [153,154].

### Antimicrobials as therapy

The ready availability of stool microbiome and SIBO breath testing has seen interest in therapies aimed at replacing missing gut microbes and/or eradicating pathogens or pathobionts.

Even though IECs are equipped with several specialized mechanisms to actively eradicate undesirable microbes, enhancement of these mechanisms does not appear to be a target of the therapies commonly employed by clinicians.

A popular therapeutic approach within the Integrative and Complementary Medicine community is that of "weed, seed and feed," wherein the first step involves the use of a plant-derived antimicrobial extract or oil to address dysbiosis. The "seed" step follows with the administration of either individual or combinations of probiotics which are "fed" by the use of prebiotic supplements.

A Google search using the term "weed seed feed" returns numerous entries but notably, none of these is from a peer-reviewed PubMed publication. As is the case with pharmaceutical antibiotics, no plant-derived antimicrobial is selective for pathogens alone, so that some degree of commensal destruction will occur with resultant compromise of the gut microbial population [155]. The uncertainties in the nature of SIBO and its diagnostic options are highlighted in this 2019 review of SIBO research over the last 3 years [156].

### Is it time for a host-centric model?

Given that there remain many unanswered questions in relation to the clinical applications of both probiotics and antimicrobials as therapy for addressing dysbiosis and consequent chronic conditions, a model that focuses on restoring homeostasis within the gut ecosystem may more reasonably coincide with the endogenous cellular mechanisms.

A diet and lifestyle that is ideal for the host may, with some specific consideration for the prebiotic needs of the commensal microbes, be similarly ideal for the microbiome. Addressing the requirements of a healthy gut ecosystem to harness the various endogenous mechanisms discussed in this chapter should simultaneously provide for all physiological processes, including the gut-brain axis.

In the words of Litvak et al., "Because our immune system already has a way to balance the colonic microbiota, harnessing this host control mechanism for therapeutic means could provide an alternative to targeting the microbes themselves for remediation of dysbiosis" [61].

## Conclusion

In our seemingly insatiable quest to manipulate the composition of the gut microbiome for the enhancement of human health, it is worth contemplating that Nature has sustained human life on this planet for millennia—and all without any of the benefits conferred by modern technology. Clearly, there are processes embedded within human cells that have allowed them to adapt to their ever-changing environments. With a better understanding of these endogenous mechanisms, it may be possible to formulate clinical strategies that resemble those used by Nature

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herself. This chapter suggests that an important piece of the gut-immune health and gut-brain puzzle has been largely overlooked and that a greater focus on restoring the function of the remarkable intestinal epithelial cells is needed in order to redress the balance.

The bidirectional interaction represented by the *Gut-Microbiome-Brain Axis* has markedly changed the way we must continue to consider aberrant function of the human nervous system, not in isolation but in integration with the GI ecosystem of the host in expectation of a favorable impact on human health and behavior.

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