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# The Leaky Gut: Mechanisms, Measurement and Clinical Implications in Humans

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## Abstract

The objectives of this review on "leaky gut" for clinicians are to discuss the components of the intestinal barrier, the diverse measurements of intestinal permeability, their perturbation in non-inflammatory "stressed states", and the impact of treatment with dietary factors. Information on "healthy" or "leaky" gut in the public domain requires confirmation before endorsing dietary exclusions, replacement with non-irritating foods (such as fermented foods), or use of supplements to repair the damage. The intestinal barrier includes surface mucus, epithelial layer, and immune defenses. Epithelial permeability results from increased paracellular transport, apoptosis, or transcellular permeability. Barrier function can be tested *in vivo* using orally administered probe molecules or *in vitro* using mucosal biopsies from humans, exposing the colonic mucosa from rats or mice or cell layers to extracts of colonic mucosa or stool from human patients. Assessment of intestinal barrier requires measurements beyond the epithelial layer. "Stress" disorders such as endurance exercise, nonsteroidal anti-inflammatory drugs administration, pregnancy, and surfactants (such as bile acids and dietary factors such as emulsifiers) increase permeability. Dietary factors can reverse intestinal leakiness and mucosal damage in the "stress" disorders. Whereas inflammatory or ulcerating intestinal diseases result in leaky gut, no such disease can be cured by simply normalizing intestinal barrier function. It is still unproven that restoring barrier function can ameliorate clinical manifestations in gastrointestinal or systemic diseases. Clinicians should be aware of the potential of barrier dysfunction in gastrointestinal diseases and of the barrier as a target for future therapy.

### Keywords

permeability; mucus; tight junctions

## INTRODUCTION

The objective of this review is to address three questions based on data almost exclusively acquired in humans: What does the "leaky gut" mean? Should clinicians diagnose leaky gut

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and, if so, how can it be diagnosed? Is the leaky gut treatable? There are vast numbers of papers that link “leaky gut” with altered microbiota in disease models in experimental animals, from allergy to non-alcoholic steatohepatitis to depression and amyotrophic lateral sclerosis. This review focuses almost exclusively on the evidence from human studies, since the clinicians need to address the three questions in the context of their patients. In addition, it focuses on the evidence of leaky gut in non-gastrointestinal diseases, which are the focus of many diseases or disorders in which leaky gut and microbiota are considered to be etiopathologically important mechanisms.

There is much folklore about the leaky gut and its relationship to microbial balance within the gut. One of the first “hits” in searching information on leaky gut on the internet provides comprehensive advice, contrasting what happens when the balance is “right” and when “out of whack”, and advice on how to get the gut microbes back into balance (BOX 1).

There is no controversy regarding barrier dysfunction in diseases resulting in intestinal inflammation and damage such as celiac or Crohn’s disease, or ulceration from nonsteroidal anti-inflammatory drugs (NSAIDs) resulting in structural abnormalities of the epithelium. The “leaky gut” is a simplistic term reflecting intestinal permeability, a function that was extensively studied in these diseases and reported in the scientific literature from 1970–1990.[1–3]

There may be several reasons for this resurgence of interest in the “leaky gut”. First, there is frustration about the lack of perceived advances in the management of common gastrointestinal symptoms such as pain, diarrhea and bloating; thus, a cause, such as leaky gut, is sought. Second, the scientific literature has promulgated “dysbiosis” in diverse states, from obesity to autism, despite evidence that this documented parabiosis[4] does not necessarily result in any metabolic or other changes in mucosal functions, including barrier function, or the role in pathogenesis of these diseases.[5] Third, there is scientific research using diverse methods that documents alterations in human intestinal barrier function in disorders such as irritable bowel syndrome or food allergy. However, there is no current gold standard with clear performance characteristics of the tests for barrier function, the diverse methods available actually measure very different endpoints, and the clinical significance and relevance are unclear. Fourth, as explained by Quigley,[6] there are popular perceptions of the barrier as a single cell thick, the epithelial layer having disruptions of intercellular connections leading to increased permeability and consequent access to the blood stream for various noxious chemicals, intact bacteria and a host of dietary and microbial components, and designation as the primary abnormality in diverse diseases such as food intolerance, fibromyalgia, chronic fatigue syndrome, and autism (all unsupported by any data).

Despite the recommendation to not self-treat, there are many resources, books and articles with recommendations on restoring the “healthy balance” including eating dirt, curing with candida, and education about the microbiome, the “human super organism” and “the good gut”.

Given the current perceptions on “leaky gut” that appear informed mostly by folklore or overreaching conclusions based on limited data, it is important to provide a balanced view of

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the scientific data to facilitate the role of clinicians in addressing the nature, diagnosis and treatment of abnormal intestinal barrier function in humans. To address this, it is important to characterize the intestinal barrier, the pathways between and through epithelial cells, and measurement of intestinal permeability in humans. This provides the basis for examination of non-intestinal diseases characterized as “stress” can really break the barrier, and to review possible treatments including diet, natural substances, and medications.

## The Intestinal Barrier

The intestinal barrier is a dynamic entity interacting with and responding to various stimuli. It consists of multiple elements. In the lumen, there is degradation of bacteria and antigens by bile, gastric acid, and pancreatic juice, and commensal bacteria that inhibit the colonization of pathogens by production of antimicrobial substances. The next element of the barrier is the microclimate consisting of the unstirred water layer, glycocalyx, and mucus layer which prevent bacterial adhesion by immunoglobulin A (IgA) secretion and by the physical barrier provided by the glycocalyx and mucus. Epithelial cells, connected by apical junctional complexes, have the ability to transport luminal content, but they also react to noxious stimuli by secretion of chloride and antimicrobial peptides. The Paneth cells in the epithelial layer, where they are most numerous in the crypts, also produce high quantities of defensins and several other antibiotic peptides and proteins when exposed to Gram positive and negative bacteria or bacterial products such as lipopolysaccharide. Beyond the epithelium, the lamina propria provides defense based on innate and acquired immunity cells secreting IgA, cytokines, chemokines, and mast cell proteases, as well as endocrine and secretomotor mechanisms mediated by the enteric nervous system which result in intestinal propulsive motility.[7] Some of the important transmitters are serotonin (5-HT), histamine, and cannabinoids.

The mucus layer consists of two components: an inner firmly adherent layer where bacteria are sparse and secreted peptides are protective with antibacterial functions (e.g., defensins, lysozyme); and a thicker and loosely adherent outer layer where bacteria and bacterial products are abundant. The mucus layer is thicker in the colon than in the small bowel and may reach a depth of over 800 microns, which is not much less than the height of an entire villus (range 500–1600 microns). There is regional variation in the barrier along the gut; in the small intestine, pore size increases from 4–5 Å at the villus tip to over 20 Å at the base of the crypt. In addition, the microbiota influences the barrier, and elements of the barrier impact the microbiota.[6] There are several examples in the literature demonstrating diverse effects of bacteria and their products on intestinal barrier structure or function. Thus, *Bifidobacteria* enhance barrier function in experimental necrotizing enterocolitis in mice,[8] the yeast *S. boulardii* has beneficial effects on altered intestinal microbiota and epithelial barrier defects in different pathologies,[9] and different strains of *E. coli* have opposite effects on the barrier: *E. coli* Nissle 1917 stimulates the TJ protein ZO-2;[10, 11] whereas, a prototypic translocating bacterium, *E. coli* strain C25, increases permeability.[12] Products of the bacteria such as bacterial toxins, or bacterial dehydroxylation producing secondary bile acids and short chain fatty acids produced by bacterial fermentation protect against bacteria or enhance the barrier function.[13–15]

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At the level of the epithelial cells, from the apical to the basal domains of enterocytes, there are three sets of intercellular junctions: tight junction [zonula occludens (ZO)], adherens junction (zonula adherens), and desmosome. Together they comprise the apical junctional complex, which supports the dense microvillus brush border and regulates epithelial barrier function and intercellular transport.[16] The anatomy and composition of the mucosal barrier and its intercellular junctions are shown in Figure 1.[17]

In general, it is recognized that there are three distinct paracellular epithelial permeability pathways: ‘leak’ and ‘pore’ pathways which are regulated by tight junctions and define intestinal permeability; and an ‘unrestricted’ pathway which is associated with apoptotic leaks in pathological states, is independent of tight junctions, and provides access of luminal antigens to the lamina propria. In the presence of erosions or ulcers, bacteria gain access to the mucosa (Figure 2).[16,18]

## The Importance of the Mucus Component of the Barrier

Mucus is secreted by the goblet cells and serves as the first physical defense in the barrier, preventing antigens, toxins and bacteria from directly contacting the epithelial cells. The elements of the mucus layer are highly glycosylated mucin proteins with a central protein core [abundant in serine (Ser), threonine (Thr), and proline (Pro) amino acid residues) and O-glycosylation with hexoses and hexosamines oriented almost perpendicular to the protein core like a bottle brush, forming a gel-like sieve overlying the intestinal epithelium.[19]

In the small and large intestine, mucin 2 (MUC2) is the most abundant mucus protein secreted by goblet cells. Intestinal epithelial cells (IECs) also express transmembrane mucins (MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17) that remain attached to the apical surface and form the glycocalyx together with glycolipids. Other major mucus proteins secreted by goblet cells are chloride channel regulator, calcium-activated-1 (CLCA1), Fc globulin binding protein (FCGBP) which covalently binds and cross-links mucus proteins, zymogen granule protein 16 (ZG16, a small lectin-like protein that binds to Gram+ organisms), and antibodies, especially IgA. Secreted mucus mixes with Paneth cell secretions containing antibacterial peptides, lysozyme, deleted in malignant brain tumors 1 (DMBT1), and also MUC2.[19]

Immune regulators, such as antimicrobial proteins (AMPs) and IgA molecules, are released in the mucus gel in a gradient from the epithelium to the lumen, thereby reinforcing the defense against the luminal microbes.[20] The composition of the mucus layer can affect the microbiota in the gut, while the microbiota also determines the properties of the mucus gel. [21] Muc2 knock-out mice spontaneously develop colitis.[22]

Surfactants, including bile salts [chenodeoxycholate (10 mM) and hyodeoxycholate (10 mM), but not cholate (10 mM), ursodeoxycholate (10mM), or Tween-20] induce secretion of mucus.[23] In fact, bile salts impact the ability of mucus to serve as a barrier to hydrophilic and lipophilic compounds.[24] Secretion of mucus is a prelude to the epithelial damage that leads to colonic secretion by the secretagogue bile acids, chenodeoxycholic acid[25, 26] and deoxycholic acid,[27, 28] and these effects can be partly inhibited by prostaglandins. Intra-

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arterial prostaglandin E2 (PGE2) evokes the secretion of intestinal mucus,[29] and PGE2 reverses NSAID-enteropathy, in part, by inducing mucus secretion.[30] Other goblet cell secretagogues are cholinergic agonists, histamine, peptide tyrosine tyrosine (peptide YY), and serotonin. Overall, there is a role for the enteric nervous system, the enteroendocrine cells, and resident immune cells in mediation of colonic mucus release.[31] Cholinergic inhibition of mucin secretion with intra-arterial atropine reduced the epithelial damage and fluid secretion secondary to 5mM sodium chenodeoxycholate in the rabbit colon in vivo.[32]

Dietary emulsifiers, like bile acids, are amphipathic, that is, they are molecules with hydrophilic and lipophilic sections that maintain fat molecules in liquid suspension or water-soluble components in a hydrophobic environment. Dietary emulsifiers interact with the multilayered endogenous mucus secretions that coat the luminal surfaces of the intestinal tract and may compromise the ability of human mucus to prevent contact between microorganisms and intestinal epithelial cells.[33] Numerous synthetic surfactant food additives (anionic, cationic or non-ionic) are used in the food industry [such as mono- and diglycerides or esters of fatty acids (E471. E473, E475)], as reviewed elsewhere.[34, 35] Some of these have been shown to increase intestinal permeability through paracellular and/or transcellular mechanisms, and some of them were also shown to inhibit P-glycoprotein or have mucolytic activity, such as the two emulsifiers, carboxymethylcellulose (CMC) and polysorbate 80 (Tween).[36]

Additionally, based on the general characteristics of surfactants, it can be predicted that they decrease the hydrophobicity of the mucus layer, which has also been shown to be associated with increased intestinal permeability.[34] Dietary emulsifiers may interact with gut microbiota and altered mucus thickness to promote colitis in *Il10* and *Tlr5* knock-out mice, which are predisposed to development of spontaneous colitis[37] and increase translocation of *E. coli* across intestinal epithelial cells.[38]

## Example of Increased Transcellular Permeability

The examples provided above focused predominantly on the intercellular barrier and mucus barrier. However, there is evidence that there may be altered expression of cellular transport mechanisms that may ultimately lead to intercellular barrier dysfunction and systemic inflammation. An excellent example of the potential impact of increased transcellular permeability without apoptosis or intestinal ulceration is provided by the demonstration, in mouse models of obesity and diabetes, that hyperglycemia drives intestinal barrier permeability through glucose transporter 2 (GLUT2)-dependent transcriptional reprogramming of intestinal epithelial cells and alteration of tight and adherence junction integrity.[39] These findings were demonstrated by reduced ZO-1 expression and increased fluorescein isothiocyanate (FITC)-dextran in serum after oral administration, indicating increased intestinal permeability, as well as an increased short-circuit current measured across the epithelial layer in Ussing chambers. There were also increased intestinal bacteria at systemic sites. Control experiments showed that these effects were due to hyperglycemia rather than obesity or alterations in leptin signaling.

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## Intestinal Permeability: Pathophysiological Mechanisms and Methods of Analysis

In this section, diverse methods of measurement and analysis are discussed, and the strengths and weaknesses are addressed. It is also important to note that intestinal permeability is influenced by several factors, which are not always considered in the publications, including the circadian cycle[40] and stress.[41]

### Orally administered probe molecules

Intestinal permeability is most commonly measured indirectly in humans by the fractional urinary excretion of orally ingested probes which cross the intestinal epithelium by the paracellular pathway, enter the bloodstream, are filtered by the glomerulus, and excreted in the urine without active reabsorption in the kidney.[42] Fractional urinary excretion can, therefore, be used as an indirect measure of intestinal permeability.

The most commonly used probe molecules are saccharides. Although regional differences for preferred absorption sites have been suggested,[43] it is important to note that sucrose is only useful in the first hour post ingestion, as it is rapidly metabolized (to glucose and fructose) and, therefore, at best, provides information about gastric and duodenal permeability. Moreover, other monosaccharides, such as mannitol and rhamnose, and disaccharides, such as lactulose and sucralose, are all absorbed in the small bowel and colon, and the timing of urinary excretion provides the best way to differentiate regional analyses: 0–2 hours reflects predominantly small intestinal permeability; and 8–24 hours reflects almost exclusively colonic permeability.[44] Also, among these saccharides, sucralose is the one not metabolized by colonic bacteria; similarly, polyethylene glycol (PEG) 400 and radioactive chromium complexed with ethylene diamine tetracetic acid ($^{51}$Cr-EDTA) are not degraded by colonic bacteria. However, the utility of these probe molecules is somewhat compromised by the “background” ingestion of some of the sugars, particularly mannitol and sucralose.

Until relatively recently, interpretation of permeability tests was based on the following assumptions. Lactulose is a relatively large molecule, can only cross via the leak pathway or at sites of epithelial damage, and is considered a marker of barrier integrity. Mannitol, which is reputedly one-third as large as lactulose, is assumed to cross the pore pathway, which allows passage of sodium ions, water and small solutes; therefore, mannitol and other monosaccharides such as rhamnose used to be regarded as measures of surface area.[42] The inference was that the lactulose: mannitol ratio might measure the sum of leak pathway permeability and epithelial damage normalized to surface area. However, a review of the molecular sizes of the sugar probe molecules (Table 1) suggests that there is no relevant difference in the reported or estimated molecular diameters and, therefore, it appears unlikely that they traverse the epithelium through different pathways.

In fact, the mass of saccharides that is absorbed during one hour in the healthy gut following ingestion orally is typically up to 2% of rhamnose and 0.07% of lactulose administered in children in USA.[45] In healthy adults, the fractional excretion over 24 hours is 31.2±3.4%

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(SEM) for $^{13}$C-mannitol, and 0.32±0.03% for lactulose.[46] Therefore, despite the similarity in molecular diameters, there is a 100-fold difference in the percent of recovery of the mono- and disaccharides; however, if the larger molecule traverses the “leak” pathway, the latter might also allow passage of the small molecule, and there is no compelling evidence that the different sugars actually traverse the intestinal barrier via different pathways.

Other factors, such as tertiary molecular structure, are likely relevant to explain the marked difference in absorption ratios of monosaccharides and disaccharides which is 30- to100-fold for the monosaccharide, mannitol, compared to the disaccharide, lactulose. In practice, modern methods of assay based on liquid chromatography-mass spectrometry accurately measure the saccharides, and the most commonly used combinations are therefore lactulose or sucralose (as disaccharides) with mannitol or rhamnose (as monosaccharides). Because of potential “contamination” by environmental exposures to mannitol and sucralose, the lactulose and rhamnose or $^{13}$C-mannitol saccharides are increasingly used for in vivo permeability measurements. In addition, as indicated above, 0–2-hour urine collection reflects predominantly small intestinal permeability, and 8–24 hour collection almost exclusively colonic permeability.

Overall, these tests still have limited validity based on uncertainty of the normal values, lack of standardization of test procedure, and lack of validation including responsiveness of a standardized test to treatment.

### *In vitro* or tissue measurements of intestinal barrier

Several methods are used to assess intestinal permeability on biopsies taken from the human gut and measured by the transfer of probe molecules across mucosal biopsies in Ussing chambers (in association with measurements of transepithelial resistance and short circuit current measurements). Other approaches quantitate the tight junction proteins in the mucosal biopsies, or they assess the fecal supernatant in cellular monolayers or rat or mouse colonic mucosa in vitro.

There are differences in *in vivo* compared to *in vitro* measurements of barrier functions. The molecular size of probe molecules that can cross the epithelial barrier in humans *in vivo* is at least 10-fold smaller than *in vitro*, which shows that molecules of approximately 4–40 kDa (e.g., dextran 4 or 40) easily traverse the intestinal mucosa in a Ussing chamber *in vitro*. There are also differences that may reflect additional functional barriers in vivo that are excluded in the in vitro studies, including the lamina propria, innervation by submucosal neurons, and permeability of end-capillaries that constitute other potential barriers impeding passage of the probe molecules into the circulation *in vivo*. For example, intercellular complexes are under neurohumoral control, such as from VIP and cholinergic neurons, from the submucosal plexus,[47, 48] and these are lost in biopsied mucosa.

### Endoscopic measurements of intestinal barriers in humans

Two techniques are available: first, confocal endomicroscopy, which shows leaks of intravenously administered fluorescein into the gut lumen during endoscopy[49] (e.g., in response to food-associated changes in the intestinal mucosa of patients with diarrhea-predominant irritable bowel syndrome); and second, endoscopic mucosal impedance, in

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which a 2-mm diameter catheter is passed through an endoscope and placed in contact with the duodenal mucosa under direct visualization, and two circumferential sensors, placed 2mm apart on the mucosa for 0.10s, in the 4 quadrants of the duodenum with a decompressed lumen, and all fluid aspirated.[50] The studies of food-associated changes during these challenge tests provide some evidence that there can be barrier changes that may indeed support the concept of transient “leakiness” of the gut.

## Abnormal Barrier Function in Intestinal Disease States

It is clear that inflammatory or ulcerating diseases result in abnormal intestinal barrier function. However, this is not the category of disease that is being associated with leaky gut, as discussed in the next section. The abnormal barrier function is well described for conditions such as inflammatory bowel disease (IBD), as well as in first degree relatives of patients with IBD,[3, 51] celiac disease and gluten sensitivity without overt celiac disease in patients with HLA-DQ2/8 (genotype associated with celiac disease,[52, 53] intestinal graft versus host disease, enteric infections and infestations, and human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDs).[42] There is also extensive literature[54] documenting abnormal intestinal permeability in irritable bowel syndrome and the association of abnormal permeability with pain in IBS, although the degree of altered barrier function is clearly lower than in inflammatory bowel or celiac diseases. Longitudinal studies in patients with IBD suggest that increased intestinal permeability preceded relapsed of Crohn’s disease,[55] suggesting a pathogenetic role of the epithelial barrier in the pathogenesis of gut inflammation; in addition, IBS is highly prevalent in first degree relatives of IBD patients,[56] suggesting that intestinal permeability could be a relevant factor in the determination of symptoms.

Several other non-gastrointestinal diseases have been associated with leaky gut, based on limited or no supporting data,[6,42] including asthma, autism, Parkinson’s disease, multiple sclerosis, eczema, psoriasis, eosinophilic esophagitis, environmental enteropathy, kwashiorkor, fibromyalgia, depression, chronic fatigue syndrome, multi-organ failure syndrome (shock, burns, trauma), non-alcoholic fatty liver disease (NAFLD), alcoholic cirrhosis, obesity, metabolic syndrome, pancreatitis, and rheumatoid arthritis. Two separate groups independently studied small bowel permeability in patients with eosinophilic esophagitis; one group documented increased small bowel permeability, though the mechanism whereby this results in the eosinophilic infiltration of the esophagus is unclear; [57] the second group did not document increased small bowel permeability, but they reported improvement in the eosinophilic esophagitis with an elemental diet.[58] All of these diseases and disorders associated with possibly altered intestinal barrier function are pathological diseases, not what is usually associated with nonspecific “leaky gut”.

## Leaky Gut: The Pro Arguments

The concept of a leaky gut in non-gastrointestinal diseases is supported by evidence of dysfunctional gut mucosal barrier in stress-associated conditions and the response of the altered barrier to non-drug interventions; associations of disease states with altered intestinal permeability and microbiome; and alterations in intestinal permeability as a result of gut-

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directed therapy in diverse conditions including healthy people or diverse groups of children in central African countries.

## Dysfunctional gut mucosal barrier due to endurance exercise and effects of non-drug intervention

Table 2 summarizes the literature on two types of conditions that result in stress to the intestinal barrier, with documented effects on intestinal permeability or biochemical evidence of mucosal damage, and restoration with a dietary, non-pharmacological intervention. These studies suggest that there are "stress" states in which there is documentation of altered barrier function and examples of normalization that would support the concept of a transient leakiness of the gut barrier. There is also recent evidence suggesting that changes in intestinal microbiota composition (characterized by increased α-diversity and changes in the relative abundance of >50% of identified genera, including increased abundance of less dominant taxa at the expense of more dominant taxa such as *Bacteroides*) and metabolism (reduced serum IL-6 and reduced stool cysteine) coincide with increased intestinal permeability (documented by increased sucralose excretion in urine after oral load) in young adults under prolonged physiological stress in the form of a 4-day cross-country ski march.[59] Discussion of the role of the microbiota in intestinal barrier function is beyond the scope of the current article. Further studies are required to explore the hypothesis that epithelial barrier dysfunction associated with mucosal enrichment of specific bacterial strains may predispose to a shift to disease-associated microbiota that eventually leads to pathological consequences such as inflammatory bowel disease in individuals with genetic predisposition.[60]

## Associations of disease states with altered intestinal permeability and microbiome

From a detailed review of the literature, it is clear that animal models of disease have documented a three-point relationship: disease phenotype, barrier change, and altered microbiota. As exemplified best with chronic liver disease, the directionality of the relationship is controversial, even from animal studies. For example, one hypothesis argues that increased endogenous production of ethanol by gut bacteria (e.g., *E. coli*) caused by small intestinal bacterial overgrowth results in increased intestinal permeability, bacterial translocation and hepatic inflammation due to the translocated bacteria or their products.[61] An alternative hypothesis is that the liver disease causes a systemic inflammatory response that leads to increased intestinal permeability, with bacterial translocation and further hepatic damage.[62, 63]

In many studies in the literature, particularly in human studies, the three focal points of the relationship are not all examined and "triangulation" is therefore based on hypothesis or inference based on the association between two of these three factors. Table 3 summarizes information garnered from studies in aging, food allergy, liver disease, parenteral nutrition (or enteral exclusion), and neuropsychiatric diseases in humans or in animal models where there are no human data available. In general, the data should be regarded as hypothesis-generating, that is, the leaky gut may be a cause or an effect of the disease (as in the case of liver disease), and there may be either normal or dysbiotic microbiota that lead to inflammatory or other consequences that have impact on the disease. In some situations,

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alteration of the microbiota may result in reduced severity of the disease in humans, as demonstrated with hepatic cirrhosis in response to treatment with *Lactobacillus casei* strain Shirota or VSL#3 which contains eight lyophilized bacterial strains.[64]

### Alterations in intestinal permeability as a result of gut-directed therapy

A third pro argument is provided by examples from the literature of *in vivo* human studies showing alterations in intestinal permeability as a result of gut-directed therapy, as summarized in Table 4. One study worthy of specific comment is the randomized, placebo controlled trial of oral glutamine in about 100 patients showing normalization of LMR in association with improvement in the IBS-SSS (symptom severity score), stool frequency and consistency [65]. However, most of the other studies are small, used different nutrients and diverse methods, and require replication.

There is also hope that probiotics or commensal organisms improve intestinal barrier function;[66] however, the evidence to date is sparse, often based on animal models rather than human studies, the beneficial effect may be through the effects of butyrate, and the documented effects have been reported for the organism *Akkermansia municiphila*.[67] There is a micro-integral membrane protein (MIMP) that is the smallest domain of surface layer protein from *Lactobacillus plantarum*. MIMP had a significant anti-inflammatory effect in an experimental model of dextran sodium sulphate (DSS)-induced colitis.[68] This was achieved through multiple mechanisms: regulating the gut barrier (appearance of FITC-dextran in serum after oral gavage, as well as up-regulation of the expression of junctional adhesion molecule (JAM)-1, occludin and ZO-1 in the colon tissues), microbiota (increased richness and diversity, including increased *Leuconostocaceae* and *Leuconostoc*, instead of *Firmicutes* and *Clostridia* which were abundant in the DSS group), and inflammatory cytokines through the toll-like receptor (TLR)4-related pathway.[68]

These cumulated observations suggest that there are non-pathological situations that may be associated with increased permeability, and these relatively minor perturbations can be reversed with dietary, nonpharmacological approaches. Further studies of such approaches, including prebiotics and probiotics, are eagerly awaited.

## Leaky Gut: The Con Arguments

As indicated by other authors,[6, 16] there are, however, important pitfalls that need to be considered and precautions to be taken in attributing biological or clinical relevance to “leakiness” of the barrier. First, altered permeability may be an epiphenomenon. For example, any inflammatory process may impair barrier integrity, and other factors such as dietary components or intraluminal factors such as bile acids can independently influence barrier function. Second, although allergens, stress, and physical activity may indeed alter intestinal barrier function, it is unclear how this predisposes to clinical consequences. Third, impaired barrier function (e.g., genetically determined defects in barrier components) does not, in isolation, lead to disease phenotype in experimental animal models of disease. Fourth, increased permeability is not necessarily deleterious, and there is no convincing evidence that an intervention that restores or improves barrier function in humans can alter the natural history of disease. Thus, for example, whereas anti-tumor necrosis factor-α

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(TNF-α) therapy reduces mucosal inflammation and restores intestinal permeability in patients with inflammatory bowel disease (IBD), and butyrate, zinc, and some probiotics also ameliorate mucosal barrier dysfunction, it is still unproven that permeability manipulation should be considered as a therapeutic target in IBD.[69]

## Conclusions

Although the ultrastructure and function of the epithelial barrier have been well characterized, the role of and interactions with other components of the barrier, especially the mucus layer and its perturbation, remain unclear. The role of gut barrier function is deemed to be important, but there are many unresolved questions as there are no validated clinical diagnostic tests. Although chemicals, nutrients, prebiotics, and even plant extracts (e.g., indigo naturalis) improve barrier function, there are no validated drug treatments yet, and the impact of restoring barrier function to ameliorate clinical manifestations in local gastrointestinal disease or systemic diseases is as yet unproven. Clinicians should be aware of the potential of barrier dysfunction in gastrointestinal diseases, and the potential as a target for future therapy.

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## Abbreviations used:

| | |
|---|---|
| **5-HT** | serotonin |
| **$^{51}$Cr-EDTA** | radioactive chromium complexed with ethylene diamine tetracetic acid |
| **AMPs** | antimicrobial proteins |
| **CLCA1** | chloride channel regulator, calcium-activated-1 |
| **CMC** | carboxymethylcellulose |
| **Da** | dalton |
| **DAO** | diamine oxidase |
| **DMBT1** | deleted in malignant brain tumors 1 |
| **DSS** | dextran sodium sulphate |
| **EoE** | eosinophilic esophagitis |
| **FABP** | intestinal fatty-acid binding protein |
| **FCGBP** | Fc globulin binding protein |

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| | |
|---|---|
| **FITC** | fluorescein isothiocyanate |
| **GLUT2** | glucose transporter 2 |
| **HIV/AIDSs** | human immunodeficiency virus infection and acquired immune deficiency syndrome |
| **IBD** | inflammatory bowel disease |
| **IECs** | intestinal epithelial cells |
| **I-FABP** | intestinal fatty-acid binding protein |
| **IgA** | immunoglobulin A |
| **JAM** | junctional adhesion molecule |
| **LC-PUFA** | long chain polyunsaturated fatty acids |
| **LMR** | lactulose mannitol excretion ratio |
| **LPS** | lipopolysaccharide |
| **LRR** | lactulose-rhamnose ratio |
| **MIMP** | micro-integral membrane protein |
| **MUC** | mucin |
| **NAFLD** | non-alcoholic fatty liver disease |
| **NASH** | non-alcoholic steatohepatitis |
| **NSAIDs** | nonsteroidal anti-inflammatory drugs |
| **PEG** | polyethylene glycol |
| **PUFA** | polyunsaturated fatty acids |
| **Pro** | proline amino acid |
| **SEM** | standard error of the mean |
| **Ser** | serine amino acid |
| **SIBO** | small intestinal bacterial overgrowth |
| **SLR** | sucralose to lactulose ratio |
| **Thr** | threonine amino acid |
| **TJ** | tight junction |
| **TLR** | toll-like receptor |
| **TNF-α** | tumor necrosis factor-α |

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|---|---|
| **TPN** | total parenteral nutrition |
| **Tween** | polysorbate 80 |
| **ZG16** | zymogen granule protein 16 |
| **ZO** | zonula occludens |

## References


1. Chadwick VS, Phillips SF, Hofmann AF. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. Gastroenterology 1977;73:247–51. [PubMed: 873125]
2. Bjarnason I, Peters TJ, Veall N. A persistent defect in intestinal permeability in coeliac disease demonstrated by a 51Cr-labelled EDTA absorption test. Lancet 1983;1:323–5. [PubMed: 6130333]
3. Hollander D, Vadheim CM, Brettholz E, et al. Increased intestinal permeability in Crohn's patients and their relatives: an etiological factor. Ann Intern Med 1986;105:883–5. [PubMed: 3777713]
4. Camilleri M, Vakil N. Microbiome: In search of mechanistic information and relevance. Am J Gastroenterol (submitted)
5. Maruvada P, Leone V, Kaplan LM, et al. The human microbiome and obesity: moving beyond associations. Cell Host Microbe 2017;22:589–99. [PubMed: 29120742]
6. Quigley EM. Leaky gut - concept or clinical entity? Curr Opin Gastroenterol 2016;32:74–9. [PubMed: 26760399]
7. Keita AV, Soderholm JD. The intestinal barrier and its regulation by neuroimmune factors. Neurogastroenterol Motil 2010;22:718–33. [PubMed: 20377785]
8. Bergmann KR, Liu SX, Tian R, et al. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. Am J Pathol 2013;182:1595–1606. [PubMed: 23470164]
9. Terciolo C, Dapoigny M, Andre F. Beneficial effects of Saccharomyces boulardii CNCM I-745 on clinical disorders associated with intestinal barrier disruption. Clin Exp Gastroenterol 2019;12:67–82. [PubMed: 30804678]
10. Kamada N, Chen GY, Inohara N, et al. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013;14:685–90. [PubMed: 23778796]
11. Ukena SN, Singh A, Dringenberg U, et al. Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. PLoS One 2007;2:e1308. [PubMed: 18074031]
12. Zareie M, Riff J, Donato K, et al. Novel effects of the prototype translocating Escherichia coli, strain C25 on intestinal epithelial structure and barrier function. Cell Microbiol 2005;7:1782–97. [PubMed: 16309464]
13. Garcia-Gutierrez E, Mayer MJ, Cotter PD, et al. Gut microbiota as a source of novel antimicrobials. Gut Microbes 2018;60:1–21.
14. Sorg JA, Sonenshein AL. Bile salts and glycine as co-germinants for Clostridium difficile spores. J Bacteriol 2008;190:2505–12. [PubMed: 18245298]
15. Peng L, Li ZR, Green RS, et al. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 2009;139:1619–25. [PubMed: 19625695]
16. Odenwald MA, Turner JR. The intestinal epithelial barrier: a therapeutic target? Nat Rev Gastroenterol Hepatol 2017;14:9–21. [PubMed: 27848962]
17. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol 2009;9:799–809. [PubMed: 19855405]
18. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol 2014;14:189. [PubMed: 25407511]

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19. Pelaseyed T, Bergström JH, Gustafsson JK, et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol Rev 2014;260:8–20. [PubMed: 24942678]
20. Johansson ME, Hansson GC. Immunological aspects of intestinal mucus and mucins. Nat Rev Immunol 2016;16:639–49. [PubMed: 27498766]
21. Jakobsson HE, Rodríguez-Piñeiro AM, Schütte A, et al. The composition of the gut microbiota shapes the colon mucus barrier. EMBO Rep 2015;16:164–77. [PubMed: 25525071]
22. Van der Sluis M, De Koning BA, De Bruijn AC, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 2006;131:117–29. [PubMed: 16831596]
23. Barcelo A, Claustre J, Toumi F, et al. Effect of bile salts on colonic mucus secretion in isolated vascularly perfused rat colon. Dig Dis Sci 2001;46:1223–31. [PubMed: 11414298]
24. Meaney C, O'Driscoll C. Mucus as a barrier to the permeability of hydrophilic and lipophilic compounds in the absence and presence of sodium taurocholate micellar systems using cell culture models. Eur J Pharm Sci 1999;8:167–75. [PubMed: 10379039]
25. Camilleri M, Murphy R, Chadwick VS. Dose-related effects of chenodeoxycholic acid in the rabbit colon. Dig Dis Sci 1980;25:433–8. [PubMed: 7379676]
26. Lewin MR, El Masri SH, Clark CG. Effects of bile acids on mucus secretion in the dog colon. Eur Surg Res 1979;11:392–9. [PubMed: 552363]
27. Rampton DS, Breuer NF, Vaja SG, et al. Role of prostaglandins in bile salt-induced changes in rat colonic structure and function. Clin Sci (Lond) 1981;61:641–8. [PubMed: 7285509]
28. Breuer NF, Rampton DS, Tammar A, et al. Effect of colonic perfusion with sulfated and nonsulfated bile acids on mucosal structure and function in the rat. Gastroenterology 1983;84:969–77. [PubMed: 6299874]
29. Plaisancié P1, Bosshard A, Meslin JC, et al. Colonic mucin discharge by a cholinergic agonist, prostaglandins, and peptide YY in the isolated vascularly perfused rat colon. Digestion 1997;58:168–75. [PubMed: 9144307]
30. Takeuchi K Prophylactic effects of prostaglandin E2 on NSAID-induced enteropathy-role of EP4 receptors in its protective and healing-promoting effects. Curr Opin Pharmacol 2014;19:38–45. [PubMed: 25063918]
31. Plaisancié P, Barcelo A, Moro F, et al. Effects of neurotransmitters, gut hormones, and inflammatory mediators on mucus discharge in rat colon. Am J Physiol 1998;275:G1073–84. [PubMed: 9815038]
32. Camilleri M, Murphy R, Chadwick VS. Pharmacological inhibition of chenodeoxycholate-induced fluid and mucus secretion and mucosal injury in the rabbit colon. Dig Dis Sci 1982;27:865–9. [PubMed: 6126321]
33. Glade MJ, Meguid MM. A glance at … dietary emulsifiers, the human intestinal mucus and microbiome, and dietary fiber. Nutrition 2016;32:609–14. [PubMed: 26899163]
34. Csáki KF. Synthetic surfactant food additives can cause intestinal barrier dysfunction. Med Hypotheses 2011;76:676–81. [PubMed: 21300443]
35. Groh KJ, Geueke B, Muncke J. Food contact materials and gut health: Implications for toxicity assessment and relevance of high molecular weight migrants. Food Chem Toxicol 2017;109:1–18. [PubMed: 28830834]
36. Lock JY, Carlson TL, Wang CM, et al. Acute exposure to commonly ingested emulsifers alters intestinal mucus structure and transport properties. Sci Rep 2018;8:10008. [PubMed: 29968743]
37. Chassaing B, Koren O, Goodrich JK, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 2015;519:92–6. [PubMed: 25731162]
38. Roberts CL, Keita AV, Duncan SH, et al. Translocation of Crohn's disease E. coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. Gut 2010;59:1331–9. [PubMed: 20813719]
39. Thaiss CA, Levy M, Grosheva I, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science 2018;359:1376–83. [PubMed: 29519916]

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40. Summa KC, Voigt RM, Forsyth CB, et al. Disruption of the Circadian clock in mice increases intestinal permeability and promotes alcohol-induced hepatic pathology and inflammation. PLoS One 2013;8:e67102. [PubMed: 23825629]
41. Vanuytsel T, van Wanrooy S, Vanheel H, et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut 2014;63:1293–9. [PubMed: 24153250]
42. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol 2013;11:1075–83. [PubMed: 23851019]
43. Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. Gut 2006;55:1512–20. [PubMed: 16966705]
44. Rao AS, Camilleri M, Eckert DJ, et al. Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. Am J Physiol 2011;301:G919–28.
45. Faubion WA, Camilleri M, Murray JA, et al. Improving the detection of environmental enteric dysfunction: a lactulose, rhamnose assay of intestinal permeability in children aged under 5 years exposed to poor sanitation and hygiene. BMJ Glob Health 2016;1(1):e000066.
46. Grover M, Camilleri M, Hines J, et al. (13) C mannitol as a novel biomarker for measurement of intestinal permeability. Neurogastroenterol Motil 2016;28:1114–9. [PubMed: 26914765]
47. Hayden UL, Carey HV. Neural control of intestinal ion transport and paracellular permeability is altered by nutritional status. Am J Physiol Regul Integr Comp Physiol 2000;278:R1589–94. [PubMed: 10848528]
48. Phillips TE, Phillips TL, Neutra MR. Macromolecules can pass through occluding junctions of rat ileal epithelium during cholinergic stimulation. Cell Tissue Res 1987;247:547–54. [PubMed: 3568101]
49. Fritscher-Ravens A, Schuppan D, Ellrichmann M, et al. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. Gastroenterology 2014;147:1012–20, e4. [PubMed: 25083606]
50. Peters SA, Edogawa S, Sundt WJ, et al. Constipation-predominant irritable bowel syndrome females have normal colonic barrier and secretory function. Am J Gastroenterol 2017;112:913–23. [PubMed: 28323272]
51. Buhner S, Buning C, Genschel J, et al. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? Gut 2006;55:342–7. [PubMed: 16000642]
52. Vazquez-Roque MI, Camilleri M, Smyrk T, et al. A controlled trial of gluten-free diet in irritable bowel syndrome-diarrhea: effect on bowel frequency and intestinal functions. Gastroenterology 2013;144:903–11, e3. [PubMed: 23357715]
53. Wu RL, Vazquez-Roque MI, Carlson P, et al. Gluten-induced symptoms in diarrhea-predominant irritable bowel syndrome are associated with increased myosin light chain kinase activity and claudin-15 expression. Lab Invest 2017;97:14–23. [PubMed: 27869798]
54. Camilleri M, Lasch K, Zhou W. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2012;303:G775–85. [PubMed: 22837345]
55. D'Incà R, Di Leo V, Corrao G, et al. Intestinal permeability test as a predictor of clinical course in Crohn's disease. Am J Gastroenterol 1999;94:2956–60. [PubMed: 10520851]
56. Aguas M, Garrigues V, Bastida G, et al. Prevalence of irritable bowel syndrome (IBS) in first-degree relatives of patients with inflammatory bowel disease (IBD). Crohns Colitis 2011;5:227–33.
57. Katzka DA, Geno DM, Blair HE, et al. Small intestinal permeability in patients with eosinophilic oesophagitis during active phase and remission. Gut 2015;64:538–43. [PubMed: 24957264]
58. Warners MJ, Vlieg-Boerstra BJ, Verheij J, et al. Esophageal and small intestinal mucosal integrity in eosinophilic esophagitis and response to an elemental diet. Am J Gastroenterol 2017;112:1061–71. [PubMed: 28417991]
59. Karl JP, Margolis LM, Madslien EH, et al. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

physiological stress. Am J Physiol Gastrointest Liver Physiol 2017;312:G559–71. [PubMed: 28336545]

60. Yu LC. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: exploring a common ground hypothesis. J Biomed Sci 2018;25:79. [PubMed: 30413188]
61. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–9. [PubMed: 23055155]
62. Luther J, Garber JJ, Khalili H, et al. Hepatic injury in nonalcoholic steatohepatitis contributes to altered intestinal permeability. Cell Mol Gastroenterol Hepatol 2015;1:222–32. [PubMed: 26405687]
63. Ponziani FR, Zocco MA, Cerrito L, et al. Bacterial translocation in patients with liver cirrhosis: physiology, clinical consequences, and practical implications. Expert Rev Gastroenterol Hepatol 2018;12:641–56. [PubMed: 29806487]
64. Meng X, Li S, Li Y, et al. Gut microbiota's relationship with liver disease and role in hepatoprotection by dietary natural products and probiotics. Nutrients 2018;10 pii: E1457.
65. Zhou Q Verne ML, Fields JZ, et al. Randomised placebo-controlled trial of dietary glutamine supplements for postinfectious irritable bowel syndrome. Gut 2018 8 14 pii: gutjnl-2017–315136. doi: 10.1136/gutjnl-2017-315136. [Epub ahead of print]
66. Hiippala K, Jouhten H, Ronkainen A, et al. The potential of gut commensals in reinforcing intestinal barrier function and alleviating inflammation. Nutrients 2018;10 pii: E988.
67. Chelakkot C, Choi Y, Kim DK, et al. Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. Exp Mole Med 2018;50:e450.
68. Yin M, Yan X, Weng W, et al. Micro-integral membrane protein (MIMP), a newly discovered anti-inflammatory protein of Lactobacillus Plantarum, enhances the gut barrier and modulates microbiota and inflammatory cytokines. Cell Physiol Biochem 2018;45:474–90. [PubMed: 29402771]
69. Michielan A, D'Incà R. Intestinal permeability in inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of leaky gut. Mediators Inflamm 2015;2015:628157. [PubMed: 26582965]
70. Karhu E, Forsgård RA, Alanko L, et al. Exercise and gastrointestinal symptoms: running-induced changes in intestinal permeability and markers of gastrointestinal function in asymptomatic and symptomatic runners. Eur J Appl Physiol 2017;117: 2519–26. [PubMed: 29032392]
71. Pires W, Veneroso CE, Wanner SP, et al. Association between exercise-induced hyperthermia and intestinal permeability: a systematic review. Sports Med 2017;47:1389–1403. [PubMed: 27943148]
72. van Wijck K, Wijnands KA, Meesters DM, et al. L-citrulline improves splanchnic perfusion and reduces gut injury during exercise. Med Sci Sports Exerc 2014;46:2039–46. [PubMed: 24621960]
73. Jonvik KL, Lenaerts K, Smeets JS, et al. Sucrose but not nitrate ingestion reduces strenuous cycling-induced intestinal injury. Med Sci Sports Exerc 2018 10 5. doi: 10.1249/MSS.0000000000001800. [Epub ahead of print].
74. Marchbank T, Davison G, Oakes JR, et al. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am J Physiol Gastrointest Liver Physiol 2011;300:G477–84. [PubMed: 21148400]
75. Bjarnason I, Scarpignato C, Holmgren E, et al. Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. Gastroenterology 2018;154:500–14. [PubMed: 29221664]
76. Moore A, Bjarnason I, Cryer B, et al. Evidence for endoscopic ulcers as meaningful surrogate endpoint for clinically significant upper gastrointestinal harm. Clin Gastroenterol Hepatol 2009;7:1156–63. [PubMed: 19362611]
77. Sigthorsson G, Tibble J, Hayllar J, et al. Intestinal permeability and inflammation in patients on NSAIDs. Gut 1998;43:506–11. [PubMed: 9824578]
78. Mahmood A, FitzGerald AJ, Marchbank T, et al. Zinc carnosine, a health food supplement that stabilises small bowel integrity and stimulates gut repair processes. Gut 2007;56:168–75. [PubMed: 16777920]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


79. Krumbeck JA, Rasmussen HE, Hutkins RW, et al. Probiotic Bifidobacterium strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. Microbiome 2018;6:121. [PubMed: 29954454]
80. Mokkala K, Pellonperä O, Röytiö H, et al. Increased intestinal permeability, measured by serum zonulin, is associated with metabolic risk markers in overweight pregnant women. Metabolism 2017;69:43–50. [PubMed: 28285651]
81. Mokkala K, Röytiö H, Munukka E, et al. Gut microbiota richness and composition and dietary intake of overweight pregnant women are related to serum zonulin concentration, a marker for intestinal permeability. J Nutr 2016;146:1694–1700. [PubMed: 27466607]
82. Mokkala K, Pussinen P, Houttu N, et al. The impact of probiotics and n-3 long-chain polyunsaturated fatty acids on intestinal permeability in pregnancy: a randomised clinical trial. Benef Microbes 2018;9:199–208. [PubMed: 29345158]
83. Man AL, Bertelli E, Rentini S, et al. Age-associated modifications of intestinal permeability and innate immunity in human small intestine. Clin Sci (Lond) 2015;129:515–27. [PubMed: 25948052]
84. Qi Y, Goel R, Kim S, et al. Intestinal permeability biomarker zonulin is elevated in healthy aging. J Am Med Dir Assoc 2017;18:810, e1-e4.
85. Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: a player in aging and a target for anti-aging intervention. Ageing Res Rev 2017;35:36–45. [PubMed: 28109835]
86. Ventura MT, Polimeno L, Amoruso AC, et al. Intestinal permeability in patients with adverse reactions to food. Dig Liver Dis 2006;38:732–6. [PubMed: 16880015]
87. Järvinen KM, Konstantinou GN, Pilapil M, et al. Intestinal permeability in children with food allergy on specific elimination diets. Pediatr Allergy Immunol 2013;24:589–95. [PubMed: 23909601]
88. Perrier C, Corthésy B. Gut permeability and food allergies. Clin Exp Allergy 2011;41:20–8. [PubMed: 21070397]
89. Katzka DA, Geno DM, Blair HE, et al. Small intestinal permeability in patients with eosinophilic oesophagitis during active phase and remission. Gut 2015;64:538–43. [PubMed: 24957264]
90. Warners MJ, Vlieg-Boerstra BJ, Verheij J, et al. Esophageal and small intestinal mucosal integrity in eosinophilic esophagitis and response to an elemental diet. Am J Gastroenterol 2017;112:1061–71. [PubMed: 28417991]
91. Leung AJ, Persad S, Slae M, et al. Intestinal and gastric permeability in children with eosinophilic esophagitis and reflux esophagitis. J Pediatr Gastroenterol Nutr 2015;60:236–9. [PubMed: 25304889]
92. Harris JK, Fang R, Wagner BD, et al. Esophageal microbiome in eosinophilic esophagitis. PLoS One 2015;10:e0128346. [PubMed: 26020633]
93. Benitez AJ, Hoffmann C, Muir AB, et al. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. Microbiome 2015;3:23. [PubMed: 26034601]
94. Arias Á, Vicario M, Bernardo D, et al. Toll-like receptors-mediated pathways activate inflammatory responses in the esophageal mucosa of adult eosinophilic esophagitis. Clin Transl Gastroenterol 2018;9:147. [PubMed: 29691386]
95. Yuan J, Baker SS, Liu W, et al. Endotoxemia unrequired in the pathogenesis of pediatric nonalcoholic steatohepatitis. J Gastroenterol Hepatol 2014;29:1292–8. [PubMed: 24372861]
96. Kapil S, Duseja A, Sharma BK, et al. Small intestinal bacterial overgrowth and toll-like receptor signaling in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2016;31:213–21. [PubMed: 26212089]
97. Bibbò S, Ianiro G, Dore MP, et al. Gut microbiota as a driver of inflammation in nonalcoholic fatty liver disease. Mediators Inflamm 2018 1 31;2018:9321643 [PubMed: 29563854]
98. Oikonomou T, Papatheodoridis GV, Samarkos M, et al. Clinical impact of microbiome in patients with decompensated cirrhosis. World J Gastroenterol 2018;24:3813–20. [PubMed: 30228776]
99. Björnsson E, Cederborg A, Akvist A, et al. Intestinal permeability and bacterial growth of the small bowel in patients with primary sclerosing cholangitis. Scand J Gastroenterol 2005;40:1090–4. [PubMed: 16211716]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


100. Tornai T, Palyu E, Vitalis Z, et al. Gut barrier failure biomarkers are associated with poor disease outcome in patients with primary sclerosing cholangitis. World J Gastroenterol 2017;23:5412–21. [PubMed: 28839442]
101. Ralls MW, Demehri FR, Feng Y, et al. Enteral nutrient deprivation in patients leads to a loss of intestinal epithelial barrier function. Surgery 2015;157:732–42. [PubMed: 25704423]
102. Demehri FR, Barrett M, Ralls MW, et al. Intestinal epithelial cell apoptosis and loss of barrier function in the setting of altered microbiota with enteral nutrient deprivation. Front Cell Infect Microbiol 2013;3:105. [PubMed: 24392360]
103. Ralls MW, Miyasaka E, Teitelbaum DH. Intestinal microbial diversity and perioperative complications. J Parenter Enteral Nutr 2014;38:392–9.
104. Mayeur C, Gillard L, Le Beyec J, et al. Extensive intestinal resection triggers behavioral adaptation, intestinal remodeling and microbiota transition in short bowel syndrome. Microorganisms 2016 3 8;4(1). pii: E16.
105. Lee WS, Sokol RJ. Intestinal microbiota, lipids, and the pathogenesis of intestinal failure-associated liver disease. J Pediatr 2015;167:519–26. [PubMed: 26130113]
106. Bulik-Sullivan EC, Roy S, Elliott RJ, et al. Intestinal microbial and metabolic alteraitons following successful fecal microbiota transplant for D-lactic acidosis. J Pediatr Gastroenterol Nutr 2018;67:483–7. [PubMed: 29901551]
107. Fang X, Wang X, Yang S, et al. Evaluation of the microbial diversity in amyotrophic lateral sclerosis using high-throughput sequencing. Front Microbiol 2016;7:1479. [PubMed: 27703453]
108. La Rosa F, Clerici M, Ratto D, et al. The gut-brain axis in Alzheimer's disease and omega-3. A critical overview of clinical trials. Nutrients 2018;10 pii: E1267.
109. Clairembault T, Leclair-Visonneau L, Coron E, et al. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol Commun 2015;3:12. [PubMed: 25775153]
110. Forsyth CB, Shannon KM, Kordower JH, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS One 2011;6:e28032. [PubMed: 22145021]
111. Keshavarzian A, Green SJ, Engen PA, et al. Colonic bacterial composition in Parkinson's disease. Mov Disord 2015;30:1351–60. [PubMed: 26179554]
112. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord 2015;30:350–8. [PubMed: 25476529]
113. Zhang R, Miller RG, Gascon R, et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol 2009;206:121–4. [PubMed: 19013651]
114. Rowin J, Xia Y, Jung B, et al. Gut inflammation and dysbiosis in human motor neuron disease. Physiol Rep 2017;5(18). pii: e13443.
115. Wright ML, Fournier C, Houser MC, et al. Potential role of the gut microbiome in ALS: a systematic review. Biol Res Nurs 2018;20:513–21. [PubMed: 29925252]
116. Stevens BR, Goel R, Seungbum K,et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut 2018;67:1555–7.
117. Liu RT. The microbiome as a novel paradigm in studying stress and mental health. Am Psychol 2017;72:655–67. [PubMed: 29016169]
118. Nguyen TT, Kosciolek T, Eyler LT, et al. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. J Psychiatr Res 2018;99:50–61. [PubMed: 29407287]
119. Maes M, Kubera M, Leunis JC, et al. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. J Affect Disord 2012;141:55–62. [PubMed: 22410503]
120. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006;27:24–31. [PubMed: 16316783]

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121. Kelly JR, Kennedy PJ, Cryan JF, et al. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front Cell Neurosci 2015;9:392. [PubMed: 26528128]

122. Hulsewe KW, van Acker BA, Hameeteman W, et al. Does glutamine-enriched parenteral nutrition really affect intestinal morphology and gut permeability? Clin Nutr 2004;23:1217–25. [PubMed: 15380916]

123. Zhou YP, Jiang ZM, Sun YH, et al. The effect of supplemental enteral glutamine on plasma levels, gut function, and outcome in severe burns: a randomized, double-blind, controlled clinical trial. J Parenter Enteral Nutr 2003;27:241–5.

124. Peng X, Yan H, You Z, et al. Effects of enteral supplementation with glutamine granules on intestinal mucosal barrier function in severe burned patients. Burns 2004;30:135–9. [PubMed: 15019120]

125. Benjamin J, Makharia G, Ahuja V, et al. Glutamine and whey protein improve intestinal permeability and morphology in patients with Crohn's disease: a randomized controlled trial. Dig Dis Sci 2012;57:1000–12. [PubMed: 22038507]

126. Russo F, Linsalata M, Clemente C, et al. Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. Nutr Res 2012;32:940–6. [PubMed: 23244539]

127. Shulman RJ, Hollister EB, Cain K, et al. Psyllium fiber reduces abdominal pain in children with irritable bowel syndrome in a randomized, double-blind trial. Clin Gastroenterol Hepatol 2017;15:712–9. [PubMed: 27080737]

128. Krawczyk M, Maciejewska D, Ryterska K, et al. Gut permeability might be improved by dietary fiber in individuals with nonalcoholic fatty liver disease (NAFLD) undergoing weight reduction. Nutrients 2018;10 pii: E1793.

129. Meng YB, Lei J, Hao ZM, et al. Influence of rhubarb on gastrointestinal motility and intestinal mucosal barrier in patients with severe burn. [Chinese] Zhonghua Shao Shang Za Zhi 2011;27:337–40. [PubMed: 22224253]

130. Del Piano M, Balzarini M, Carmagnola S, et al. Assessment of the capability of a gelling complex made of tara gum and the exopolysaccharides produced by the microorganism Streptococcus thermophilus ST10 to prospectively restore the gut physiological barrier: a pilot study. J Clin Gastroenterol 2014;48(Suppl 1):S56–61. [PubMed: 25291130]

131. Sequeira IR, Kruger MC, Hurst RD, et al. Ascorbic acid may exacerbate aspirin-induced increase in intestinal permeability. Basic Clin Pharmacol Toxicol 2015;117:195–203. [PubMed: 25641731]

132. Willumsen JF, Darling JC, Kitundu JA, et al. Dietary management of acute diarrhoea in children: effect of fermented and amylase-digested weaning foods on intestinal permeability. J Pediatr Gastroenterol Nutr 1997;24:235–41. [PubMed: 9138166]

133. Agapova SE, Stephenson KB, Divala O, et al. Additional common bean in the diet of Malawian children does not affect linear growth, but reduces intestinal permeability. J Nutr 2018;148:267–74. [PubMed: 29490090]

134. Mullen A, Gosset L, Larke N, et al. The effects of micronutrient-fortified complementary/replacement food on intestinal permeability and systemic markers of inflammation among maternally HIV-exposed and unexposed Zambian infants. Br J Nutr 2012;107:893–902. [PubMed: 21899803]

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**BOX 1.**

### Leaky Gut

**https://www.healthyway.com/content/the-truth-about-leaky-gut-syndrome-what-it-is-and-why-you-want-to-avoid-it**

BALANCE When the microbial balance in your gut is right, your whole body functions the way it's supposed to. But when that balance gets out of whack—say because of chronic stress, chronic constipation, exposure to environmental toxins like pesticides, eating a poor diet, or taking an antibiotic that wipes out a lot those microbes—the "bad" bacteria cut holes in the fence and some of them, along with food particles and toxins, leak into the bloodstream. When your immune system sees organisms where they don't belong, it attacks, causing irritation and inflammation.

CAUSES: Leaky gut has so many possible causes – so many possibly symptoms

CONSEQUENCES: "The leakage in leaky gut may be responsible for a huge variety of health issues, ranging from minor (bloating, cramps, fatigue, food allergies and sensitivities, gas, and headaches) to "bigger things": autoimmune conditions, depression and other mood disorders, diabetes, inflammatory bowel disease, and multiple sclerosis".

TREATMENT: "Functional medicine": get gut microbes back into balance: *Multi-Step program*

- Remove foods that create problems e.g. gluten, sugar, and dairy.
- Replace with foods less likely to irritate gut: Fermented foods e.g. sauerkraut, kimchi, yogurt, kefir, and pickles, are healing foods
- Repair the damage with supplements: L-glutamine (heal the intestinal lining), vitamin D, zinc, and omega-3 fatty acids (such as fish oil).
- Repopulate your good gut bacteria: probiotics or get a transplant from another person
- One of the biggest leaky gut red flags is having issues with a variety of foods.
- Talk with your healthcare provider: You might have leaky gut syndrome.
- Do not, however, try to treat it yourself

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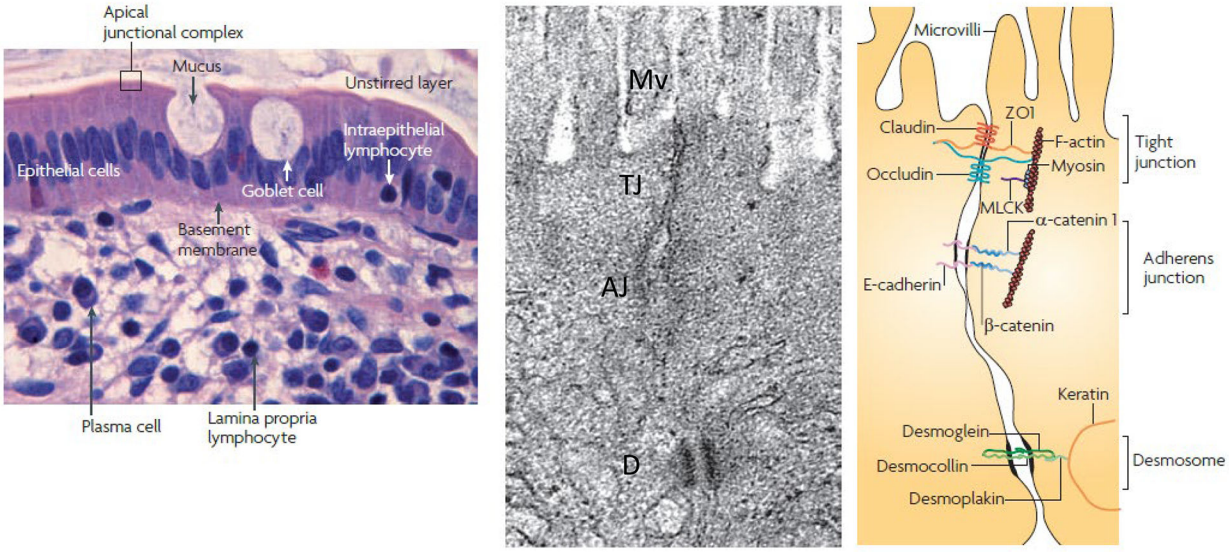


**Figure 1: Anatomy of the mucosal barrier.**

Left panel: In the human intestinal mucosa, composed of columnar epithelial cells, lamina propria (with its immune cells) and muscular mucosa, the goblet cells synthesize and release mucin, and the unstirred layer is immediately above the epithelial cells. The tight junction is a component of the apical junctional complex, and it seals paracellular spaces between epithelial cells.

Middle and Right Panels show an electron micrograph and the corresponding line drawing of the junctional complex of an intestinal epithelial cell. The key elements of the tight junction are the zona occludens and zona adherens, each of which is made up of different components. Just below the base of the microvilli (Mv), the plasma membranes of adjacent cells seem to fuse at the tight junction (TJ), where claudins, zonula occludens 1 (ZO1), occludin and F-actin interact. E-cadherin, α-catenin 1, β-catenin, catenin δ1 (also known as p120 catenin; not shown) and F-actin interact to form the adherens junction (AJ). Myosin light chain kinase (MLCK) is associated with the peri-junctional actomyosin ring. Desmosomes, located beneath the apical junctional complex, are formed by interactions between desmoglein, desmocollin, desmoplakin and keratin filaments.

In general, diffusion through claudins and occludin within the membrane is energy-independent, whereas ZO-1 facilitates exchange between tight junction and cytosolic pools through energy –dependent mechanisms

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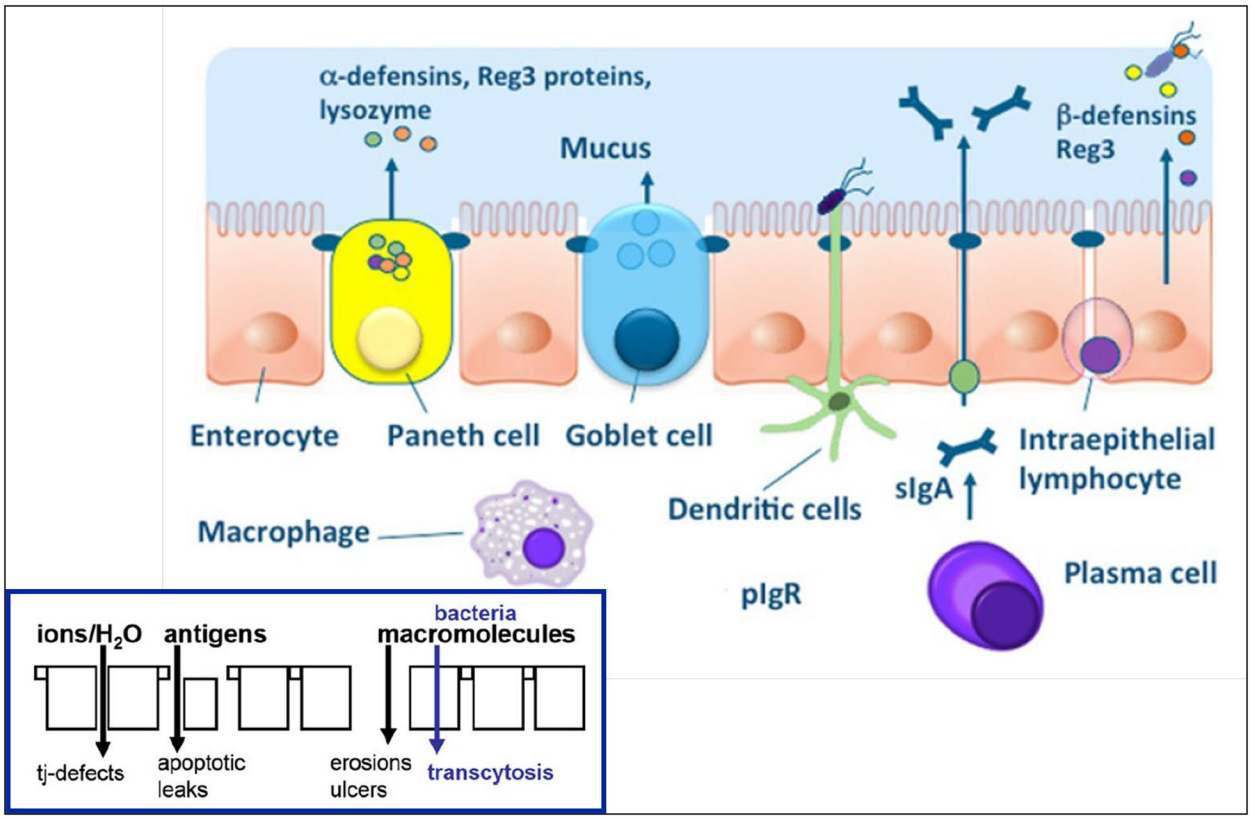


**Figure 2.**

Intestinal barrier and its dysfunctions. The intestinal barrier includes the mucus layer preventing bacterial adhesion by secretion of chemicals such as α-defensins and IgA secretion, epithelial cells, connected at the tight junctions (tj) by junctional complexes, having the ability to transport luminal content and react to noxious stimuli by secretion of chloride and antimicrobial peptides, and the lamina propria innate and acquired immunity cells secreting Ig and cytokines. Intestinal permeability measurements are determined by the marker molecules used for measurement, since the type of molecules that pass the intestinal barrier depends on the type of lesion. Reproduced from ref. 18.

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**Table 1.**

Molecular sizes of the sugar and other probe molecules

| Probe molecule | Mol. weight, Da | Mol. diameter, Å, reported | Mol. diameter, Å, estimated* |
|---|---|---|---|
| **Rhamnose** | 164 | 8.2 | 6.9 |
| **Mannitol** | 182 | 6.7 | 7.2 |
| **Lactulose** | 342 | 9.5 | 9.7 |
| **Cellobiose** | 342 | 10.5 | 9.7 |
| **Sucralose** | 398 | NA | 10.4 |
| **Cr-EDTA** | 340 | 10.5 | 9.6 |
| **Dextran 4 kDa** | 4,000 | NA | 30.0 |

* calculated based on the formula: radius=0.33*(MM^0.46), where MM=molecular mass

Å= ångström; Cr-EDTA=chromium complexed with ethylene diamine tetracetic acid, Da=dalton

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**Table 2.**

Summary of the literature on conditions that result in stress to the intestinal barrier, with documented effects on intestinal permeability or biochemical evidence of mucosal damage, and restoration with a dietary, non-pharmacological intervention

| Barrier stressor; Clinical scenario | Specific Study | Effects on Barrier Function | | Dietary Intervention and Its Effects | Reference # |
|---|---|---|---|---|---|
| | | Intestinal permeability | Mucosal Damage | | |
| **Endurance exercise Marathon runners with fecal occult blood or bloody diarrhea** | Biking challenge | Urine iohexol (MW 821Da) ↑ | Serum I-FABP ↑, zonulin ↓ | | 70 |
| | Running challenge | LRR ↑ and correlated with core temperature (e.g. >39°C) | ND | | 71 |
| | Biking challenge | LRR ↑ | Serum I-FABP ↑ | Citrulline (vs alanine) reversed ↑serum I-FABP and gastric hypoperfusion without effect on LRR | 72 |
| | Biking challenge | ND | Serum I-FABP ↑ | Sucrose (vs. nitrate) reversed ↑ serum I-FABP no Δ gastric hypoperfusion | 73 |
| | 7 runners, 2 boxers, 3 rugby | LRR ↑ | ND | Nurticeutical colostrum vs. placebo reduced LRR ↑ and apoptosis of cell lines in vitro | 74 |
| **NSAID enteropathy: NSAIDs cause small bowel ulcers and inflammation** | Diverse NSAIDs e.g. indomethacin | $^{51}$CrEDTA, saccharides | ND | | 75–77 |
| | Indomethacin | LRR ↑ | ND | Zinc carnosine (vs. placebo) reduced LRR and increased HT29 cell proliferation (vs. ZnSO4) | 78 |
| | Aspirin | increased colon permeability: urine SLR and sucralose | ND | Bifidobacterium BB-12 and adolescentis (IVS-1) and galacto-oligosaccharide prebiotic reduce colon permeability | 79 |
| **Pregnancy with or without obesity** | | Increased serum zonulin and increased serum LPS, associated with metabolic risk markers | ND | | 80 |
| | | High serum zonulin | ND | n-3 PUFAs, fiber, and a range of vitamins and minerals: reduced high serum zonulin; greater richness of gut microbiota | 81 |
| | | No effects on serum zonulin or LPS | ND | Probiotics and/or LC-PUFA | 82 |

Da=dalton; I-FABP=intestinal fatty-acid binding protein; LC-PUFA=long chain polyunsaturated fatty acids; LPS=lipopolysaccharide; LRR=lactulose-rhamnose ratio; ND=not determined; NSAIDs=nonsteroidal anti-inflammatory drugs; PUFA=polyunsaturated fatty acids; SLR=sucralose to lactulose ratio

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**Table 3.**

Summary of diseases or disorders with increased intestinal permeability and altered microbiota. In each category, it is infrequent for the altered barrier dysfunction and microbiota to be documented in the same human study.

| Condition | Small Intestinal or Colonic Barrier Function | | Microbiota Changes | Other Effects | Effects of Treatment |
|---|---|---|---|---|---|
| | IP probe molecules or epithelial damage | Serum biomarkers | | | |
| **Aging** | No difference in LMR or most TJ protein expression, but increased claudin 2 expression and decreased transepithelial resistance in ileal biopsies ex-vivo[83] | ↑ zonulin[84] | ↓ *Firmicutes, Bifidobacteria, Faecalibacterium prausnitzii*<br>↑ *Bacteroidetes, Clostridia and facultative anerobes*[85] | | |
| **Food allergy** | ↑ LMR 3 fold vs health[86]<br>↑ LMR 38% in children with food allergy[87] | | | Postulated mast cell and IgE-mediated increase in inflammatory cytokines[88] | Increased LMR in children with food allergy despite dietary exclusion[87] |
| **Eosinophilic esophagitis** | Increased small bowel IP based on lactulose absorption[89] but not LMR in adults[89, 90] or in children;[91] ex-vivo assessment of duodenal mucosal integrity was normal[90] | | Esophageal microbiome: increased hemophilus[92] or *Neisseria* and *Corynebacterium*in in active EoE[93] | Bacterial load and TLR1, TLR2, TLR4, and TLR9 were overexpressed and mucin genes under-expressed on biopsies with active EoE[94] | No effect of elemental diet on duodenal mucosa or LMR or tight junction protein expression;[90]<br>No effect of exclusion diets on esophageal microbiome[93] |
| **Liver Diseases** | | | | | |
| ***NAFLD/ NASH*** | ↑ LMR or $^{51}$Cr-EDTA in 39% of 139 pts with NAFLD (SRMA 5 studies)[62] | ↑ LPS in 42% of NASH;[95]<br>↑ LPS in NAFLD associated with SIBO[96] | ↑ SIBO (37.5%) in pts with NAFLD, especiallygram –ve bacteria and *E.coli*;[96]<br>Review documents show diverse microbiota changes (variable in different studies)[97] | Increased endogenous ethanol production by gut bacteria in NAFLD[61] | |
| ***Cirrhosis*** | | | Significant microbiota change in liver cirrhosis[98] | | Reduced cirrhosis severity with *Lactobacillus* and VSL#3 probiotics[64] |
| ***Sclerosing cholangitis*** | LRR normal [83% (19/22) with quiescent IBD][99] | Higher serum I-FABP associated with IgA antibodies against F-actin[100] | 1/22 had SIBO (*Enterobacter*);[94]<br>Enhanced mucosal immune response to various microbial antigens associated with IgA antibodies against F-actin[99] | IgA antibodies against F-actin, independent predictor of poor disease outcome [100] | |
| **TPN or enteral deprivation** | ↑ FITC-Dextran I.P ex-vivo and ↓ZO-1, E-cadherin, and claudin-4 in unfed segments in pediatric patients;[101]<br>↓ZO-1 and villus height in mice[102] | | Wide variability in microbial diversity in patients with small bowel resections;[103]<br>Patients with short bowel on TPN have "lactobiota" enriched in the *Lactobacillus/ Leuconostoc* group, depleted in anaerobic micro-organisms (especially *Clostridium* and *Bacteroides*)[104] | In TPN-liver disease, microbes or LPS reaching liver and activating Kupffer cells;[105]<br>*Lactobiota* fermentation leads to increased risk of d-encephalopathy[104] | Successful use of fecal microbial transplant for the treatment of recurrent D-lactic acidosis[106] |
| **Neurological Diseases** | | | | | |

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| Condition | Small Intestinal or Colonic Barrier Function | | Microbiota Changes | Other Effects | Effects of Treatment |
|---|---|---|---|---|---|
| | IP probe molecules or epithelial damage | Serum biomarkers | | | |
| ***Alzheimer*** | | | Differences in *Bacteroides, Faecalibacterium, Anaerostipes, Prevotella, Escherichia*, and *Lachnospira* at genus level and decreased *Firmicutes/ Bacteroidetes* ratio at phylum level[107] | | Only 2/6 trials of omega-3 FA showed any benefit on cognitive decline, typically in those with mild cognitive impairment[108] |
| ***Parkinson*** | Down-regulation of occludin not ZO-1 in colonic mucosa; however, flux of sulfonic acid and horseradish peroxidase not abnormal with or without Lewy bodies;[109]<br>LMR normal, but ↑ 24h urinary sucralose (marker of total intestinal permeability)[110] | Lower plasma levels of LPS binding protein, an indirect measure of systemic endotoxin exposure[110] | Significantly more intense staining of *E. coli* in epithelium and lamina propria of sigmoid mucosa;[110]<br>Reduced butyrate-producing bacteria from the genera *Blautia, Coprococcus* and *Roseburia*; putative "proinflammatory" *Proteobacteria* of the genus *Ralstonia* significantly more abundant in mucosa of Parkinson's patients[111] | Correlation of increased intestinal permeability in Parkinson disease with intestinal alpha–synuclein;[109]<br>Relative abundance of *Enterobacteriaceae* positively associated with severity of postural instability and gait difficulty[112] | |
| ***ALS*** | ↑ LPS in most severe amyotrophic lateral sclerosis[113] | | Low diversity of the microbiome compared to healthy cohorts; low *Ruminococcus* spp. in 3/5 patients with low *Firmicutes/ Bacteroidetes* (F/B) ratio[114] | Decreased levels of butyrate-producing bacteria; decreased levels of micro-organisms of the genera *Oscillibacter, Anaerostipes, and Lachnospira*;[115]<br>3/5 patients had elevated inflammatory markers in stool[114] | |
| ***Psychiatric diseases*** | Plasma levels of LPS, zonulin and FABP2 were each significantly elevated in depression/anxiety patients compared to non-depressed or anxious controls.[116] | | A review documents extensive literature on cross-sectional and longitudinal studies documenting association between stool microbiota and anxiety and depression.[117]<br>A review documents studies of the microbiome and microbial translocation in patients with schizophrenia and bipolar disorder.[118] | Elevated serum IgM and IgA against LPS in depression;[119]<br>Psychological stress increases pro-inflammatory cytokines (extensive literature reviewed in ref. 120). | Probiotics reduce depression scores in 6 randomized, placebo-controlled trials (reviewed in ref. 121). |

EoE=eosinophilic esophagitis; FABP= fatty-acid binding protein; FITC=fluorescein isothiocyanate; IBD=inflammatory bowel disease; I-FABP=intestinal fatty-acid binding protein; IgA=immunoglobulin A; IP=intestinal permeability; LMR=lactulose mannitol excretion ratio; LPS=lipopolysaccharide; LRR=lactulose-rhamnose ratio; NAFLD=non-alcoholic fatty liver disease; NASH=non-alcoholic steatohepatitis; SIBO=small intestinal bacterial overgrowth; TJ=tight junction; TLR=toll-like receptor; TPN=total parenteral nutrition; ZO=zonula occludens

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**Table 4.**

Examples from the literature of *in vivo* human studies showing alterations in intestinal permeability as a result of gut-directed therapy with nutrient, supplement or fiber

| Therapy | Patients with | Comments | Reference # |
|---|---|---|---|
| **Glutamine i.v.** | Nutritional depletion pre-surgery | Patients with increased intestinal permeability did not improve after glutamine-enriched total parenteral nutrition | 122 |
| **Enteral glutamine** | 50–80% burns | Urinary LMR in enteral glutamine group lower than standard enteral formula | 123 |
| **Enteral glutamine** | 30–75% burns | Plasma DAO activity and urinary LMR in enteral glutamine group were lower than in untreated burn group | 124 |
| **Enteral glutamine** | Crohn's disease patients | Glutamine and active control (whey) both reduced LMR | 125 |
| **Oral glutamine** | Post-infectious IBS | Elevated urinary LMR was normalised in the glutamine but not the control group | 65 |
| **Galactooligo-saccharides** | Obesity | Post-aspirin sucralose:lactulose ratios and sucralose excretion reduced indicating improvements in colonic permeability | 79 |
| **Inulin- enriched pasta** | Healthy young volunteers | Reduced serum zonulin and urine LMR but not mannitol excretion | 126 |
| **Psyllium** | Children with IBS | No effect on sucrose and sucralose recovery, and LMR | 127 |
| **Fiber** | NAFLD patients undergoing diet Rx for obesity | Increasing nutritional fiber from 19 to 29g/day reduced serum ZO levels, and improved hepatic steatosis | 128 |
| **Rhubarb** | Day 3 post-burns | Plasma DAO activity in rhubarb-treated group were lower than in controls | 129 |
| Gelling complex of **tara gum + exopoly- saccharides of *Strep. Thermos-philus* ST10** | Healthy participants | Reduced LMR and redcued sucralose concentration suggesting improvement in both small intestinal and colonic permeability | 130 |
| **Ascorbic acid** | Healthy female participants | Excretion of lactulose over 6-hr augmented after consumption of either aspirin or ascorbic acid compared with that after consumption of placebo | 131 |
| **Fermented and amylase-digested weaning foods** | Tanzanian children aged 6–25 months with acute diarrhea | Reduction in L/M ratio between admission and day 3 of hospitalization was significantly greater in the fermented and amylase-digested weaning group (89%) than the conventional or high-energy density amylase digested porridge groups (44% and 75%, respectively) | 132 |
| **Common beans** | Rural children in second year of life (Malawi) | Additional beans in diet reduced lactulose excretion without effect on LMR | 133 |
| **Micronutrient-fortified complementary/replacement food** | Zambian infants from 6 to 18 months old | LMR (adjusted for baseline urinary L:M ratio, socio-economic status, mother's education, season of birth and baseline stunting, and stratified by maternal antenatal HIV status, child's sex, concurrent breast-feeding status and anemia at baseline) worse in group with fortified diet especially among boys and, among the infants of HIV-negative mothers | 134 |

DAO=diamine oxidase; LMR=lactulose mannitol excretion ratio

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