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Intestinal Barrier Dysfunction in Inflammatory Bowel Disease: Underpinning Pathogenesis and Therapeutics

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Abstract

The intestinal barrier is composed of several essential elements including luminal enzymes, bile acids, water layer, epithelial layer, and enterocyte layer. It acts as a dynamic interface between the luminal contents of food, commensal and pathogenic bacteria, and the gastrointestinal tract. The role of barrier dysfunction is of significant research interest in the development and targeted treatment of chronic inflammatory gastrointestinal conditions, such as inflammatory bowel disease. This review aims to examine the role of intestinal barrier dysfunction in the development of inflammatory bowel disease, the pathophysiology of increased barrier permeability in inflammatory bowel disease, and to explore potential treatment targets and clinical applications.

Keywords

Intestinal permeability; Inflammatory bowel disease; Mucus; Tight junctions

Introduction

The objectives of this review are to examine the potential role of barrier dysfunction as a risk factor for the development of inflammatory bowel diseases (IBD), the molecular mechanisms involved in the leakiness of the barrier, and opportunities for treatment of the abnormal barrier to aid in the management of IBD. The assessment of human intestinal barrier function in vitro and in vivo and the interaction of microbiota with intestinal permeability have been addressed in prior reviews [1, 2]. The importance of the diverse components of the barrier is evident through barrier effects of fecal metabolites, and the role of cellular mechanisms in maintaining the barrier integrity. Recent data have documented deficiencies in the commonly used disaccharide to monosaccharide ratios to

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measure intestinal permeability. In order to appraise intestinal barrier in human disease states, it is essential to use more valid endpoints for in vivo measurements, and refining these measurements is necessary to (further elucidate the altered barrier function in IBD and its potential treatment through targeting the cellular and molecular mechanisms putatively involved in IBD [3].

Intestinal Barrier

There are several important elements of the intestinal barrier (Fig. 1). In the lumen, there is degradation of bacteria and antigens by bile, gastric acid, and pancreatic enzymes, as well as commensal bacteria that produce antimicrobial substances and inhibit colonization by pathogens. Adjacent to the epithelial surface, the microclimate is composed of the unstirred water layer, glycocalyx, and mucus layers, which prevent bacterial adhesion through their physical properties and by the effects of immunoglobulin (Ig) A secreted by immunocytes in the lamina propria. The epithelial cells (enterocytes) are connected by junctional complexes; the enterocytes transport luminal content and react to noxious intraluminal stimuli by secretion of chloride and antimicrobial peptides including lysozyme, and α defensins from Paneth cells. Beneath the epithelium, the lamina propria includes innate and acquired immune cells secreting Ig and cytokines. Products of the enteroendocrine cells and the enteric nervous system can stimulate intestinal propulsive motility to clear potentially injurious elements downstream [4].

Although the enterocyte layer constitutes only one component of the barrier, it has been extensively studied and includes elements that are relevant to the potential role of barrier dysfunction in IBD. The main components of epithelial intercellular junctions are the apical junctional complex which includes adherens junctions and desmosomes [5–7]. First, the apical junctional complex is characterized by interactions between F-actin, myosin II, zonula occludens-1 (ZO-1), claudins, tight junction–associated marvel proteins (TAMPs), and immunoglobulin superfamily members such as junctional adhesion molecules (JAM) and the coxsackie adenovirus receptor (CAR) [8]. Claudin-2 expression is limited to crypt, rather than surface, epithelial cells in the normal colon [9]. In fact, claudin-2 expression is greater in the Brunner’s glands of the small bowel where its effects may be more important [9–11]. Claudins 1 and 2 are associated with increased permeability, and claudin-2 expression is increased by IL-6 in inflammatory states [12]. Claudin-1 and claudin-2 expression may even be involved in early stages of IBD-associated neoplasia[11]. Second, the adherens junctions, specifically catenins (intracellular components) and e-cadherins which are calcium-dependent transmembrane proteins, are responsible for cell–cell adhesion and the maintenance of the cell layer structure. Third, the desmosomes including desmoglein, desmoplakin and desmosomal cadherins, represent the strongest link between epithelial cells, maintaining epithelial structural integrity in case of mechanical stress [5].

Transport Across the Intestinal Barrier

Paracellular transport or permeability is the passage of molecules between adjacent epithelial cells. Drugs and other molecules pass through the tight junctions (TJ) via two distinct pathways based on different sizes and selectivities. The high capacity, charge-

selective *pore pathway* allows for passage of small ions and uncharged molecules (typically < 8 Å). The paracellular pathway of absorption involves simple passive diffusion along a concentration gradient. The pathways can also be distinguished based on their capacity, as the pore pathway can allow for absorption of large quantities of small uncharged solutes and ions. Paracellular permeability is largely determined by claudin proteins and their role in the pore pathway, as the pore is formed by interactions between β-sheets within extracellular loop 1 of claudins on adjacent cells. Claudins are a group of > 25 types of proteins that are involved in TJ formation, with molecular weights ranging from 21 to 34 kDa (207–305 amino acids) [5]. The permeability of these pathways can be measured using several complementary methods such as the in vitro measurement of Trans-Epithelial Electrical Resistance (TEER), which measures the flux of all ions especially Na^+ and Cl^- across the epithelium and is most useful as a measure of permeability when ion conductance across the junction is far greater than the conductance across apical and basolateral membranes, as in leaky epithelia.

The low capacity, *leak pathway* allows flux of larger ions and molecules (typically < 100 Å), regardless of charge, though to a lesser capacity than the pore pathway [13]. Active (energy-dependent) uptake at epithelia involves several mechanisms including brush border transmembrane proteins, membrane fusion, transcytosis via vesicle carriers, or drug absorption into the cell. After entry into the cell, absorbed molecules are secreted on the basolateral side of the enterocyte into the systemic circulation [5]. For example, sodium-glucose cotransport-1 (SLGT-1) is a well-known transmembrane protein, which mediates most sodium-dependent glucose uptake in the small intestine [14]. Leak pathway permeability can be assessed by directly measuring macromolecular flux of tracers of relatively low molecular weight and molecular diameter across the epithelium. Commonly used tracers are mannitol, sucrose, lactulose, sucralose, inulin, ora polydisperse of individual molecules of varying sizes as in polyethylene glycol (PEG) 400) [8]. Larger molecules, such as dextran 4 or 10 kDa, tend to be used to assess the leak pathway in studies of mucosa evaluated in vitro. However, it is important to note that several of these probe molecules have estimated diameters of < 8 Å (especially the monosaccharides) and, therefore, they also reflect pore pathway permeability (e.g., erythritol 3.2–6.0 Å and mannitol 6.7–7.2 Å) due to their reported diameters or mathematically estimated diameters based on molecular mass [13, 15]. Leak pathways are more permeable with downregulation of occludin, non-muscle myosin II and ZO-1 [16–19]. Occludin is a 65 kDa protein whose expression is regulated by different kinases, and localization follows that of ZO protein because of its linkage.

Considerations in Measuring “Leaky Gut” in vivo in Humans

Although there are in vitro methods to measure mucosal permeability, histological measurements of barrier proteins in mucosal biopsies, and confocal endomicroscopy of the duodenum, all these methods are invasive, and cannot be easily repeated longitudinally on large patient cohorts. Thus, most observations on GI barrier function use administration of oral probes, monosaccharides and disaccharides, with measured urinary excretion. However, criteria for non-invasive methods to measure permeability are not established. The urinary excretion of orally ingested probe molecules for the in vivo measurement of intestinal permeability includes probes of diverse molecular weight and measurement

of mathematically estimated molecular diameters [15, 20]. In relation to studies without overt ulceration or epithelial cell apoptosis (e.g., functional diarrhea, or relatives of patients with IBD), it is important to note that the absorption of the disaccharide probes (lactulose, sucralose), EDTA, DTPA, and PEG is limited from the perspective of % of mass administered. Thus, even though studies typically administer 2.5–5 times as much lactulose as mannitol, there is usually a higher amount of mannitol than lactulose excreted with concomitant administration of the two sugar probes. In fact, studying the individual sugar excretions (rather than the ratio) shows more mannitol excreted during 0–2 h after oral administration in the fasting state, than after 4 h; this suggests that the small bowel is more permeable than the colon, as has been documented in many studies [21].

The timing of urine collection is also critical to assess the relative contributions of small intestine and colon to measurements of permeability. By conducting simultaneous gamma-radiolabeled imaging and timed urine collections (in 2-h aliquots for the first 8 h, followed by a collection from 8 to 24 h), it was demonstrated that urine collections at 0–2 h reflect predominantly small bowel, 2–8 h reflect both small bowel and colon, and 8–24 h exclusively colon [21, 22].

Another pitfall in measurements of permeability stems from contamination with the oral probes from baseline or during the studies. The prototypical contamination is with sucralose and mannitol which is identified in a range of fruits and vegetables (e.g. watermelon, clingstone peaches, button mushrooms, cauliflower, celery, snow peas, butternut squash, and sweet potato), ‘sugar free’ products (listed as mannitol, or under its food additive number, e421), and in cosmetics and beauty products where they are used primarily as a humectant or as a binder, masking agent, moisturizing agent, flavoring agent (lip balms, etc.) and skin conditioner.

The potential confounders with oral probe molecules were extensively documented in 60 adults studied on three occasions, with significant quantities of mannitol in 70% of participants, and rhamnose and sucralose in 6–7% [23]. Evidence of contamination with rhamnose was also documented in a pediatric study of celiac disease [24]. This contamination may also impact the estimated ratios of lactulose to mannitol. For example, in the same healthy individuals, there was higher LMR beyond 4 h after oral ingestion, suggesting colonic permeability is greater than small bowel permeability, which is clearly incorrect [21]. Moreover, given the relatively small mass of disaccharide absorbed normally, a very small increase in lactulose absorption has a marked impact on the calculated ratio of disaccharide to monosaccharide.

Mucus

The gut mucus layer consists of mucins, a family of large, complex, glycosylated proteins vital for maintaining intestinal health, which creates a coat that covers the intestinal cells protecting them from contact with external and toxic substances, digestive enzymes, and bacteria. The mucins consist of a protein core composed of sequences containing the amino acid residues, proline (Pro), threonine (Thr), and serine (Ser), called PTS-rich sequences. These sequences are often repeated in tandem, in which the Ser and Thr are extensively

O-glycosylated and confer a ‘bottle brush’-like conformation. More than 80% of the mucin mass is made up of O-glycans. This leads to the creation of a glycan coat, hiding the protein core of the mucins and protecting it from endogenous protease degradation, in addition to conferring the capacity to bind and be soluble in water and to form a gel [25].

The regulation of the intestinal mucus barrier is complex, and there is bidirectional interaction between host glycans and gut microbes whose composition contributes to the regulation of the intestinal mucus barrier function. Mucin glycans bind water, conferring moisturizing and lubricant properties, protecting the epithelial cells from dehydration and mechanical stress during the passage of luminal content and peristalsis forces. Mucus thickness and types of mucins in the intestinal tract in mice and humans have been documented [25].

Transmembrane mucins form the glycocalyx, whereas secreted mucins from sentinel goblet cells form the gel overlying the epithelium. When sentinel goblet cellsfinish emptying mucus, they are expelled from the colonic crypt and are often replaced by bacteria [26]. In humans, the goblet cell-to-enterocyte ratio changes along the intestinal tract: 4% in the duodenum, 6% in the jejunum, 12% in the ileum, and 16% in the distal colon, reflecting the proportion of goblet cells which increase proportionally to the increase in the number of microorganisms in each region (10^3 in the duodenum, 10^4 in the jejunum, 10^7 in the ileum, and 10^{12} in the colon) [25]. The mucus of the small intestine is non-attached, easily removed, discontinuous, and relatively porous and penetrable to different components such as bacteria. In the small intestine, peristalsis leads to decreased contact time for luminal contents and the epithelium, compared to the colon. In comparison, the colon has both an inner adherent and outer non-attached mucus layer accounting for differences in mucus thickness [27, 28]. The combination of transit time, and structural differences in the mucus layers of the small intestine and colon contribute to regional differences in permeability [25].

The proportion of mucus-degrading bacteria increases when the diet is deprived of specific dietary fibers, since mucus then becomes an energy source for the gut microbiota [23]. In addition to supplying attachment sites for the microbiota, mucin glycans also serve as nutrients for microorganisms called ‘mucolytic bacteria’, favoring their replication. Bacteria digest glycans through their glycosidase enzymes. Table 1 summarizes mucus regulation by specific pathogenic or commensal microorganisms and microbial metabolites [25].

Dietary Perturbations of Mucin and Microbiota Drive Intestinal Inflammation

Among the most important dietary components capable of perturbing the intestinal barrier, emulsifiers and fat have been shown to drive intestinal inflammation, especially if there is a genetic predisposition as shown in interleukin-10 (IL-10) knockout mice [29, 30].

First, exposure of the microbiome and mucus to food additive emulsifiers in the intestinal lumen leads to decreased diversity of the microbiome and increased pro-inflammatory potential. Some emulsifiers increase bacterial expression of flagellin and lipopolysaccharide (enhancing the ability of bacteria to translocate through the mucus layer to the epithelial cell) and thinning of the mucus, thereby increasing penetrability through alterations in

membrane-associated proteins such as ZO-1. However, this is controversial as data of intestinal epithelial specific ZO-1 knockout mice showed intact barrier function [31]. Bacterial translocation activates inflammatory pathways through the B-cell lymphoma/leukaemia-10 (Bcl-10) and Toll-like receptor-4 (TLR-4), which in turn activate the nuclear factor kappa light-chain-enhancer of activated β cells (NF-kβ) cascade, secretion of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and IL-6, and the development of colitis [29].

Second, a high fat diet is associated with changes in the microbiota such as increases in sulfur-reducing bacteria (SRB), with increased production of hydrogen sulfide (H_2S) leading to decreased butyrate oxidation or reduced *Bifidobacteria* leading to increased inflammation and barrier dysfunction [30, 32].

The type of fat in the diet may exert different effects on microbiota and permeability, as shown by Devkota et al. [30] Thus, a diet high in saturated fat (milk) exerted different effects on microbiota compared with a diet high in polyunsaturated fat (safflower oil) [30]. A high milk fat diet promoted an increase in *Bilophila wadsworthia*, a SRB, that is present in inflammatory conditions where by it promotes changes in the host bile acid composition resulting in dysbiosis and possibly leading to intestinal damage in genetically susceptible hosts. Another fat, corn oil [a source of omega-6-polyunsaturated fatty acids (PUFA)] decreased integrin-linked kinase which is indispensable for barrier function, and decreased expression of several TJ proteins of the intestinal barrier [33]. Paradoxically, a high fat diet is associated with lower abundance of the mucin-degrading bacteria *A. muciniphila* in healthy subjects, and it was associated with restoration of mucus thickness, suggesting that fat may disrupt epithelial components while enhancing the contribution of mucus to barrier function [34].

Gastrointestinal Disease Impairs Barrier Function

Diseases and disorders associated with altered intestinal barrier function and natural surfactants such as bile acids, in addition to food emulsifiers, can dissolve mucus and increase mucosal permeability [35]. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) provide an example of an inflammatory diathesis leading to a range of disorders, from enteropathy or erosions to altered barrier function [36]. Traditionally, NSAIDs have been used to study a perturbed barrier in experimental models. The increased permeability due to NSAIDs has been measured by urinary excretion of orally administered ^{51}Chromium-EDTA or saccharide probes [37–39]. In an experimental model, the presence of ischemia and cytokines increased permeability, which were reversible pharmacologically with lubiprostone [40].

Barrier defects have been described in association with intestinal diseases including Crohn's disease (CD) and ulcerative colitis (UC) (Table 2) [41–46]. Fecal metabolomics in IBD have also been extensively reviewed; based on current knowledge, the most significant factors affecting intestinal epithelial barrier are short chain fatty acids, tryptophan metabolites, and bile acids [47]. The biological effects of metabolites are summarized in Table 3, based on the extensive review by Iyer and Corr [47]. As researchers gain a greater understanding of

the role of metabolites in controlling immune metabolism, this may become a significant therapeutic target for autoimmune diseases such as IBD.

Interestingly, prior meta-analyses in IBD have shown that achieving endoscopic remission can lead to improved outcomes in both CD and UC, including steroid-free clinical remission, colectomy-free survival and hospitalizations [48, 49]. This has led to consensus of the therapeutic goal of mucosal healing as outlined in the STRIDE-II initiative [50]. Additionally, intestinal barrier healing has been associated with disease risk of progression of IBD using confocal endoscopy, when compared to standard of endoscopic and histologic remission [51, 52]. Despite immune modulating treatments, over 30% of patients with IBD still have persistent symptoms in the absence of endoscopic inflammation [53]. Prior research alludes to the barrier as key in understanding this cohort of patients and makes the intestinal barrier an appealing target for future therapeutic modalities.

Permeability as a Risk Factor in Development of IBD

Intestinal barrier dysfunction has been proposed as a risk factor for the development of IBD. It is hypothesized that prior to the onset of IBD, barrier dysfunction and resultant increased intestinal permeability leads to an ingress of luminal contents into the lamina propria resulting in innate and adaptive immune cell activation, eventually leading to intestinal inflammation (Fig. 2) [54]. Several earlier studies had documented altered intestinal permeability in both CD and UC, even as a marker of clinical course of disease [45, 55–58]. Cytokines have been shown to modify the expression of TJ proteins [59]. In active IBD, claudin-2 expression and myosin light chain kinase (MLCK) activity are both increased, suggesting that both pore and leak pathways are involved in disease-associated impairment of barrier function [11, 60, 61]. Additionally, in UC, mucus barrier structural weakening can be found prior to the onset of inflammation which may account for the sentinel goblet cells weakened response to pathogens, and the histological hallmark of goblet cell and mucin depletion in colonic mucosal biopsies in patients with UC [62].

More recent large studies conducted in first-degree relatives of patients with CD suggest that increased intestinal permeability is a factor predisposing to the development of CD [54]. In a study of 1,420 asymptomatic, first-degree relatives (aged 6 to 35 years) of patients with CD, followed over an average of 7.8 years, urine lactulose to mannitol ratio (LMR) at baseline was significantly higher in the individuals who went on to develop CD and this effect was independent of subclinical intestinal inflammation (measured by fecal calprotectin > 100 μg/g) [63]. Among the 50 relatives who developed CD, the median time to diagnosis during follow-up was 2.7 years. The test of intestinal permeability involved ingestion of lactulose (5 g), mannitol (2 g), sucrose (100 g), and flavored drink crystals (1.5 g) in 500 mL of tap water before bed, and then urine collection the following morning. Abnormal LMR was associated with diagnosis of CD during follow-up [63].

In a subsequent extension of the study, the cohort was divided into a discovery cohort (n = 2,472) and a validation cohort (n = 655). A regression model used to assess microbial associations with abnormal LMR (> 0.025) showed reduced alpha-diversity (Chao1 index) and altered beta-diversity (Bray–Curtis dissimilarity index) compared to subjects with LMR

≤ 0.025. Random forest approach revealed that bacterial community was associated with gut barrier function: 4 genera (decreased prevalence of *Adlercreutzia, Clostridia UCG 014, and Clostridium* sensu stricto *1* and increased abundance of *Colidextribacter*) and eight pathways (including decreased biosynthesis of glutamate, tryptophan and threonine) were replicated in the validation cohort [64].

Mechanisms to Protect or Modulate the Intestinal Barrier in Inflammatory Bowel Disease

Several mechanisms may protect or modulate the intestinal barrier in IBD. These mechanisms are summarized below.

A. Urokinase Interaction

Urokinase-type plasminogen activator (uPA) interacts with its receptor uPAR to mediate cell migration and activation. Recent human/mouse intestinal organoid studies have investigated the role of uPA-uPAR interaction which may be upregulated in biopsies of IBD mucosa [65]. Through simulated barrier damage using pathogenic cytokines, this study evaluated how the inhibition of uPA-uPAR impacted permeability, which was measured using TEER. The findings suggest that pharmacologic inhibition of the uPA-uPAR interaction may protect the epithelial barrier from intestinal damage associated with inflammation. Further investigation is needed to determine if this is a potential therapeutic target for IBD.

B. Cytokines

B1. Exogenous **tumour necrosis factor (TNF)** increases MLCK1 activity in cultured intestinal epithelial cells, which causes perijunctional myosin II regulatory light chain phosphorylation (pMLC) and condensation, and triggers occludin endocytosis [66, 67]. In this sequence of reactions, MLCK1 requires binding to the tacrolimus-sensitive protein FK506 binding protein 8 (FKBP8) to be recruited to the perijunctional actomyosin ring. MLCK1 can then induce phosphorylation of MLC (pMLC), which drives internalization of the transmembrane TJ protein occludin. This increases flux across the TJ leak pathway and enhances paracellular permeability to large solutes because of immune-mediated barrier loss. Tacrolimus has no effect on TNF induction of MLCK1 expression, but it causes dissociation of FKBP8 from MLCK1 thereby inhibiting MLCK1 recruitment to the perijunctional actomyosin ring, inhibition of MLCK1-mediated p-MLC, occludin internalization, and TNF-induced increased permeability [68]. In summary, FKBP8 can be blocked by tacrolimus, which therefore prevents TNF-induced perijunctional MLCK1 recruitment, MLC phosphorylation, and FKBP8 interactions. These actions appear to counter perijunctional MLCK1 recruitment and MLCK1-FKBP8 interactions that are increased in CD [69]. Another approach to targeted inhibition of MLCK-mediated TJ barrier regulation was tested to inhibit the specific induction of long MLCK1 and its recruitment to the perijunctional actomyosin ring. Such specific targeting of long MLCK1 (by divertin) prevents TNF-induced barrier loss and reverses immune colitis without the potential of unacceptable toxicities resulting from loss of MLCK enzymatic activity in smooth muscle and non-muscle cells [70]. These exciting data require further replication in IBD models to show impact on disease.

B2. Interleukin-8 is a cytokine used for recruitment and activation of neutrophils acting as a pro-inflammatory role. In situ hybridization of IBD patients' mucosa showed strong signals with IL-8 anti-sense RNA probes [71]. It was found that quantity of cells expressing IL-8 was proportional to severity of inflammation. Prior studies have also shown an association with adherent E coli in IBD triggering mitogen-activated protein kinase IL-8 release, which may be inhibited by mesalamine. The role of IL-8 may play a role in the pathogenesis of IBD, though it is unclear the distinct role in permeability of the membrane [71].

B3. Interkeukin-10 works by suppressing cytokine synthesis by Th1 helper cells, through impaired macrophage dependent stimulation of antigen reactive Th1 cells [72]. This was elucidated in IL-10 deficient mice models which developed chronic enterocolitis [73]. Further studies have no demonstrated successful decrease in established inflammation when IL-10 is administered systemically. Though it is considered that IL-10 downregulation may prevent the development of IBD [74].

B4. Interleukin-13 can stimulate claudin-2 expression in surface epithelial cells (where the TJ protein is not normally expressed). This increases flux across small TJ pores, thereby enhancing paracellular cation permeability [41]. There is evidence that glutamine is able to reverse the deleterious effect of IL-13 on intestinal permeability by increasing claudin-1 expression via disruption of the phosphatidylinositol-3-kinase/Akt signaling pathway in Caco-2 cells [75].

B5. Interleukin-22 critically regulates epithelial homeostasis through regulation of epithelial cell permeability and mucus production. IL-22 is expressed in response to pro-inflammatory cytokines and is required to activate the DNA damage response of the intestine [76]. Paradoxically, IL-22 has also been shown to promote inflammation [77]. Through induced expression of mucin genes via STAT3-dependent signaling and increase in number of intestinal goblet cells, IL-22 helps with the clearance of pathogens penetrating the barrier [78, 79]. In IBD, the role of IL-22 is complex and dependent on context. Without a preclinical model, investigating the role of IL-22 has been based on studies of the effects of pharmacologic IL-22 agonists in patients with active IBD [76]. For example, UTTR1147A is a IL-22 IgG4 Fc fusion protein which is a IL-22 pathway agonist. It has been shown to safely promote tissue regeneration in phase I studies of healthy patients and UC [80]. UTTR1147A is currently being tested in patients with active IBD [81].

B6. Interferon-gamma—Gene expression of NOD2 in human intestinal epithelial cells is upregulated synergistically by TNF-α and interferon-gamma (IFN-γ) [82]. IFN-γ increases paracellular permeability in intestinal epithelial cells through the redistribution and expression of TJ proteins and the rearrangement of the actin cytoskeleton [83]. There is evidence that the deleterious effect of IFN-γ on intestinal barrier function can be reduced by the product of protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene, called T cell protein tyrosine phosphatase (TCPTP). Thus, TCPTP protected against intestinal barrier dysfunction induced by IFN-γ by two mechanisms: It maintained localization of ZO-1 and occludin at apical TJs and restricted the expression and insertion of the cation pore-forming transmembrane protein, claudin-2, at TJ through upregulation of the inhibitory cysteine protease, matriptase [84]. A consistent barrier-protective effect of TCPTP was

documented in other studies: Cells expressing TCPTP effectively dampen immune responses to limit changes in flux across the pore and the leak pathways, whereas TCPTP loss in intestinal epithelial cells leads to upregulation of claudin-2 expression, triggering of excessive cytokine secretion in TCPTP-deficient immune cells, activation of MLCK to cause occludin internalization, and increased leak pathway permeability triggering further immune activation and creating a vicious cycle that leads to disease [85]. Similarly, TCPTP deficiency increases intestinal expression of claudin-2 in vitro, in vivo, and in *PTPN2*-genotyped patient with CD [84].

B7. T regulatory (Treg) cell dysfunction is found in patients with IBD, as these cells play a critical role in limiting an autoimmune response and may be a future therapeutic target using antigen-directed Treg therapy [86]. **Th17 cells** are thought to be closely related to the development of IBD and other autoimmune conditions. The presence of mucosal Th17 helps regulate barrier integrity by stimulating the formation of tight junctions and antimicrobial peptides. In contrast, the role of Th17 is dichotomous and can also be pro-inflammatory under pathological conditions [87]. **Innate lymphoid cells (ILCs)** remain understudied in IBD, though are a distinct immune cell different from T or C cells. Studies show that ILCs can contribute to host defense via epithelial barrier function by integrating both pro and anti-inflammatory signals. Additionally, consumption of ILCs can allow for influx of commensal bacteria leading to infection and systemic inflammation [88].

Potential of Dietary Modulation to Impact Intestinal Permeability

Patients may appreciate efforts to study how specific key dietary factors influence the intestinal barrier such as a high fat diet, emulsifiers, and alcohol, or specific dietary supplementations (Table 4) [3, 89, 90].

Fiber has been found to improve intestinal barrier health, through alteration of the gut microbiota and mucus layer through fermentation products, mainly short chain fatty acids (SCFAs) [91]. The *SCFAs*, acetate, propionate and butyrate, regulate neuroimmune functions as a protective measure for the intestinal barrier. For example, butyrate regulates hypoxia-inducible factor-1 to modulate the efficiency of genes coding for claudin-1 [92]. *Butyrate* can enhance TEER and affect relocation of ZO-1 and occludin. A study of fermentable fiber in dextran sulfate sodium (DSS)-induced colitis mice, showed a protective effect on TJs leading to suppression of colitis [93]. Paradoxically, when Kaiko et al. administered mice with DSS, they found an increased exposure of the stem cells to luminal butyrate, which did not allow for healing of the ulceration [93]. *Emulsifiers are food additives such as* carboxymethylcellulose and polysorbate-80, found in most processed food items to extend shelf stability. When emulsifiers were given to mice, the microbiota composition became more pro-inflammatory thereby increasing permeability [29, 94]. In humans, data should be interpreted with caution as permeability was not directly measured and prior studies restricted emulsifiers in combination with other dietary interventions making it difficult to attribute inflammatory affects to emulsifiers alone [95]. *Fat-soluble vitamins* may also impact permeability. In CD, vitamin D supplementation was shown to improve gastroduodenal permeability compared to placebo, though small bowel and colonic permeability was not impacted [96]. Whereas, a *high fat diet* is known to alter TJ

proteins and microbiota, thereby inducing inflammation in rat studies leading to increased permeability; prior UC cohorts have failed to show clear associations [97, 98]. *Polyphenols* are secondary metabolites found in fruits and vegetables, and mice studies have shown a reduction in colonic damage in the setting of indomethacin through the assembly of ZO-2, occludin and claudin-1; human studies have not yet been completed [3, 99]. *Glutamine*, an l-alpha amino acid abundant in human blood, has been shown to reduce intestinal permeability in patients with CD when given enterally [100]. *Acute and chronic alcohol use* in patients with concomitant liver disease can lead to increased permeability via cell damage and TJ protein alteration [101, 102]. Further research is needed to understand how dietary interventions impact patients with IBD.

Summary of Potential Pharmacologic Restoration of Normal Barrier Function in IBD

Given the pivotal role of TNF-α and MLCK as the main regulators of the leak paracellular pathway, there is evidence for:

a. *Adalimumab* was tested in two in vitro models of mucosal inflammation, stimulation of Caco-2 and T-84 cells with interferon-γ and TNF-α; it had beneficial effects on TJ proteins, reduced MLCK phosphorylation as well as reversing the decreased transepithelial resistance [103]. Based on studies of endoscopic biopsies from non-inflamed colon of seven patients with CD before and after *infliximab* infusions, ^{51}Cr-EDTA permeability was increased in CD and restored to control levels by infliximab. In addition, transmucosal uptake of adherent-invasive E. coli strain was reduced through a mechanism that involves blocking lipid rafts in epithelial cells [104]. Treatment of patients with infliximab resulted in a marked decrease of intestinal permeability as measured by the LMR, and mucosal mRNA expression associated with increased intestinal permeability (e.g., claudin, collagen, and laminin genes) was reversed by infliximab in patients with UC [105, 106]. These data suggest that barrier protection might therefore constitute a novel mechanism of how anti-TNF-α therapy contributes to epithelial restitution and tissue repair in IBD.

b. A *small molecule, divertin*, prevents MLCK1 recruitment by proinflammatory cytokines including TNF-α, interleukin-1β, and several related molecules to the perijunctional actomyosin ring [70]. Such recruitment of MLCK1 leads to molecular reorganization of TJs' structure and composition, including occludin endocytosis. Ultimately, divertin restores barrier function after TNF-induced barrier loss and prevents disease progression in IBD [70].

c. Older studies found some anti-inflammatory effects of *phosphatidyl choline phospholipids* on the colonic barrier [107]. More recently, a double-blinded, placebo-controlled randomized controlled trial found an improvement in Simple Clinical Colitis Activity Index in patients who received a newly designed phosphatidylcholine formula [108]. Further studies have yet to be completed to evaluate this unique therapeutic target in IBD patients with permeability measures.

d. *Specific drugs and targets* may regulate intestinal permeability and are under investigation. These include *monoclonal antibodies to IL-13, IL-22 and IFNγ, and drugs targeting receptors* (e.g., α2-adrenergic receptors with dexmedetomidine, glucagon-like peptide 2, glucocorticoids), or *ion channels* (e.g., chloride channel with lubiprostone), or *mast cell stabilizers* [89]. *MicroRNAs* are small non-coding RNAs that regulate several pathways in IBD including dysregulation of autophagy and Th17 signalling. In the future, microRNAs may show promise in treating IBD through mechanisms to fix pathology instead of targeting symptomology [109, 110].

Conclusion

There is compelling evidence of altered barrier function in both established IBD and as a risk factor in first-degree relatives of patients with IBD, particularly CD. Mechanistic studies have allowed for early investigations of unique IBD treatments by restoring the barrier function. However, it is essential to consider the complexity and multidimensional nature of the intestinal barrier that cannot be fully characterized using in vitro measurements or morphological description of TJ elements in the epithelial layer. It is important to apply valid measurements to document breaching of the barrier and responses to experimental approaches to treatment. The demonstration of environmental exposures to probe molecules in the diet and cosmetic preparations (e.g., mannitol, rhamnose, sucralose) suggests significant measurement bias may have occurred in prior studies, and thus there is the need for replication and validation of observations on barrier function in IBD. The validated methods are now available and should promote further research on the potential role of barrier function in the etiopathogenesis and management of IBD.

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Data availability

The data that support the findings of this review are available on request from the corresponding author. The data are not publically available due to privacy or ethical restrictions.

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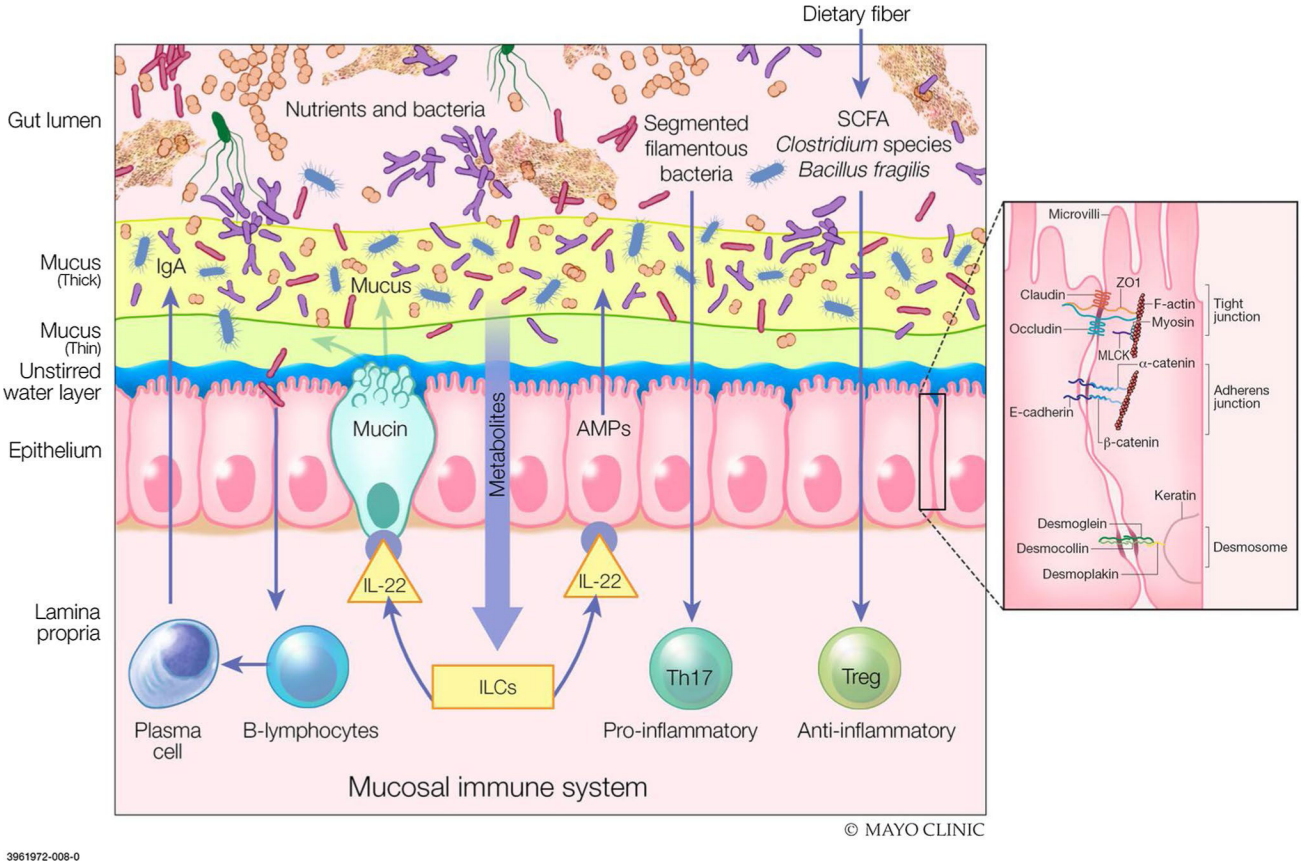

Fig. 1.

Components of intestinal barrier function. The gut lumen is where the degradation of bacteria and antigens by bile, gastric acid and pancreatic juice occurs. Commensal bacteria inhibit the colonization of pathogens through production of defense antimicrobial substances. The unstirred water layer, glycocalyx and thick/thin mucus layer works to prevent bacterial adhesion by immunoglobulin A (IgA) secretion. Within the epithelium, tight junctions (TJs) connect the epithelial cells to transport luminal contents. TJ-associated proteins (occludin, zonula occludens-1, claudin-1/4, junctional adhesion molecules) are vital to barrier integrity. Myosin light chain activation regulates the contraction and tension of actin which allows for opening of the paracellular pathways. The lamina propria is home to innate and acquired immune cells which secrete immunoglobulins and cytokines for use within the endocrine and enteric nervous system. Disruption of the intestinal barrier with permeation of noxious molecules to the lamina propria, induces mucosal immune system activation and inflammation. *SCFA* short chain fatty acids, *IgA* immunoglobulin A, *IL* interleukin, *ILCs* innate lymphoid cells, *Th* t helper cells, *Treg* T regulatory cells, *ZO-1* zonula occludens-1, *MLCK* myosin light-chain kinase (From: Camilleri M, Lyle BJ, Madsen KL, Sonnenburg J, Verbeke K & Wu GD. Role for diet in normal gut barrier function: developing guidance within the framework of food-labeling regulations. Am J Physiol Gastrointest Liver Physiol. 2019 Jul 1;317(1):G17-G39; used with permission of Mayo Foundation for Medical Education and Research, all rights reserved)

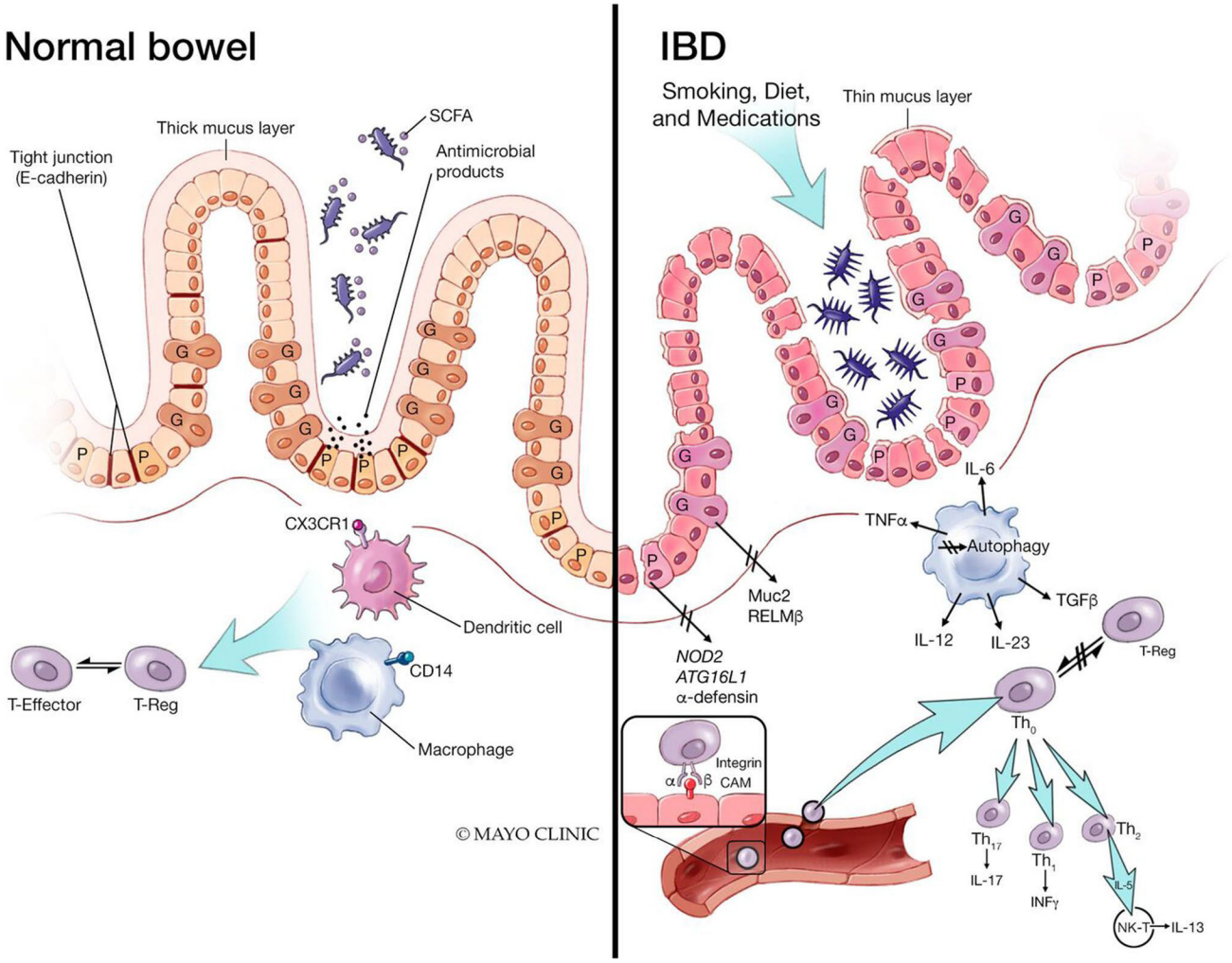

Fig. 2.

Impact of inflammatory bowel disease on the intestinal barrier. The pathogenesis of inflammatory bowel disease (IBD) is multifactorial due to genetic susceptibility, environmental factors, and immune dysregulation. The cytokine-mediated dysfunction of the TJ barrier via IL-6 and TNFα results in immune activation and mucosal inflammation, an essential component of IBD. Additionally, patients with IBD have increased intestinal permeability reflecting decreased epithelial barrier function. *SCFA* short chain fatty acids, *CXCr1* C-X-C motif chemokine receptor 1, *CD14* cluster of differentiation 14, *IL* interleukin, *TNFα* tumor necrosis factor alpha, *Muc2* Mucin 2, *RELMβ* resistin-like molecule beta, *ATG16L1* autophagy related 16 like 1, *Th* T helper cells, *INFγ* interferon gamma, *NK-T* natural killer cell (From: Ramos GP & Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019 Jan;94(1):155–165; used with permission of Mayo Foundation for Medical Education and Research, all rights reserved)

Table 1

Specific pathogenic or commensal microorganisms and microbial metabolites regulating mucus [25]

Microorganism or metabolite class	Specific microorganism or metabolite	Effect on mucins or mucus	Citations
Pathogenic	*Vibrio cholerae Giardia lamblia Entamoeba histolytica Trichuris muris*	Induce mucins degradation	[111, 112]
	Listeria monocytogenes Entamoeba histolytica Nippostrongylus brasiliensis Trichinella spiralis	Inhibit mucus production Regulate Goblet cell function Regulate mucin expression	[113, 114]
Commensals	*Lactobacillus spp.*	Stimulate MUC3 expression and MUC2 production and secretion	[115]
	Bifidobacterium longum	Restore mucus growth	[116]
	Lactobacillus reuteri	Increase mucus layer thickness	[117]
	Akkermansia muciniphila	Restore/increase mucus layer thickness Increase Goblet cell number	[34]
Microbial metabolites	Short chain fatty acids: acetate and butyrate	Stimulate MUC2 expression Increase mucus production Increase mucus secretion	[118]

Modified from Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut.* 2020;69(12):2232–2243

MUC mucins

Table 2

Barrier defects associated with inflammatory diseases of the intestine [41]

Disease	Cause of barrier defect	Timing of barrier defect	Effects on Tight Junction proteins and cytoskeleton	Citations
Crohn's disease	Unknown; Association with frameshift insertion at nucleotide 3020 of NOD2	Before clinical onset or clinical relapse	Claudin-2 upregulation; MLCK activation;	[61, 119, 120]
Ulcerative colitis	Unknown	Unknown	Occludin downregulation	[61, 119, 120]
C. difficile colitis	Actomyosin disruption; glycosylation of Rho proteins	Toxin release; disease onset	Loss of ZO-1 and ZO-2	[121]
GVHD	Associated t TNF production	After clinical onset	Unknown	[122]

Modified from Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009;9(11):799–809.

GVHD graft-versus host disease, *MLCK* myosin light chain kinase, *ZO* zonula occludens

Table 3

Effects of metabolites on immune, tight junction, and cellular elements of intestinal barrier [47]

Metabolite	Microbial sources of metabolites	Receptors	Effects of metabolites			Citations
			Immune	TJ / cellular	Microbial	
Butyrate	*Clostridium* clusters	GPR41, GPR109A, GPR65 (?)	↑ TGF-β	↓ proliferation of crypt stem cells ↑ TJ proteins (occlu- din, ZO-1)		[123]
Acetate	*Bacteroides spp. Prevotella spp.*	GPR43				[124]
Propionate	*Bacteroides spp., Veillonella spp., Dialister spp. Ruminococcus spp.*	GPR41, GPR43				[125]
Bile acids	*Bacteroides spp., Eubacterium spp., Lactobacillus spp. Clostridium spp.*	FXR, TGR5, pregnane X receptor (PXR), vitamin D (VDR)	↓ by UDCA, LCA	Permeability ↑ by DCA, CDCA ↓ IL-8 production by DCA, LCA		[126]
Tryptophan metabolites: kynurenic acid, HT, indole derivatives	*Bacteroides spp., Lactobacillus spp. Clostridium spp.*	GPR35 (?), Aryl hydrocarbon receptor (AHR), PXR	Indoles ↑ IL-10 to ↑ goblet cell differentiation	Indoles regulate epithelial repair and differentiation and reduce disassembly of adherens junction	Indole derivatives promote expression of anti-micro-bials	[127]

Modified from Iyer N, Corr SC. Gut Microbial Metabolite-Mediated Regulation of the Intestinal Barrier in the Pathogenesis of Inflammatory Bowel Disease. *Nutrients.* 2021;13[12]

GPR G protein receptors, *FXR* Farnesoid X receptor, *TGR* Triterpene glycoside receptor, *TGF-B* Transforming growth factor beta, *UDCA* Ursodeoxycholic acid, *LCA* Lithocholic acid, *IL* interleukin, *ZO* Zonula occludens, *DCA* Deoxycholic acid, *CDCA* Chenodeoxycholic acid, *HT* hydroxytryptamine

Table 4

Key dietary components associated with potential to have positive effects on overall gut integrity [3]

Component[a]	Common food sources
Prebiotic fiber	
Beta Glucan	Barley, mushroom, oat
Fructans	Asparagus, banana, barley, chicory root, garlic, honey, Jerusalem artichoke, leek, nectarine, onion, scallion, rye, wheat
Galacto-oligosaccharide	Cashew, legume (chickpea, red kidney bean, soybean, split pea), milk, pistachio, squash (butternut, pumpkin)
Pectin	Apple, banana, broccoli, carrot, dried pea, grapefruit, lemon, orange, potato, tomato
Resistant starch	banana, legume (black bean, dried pea, fava bean, lentil, pinto bean, soybean), whole Grain (barley, oat)
Xylo-oligosaccharide	Bamboo shoots and other vegetables, fruit, honey, milk
Polyphenol: subclass	
Flavonoid: Anthocyanin	Black bean, blackberry, black currant, black raspberry, blueberry, cherry, cranberry, eggplant, pecan, purple sweet potato, red cabbage, red grape, red (or blood) orange, red radish, red raspberry
Tannin: Ellagitannin	Almond, blackberry, blueberry, cranberry, pecan, pomegranate, raspberry, strawberry, walnut
Probiotic: bacteria/yeast	
Bifidobacterium Escherichia coli Lactobacilli Saccharomyces	Fermented dairy and nondairy sources: kefir, kimchi, kombucha, miso, sauerkraut, tempeh, yogurt
Amino Acid	
Glutamine	*Animal-based source*: dairy (cheese, milk, yogurt), egg, meat, poultry, seafood *Plant-based source*: almond, cashew, kale, legume (chickpea, kidney bean, lentil, peanut, soybean), mushroom (shiitake), pistachio, seed (pumpkin, sunflower), red cabbage, spinach, tomato, whole grain (oat, quinoa, wheat) **L**-glutamine used as a food additive and nutritional supplement
Mineral	
Zinc	*Animal-based source*: dairy (cheese, milk, yogurt), egg, meat (red), poultry (dark), shellfish (crab, lobster, oyster) *Plant-based source*: almond, legume (bean, lentil, pea), potato, seed (chia, pumpkin, sunflower), walnut, whole grain (oat, quinoa, wheat) Zinc-**L**-carnosine used as a nutritional supplement
Macronutrient	
Fat	Modify a typical Western-style diet high in fat (e.g. saturated fat). A Mediterranean diet eating pattern with focus on healthy unsaturated fats (olive oil, nuts), plant- based choices (fruits, vegetables, whole grains) and lean protein sources (fish, legumes) may favorably impact intestinal gut function

The U.S. Department of Agriculture (USDA) food composition data resource at https://fdc.nal.usda.gov/about-us.html was accessed to obtain information on food sources

[a]Commercially produced components (e.g., inulin, oligosaccharides, pectin, resistant starch, bacteria/yeast strains, l-glutamine, zinc-l-carnosine) are used as food additives/nutritional supplements