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Macronutrients as Regulators of Intestinal Epithelial Permeability: Where Do We Stand?

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ABSTRACT

The intestinal barrier function (IBF) is essential for intestinal homeostasis. Its alterations have been linked to intestinal and systemic disease. Regulation of intestinal permeability is key in the maintenance of the IBF, in which the intestinal epithelium and tight junctions, the mucus layer, sIgA, and antimicrobial peptides are important factors. This review addresses the concept of IBF, focusing on permeability, and summarizes state-of-the-art information on how starvation and macronutrients regulate it. Novel mechanisms regulate intestinal permeability, like its induction by the normal process of nutrient absorption, the contribution of starvation-induced autophagy, or the stimulation of sIgA production by high-protein diets in a T-cell-independent fashion. In addition, observations evidence that starvation and protein restriction increase intestinal permeability, compromising mucin, antimicrobial peptides, and/or intestinal sIgA production. Regarding specific macronutrients, substantial evidence indicates that casein (compared to other protein sources), specific protein-derived peptides and glutamine reinforce IBF. Dietary carbohydrates regulate intestinal permeability in a structure- and composition-dependent fashion; fructose, glucose, and sucrose increase it, while nondigestible oligosaccharides (NDOs) decrease it. Among NDOs, human milk oligosaccharides (HMOs) stand as a promising tool. NODs effects are mediated by intestinal microbiota modulation, production of short-chain fatty acids, and direct interactions with intestinal cells. Finally, evidence supports avoiding high-fat diets for their detrimental effects on IBF. Most studies have been carried out in vitro or in animal models. More information is needed from clinical studies to substantiate beneficial effects and the use of macronutrients in the treatment and prevention of IBF-related diseases.

1 | The Intestine and the Intestinal Barrier Function

The intestine is an organ with high plasticity, understood as the capacity to dynamically adapt to extrinsic factors. This plasticity is manifested in the response to dietary changes, inflammation, or tissue damage (Le Gall et al. 2019; Meyer et al. 2022). Intestinal barrier function (IBF) is the capacity of the intestine to absorb nutrients while regulating the passage of microorganisms and molecules present in the lumen (Salvo Romero et al. 2015; Sanchez de Medina et al. 2014). IBF and intestinal plasticity are closely related, and strict regulation is

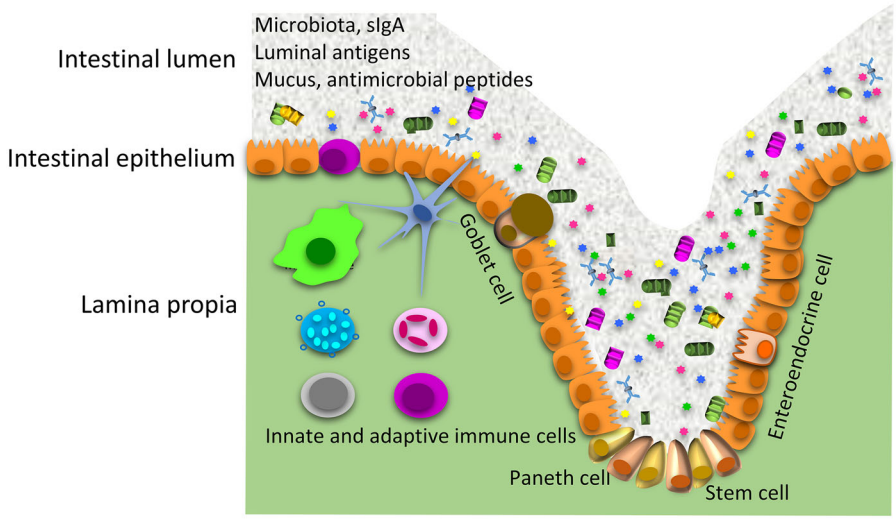

FIGURE 1 | Intestinal barrier function elements. IEL: intraepithelial lymphocyte; sIgA: secretory immunoglobulin A.

required to avoid disease. Several elements contribute to IBF, including the intestinal epithelium, the cells of the immune system, mainly located in the lamina propria underlying the intestinal epithelium, the mucus layer and antimicrobial peptides (AMP), secretory immunoglobulin A (sIgA), the microbiome, and intestinal motility (Di Tommaso et al. 2021) (Figure 1). The epithelium is the central element in IBF. This monolayer serves as a physical barrier to the passage of microorganisms and antigens from the lumen to the inner mucosa, while exerting absorptive functions (Chelakkot et al. 2018). Intestinal epithelial cells (IECs) include absorptive enterocytes, mucus-producing cells (also called goblet cells), and Paneth cells, which produce AMPs and regulate intestinal proliferation (Mei et al. 2020). Interestingly, pancreatic exocrine secretion may significantly contribute to AMP as well (Ahuja et al. 2017). In addition, the intestinal epithelium contains enteroendocrine cells that produce hormones to regulate intestinal motility, satiety, insulin secretion, or the release of digestive enzymes (Vancamelbeke and Vermeire 2017). Tuft cells are intestinal epithelial chemosensory cells closely related to the taste receptor cells in the oral cavity (Silverman et al. 2024). Thanks to their chemosensory capacity, they detect parasites, such as helminths and protozoa, and can initiate innate immune responses (Howitt et al. 2016). Finally, stem cells, located at the base of the intestinal crypts, are responsible for the generation of all epithelial cell types (Barker et al. 2012). Stem cells give rise initially to amplifying cells, which are in the immediately adjacent region upward and are characterized by high proliferative capacity. These cells undergo differentiation as they ascend toward the surface (Sanman et al. 2021).

The intestinal epithelial layer must be continuously renewed, ensuring prompt recovery from injury. In humans, this process takes place in 5–7 days. Proliferation arises in the crypts and is regulated by crypt cells (particularly Paneth cells, via Notch ligands and epidermal growth factor), mesenchimal cells, and the microbiota. This system is extremely efficient and at the same time is adaptable to different circumstances (fasting, inflammation, cell injury). This is of particular relevance considering the high cost of epithelial homeostasis.

From a metabolic point of view, absorptive enterocytes and colonocytes use mainly oxidative phosphorylation as source of energy, but with different preferred substrates. Small intestinal enterocytes depend mainly on D-glucose, L-Gln, and L-Glu, obtained from plasma, while colonocytes use luminal butyrate generated by the microbiota. In turn, stem cells at the crypt base depend mainly on glucolysis to obtain energy at a fast pace.

2 | Intestinal Permeability

The regulation of epithelial permeability is of utmost importance for IBF plasticity (Y. Liu and Chen 2020). Control of intestinal permeability is pivotal for the absorption of nutrients and to avoid the undesirable effect of the uptake of harmful molecules. Inflammation, bacteria, or dietary antigens may alter intestinal permeability, enabling the transfer of luminal contents to the lamina propria and the systemic circulation, and triggering immune reactions. Consequently, impairment of intestinal permeability has been related to both intestinal and systemic diseases.

Flux across the epithelial barrier occurs either across the cells (transcellular pathway) or between cells (paracellular pathway). Epithelial permeability is regulated primarily at the level of the epithelium itself, but it also depends on various elements of the intestinal barrier, such as mucus, IgA or AMP. Mucus, permeable to solutes but not luminal microorganisms, is produced mainly by goblet cells, and constitutes the first defense layer of the mucosa (Fekete and Buret 2023). It is mainly composed of mucins, strongly O-glycosylated proteins that form polymeric networks (S. Yang and Yu 2021). In the small intestine, there is a single layer of easily penetrable (lax) mucus, whereas in the colon, the mucosal barrier comprises a similar lax outer layer, which features degraded MUC2 (the main mucin), and a denser inner layer (Arike et al. 2017). Access of the microbiota is normally limited to the outer layer. In pathologic conditions, particularly in an inflammatory context, microbial penetration is facilitated and contact with the epithelium is enhanced (Johansson et al. 2011).

IgA, an antibody isotype naturally resistant to proteolysis, is the main antibody isotype in the intestinal mucosa. It is actively

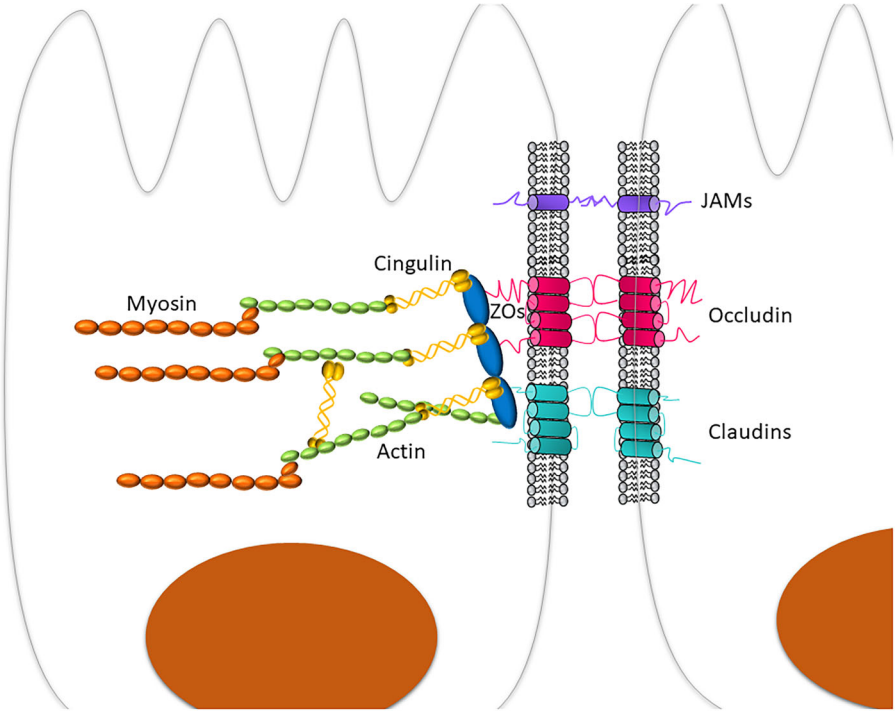

FIGURE 2 | Tight junction structure and proteins involved. JAM: junction adhesion molecules.

secreted by IECs through transcytosis as a dimer, associated with the J chain and the secretory component. sIgA reaches the gut lumen in substantial amounts (~3 g/day) (Kaetzel 2014). Interestingly, newborn children lack intestinal IgA producing cells, while IgA is a major immunoglobulin in colostrum (Brandtzaeg 2010). IgA targets a diverse range of antigens, including toxins, and prevents microbial attachment and motility (Han et al. 2023; Siniscalco et al. 2024). In addition, sIgA has the capacity to aggregate rapidly dividing bacteria, to inhibit biofilm formation, and to bind environment-sensing specific molecules on microbes, regulating microbe response. In addition, DNAase and Rnase activities have been reported for human milk sIgA, with the heavy chain featuring the binding DNA site and the light chain the catalytic site (Nevinsky et al. 2000). Through these and other mechanisms IgA modulates the composition of the microbiota. For instance, sIgA-deficient mice exhibit increased amounts of segmented filamentous bacteria (Suzuki et al. 2004).

AMP (cathelicidin, a- and b-defensins, regenerating islet-derived protein (REG) IIIg, elafin, and so forth) are secreted by immune cells but also by Paneth cells and absorptive enterocytes. They contribute directly to antimicrobial defense in the mucus layer and the crypt space, but also exert other biological activities, such as immunomodulation, antiapoptotic effects, protease inhibition, and so on, and may modulate the microbiota.

Epithelial permeability is regulated chiefly by junctional protein complexes at the apical side called tight junctions (TJs). Other complexes (adherent junctions, desmosomes) contribute to cell adhesion but do not influence permeability (Groschwitz and Hogan 2009). They include transmembrane proteins and cytosolic scaffolding proteins (Figure 2). The former include junction adhesion molecules (JAMs), claudins, occludin, and tricellulin. JAM-A plays a significant role in the regulation of permeability to macromolecules. Claudins are tetraspanning proteins that regulate permeability by forming strands via homo- or heteromeric interactions in antiparallel orientation, thus generating pore-like structures. Occludin is also a tetraspanning protein whose distribution and phosphorylation modulate intercellular adhesion, TJ strand complexity, and permeability to macromolecules (Srivastava et al. 2022). Scafolding proteins, like zonula occludens (ZOs) 1–3 and cingulin, bind to intracellular motifs of TJ transmembrane proteins and to the cytoskeleton. ZO-1 interacts with multiple proteins through several PDZ domains; the first one interacts with claudins, the second dimerizes with ZO proteins 2 and 3, and the third participates in the oligomerization with JAM-A. ZO-1 also forms oligomers with other proteins, like occludin or catenin, through other oligomerization domains. Finally, ZO-1 binds cytoskeletal proteins like F-actin through an actin binding region located at its carboxy-terminal end. Cingulin has also been shown to bind JAM-A, and to exhibit high affinity for F-actin and myosin (Horowitz et al. 2023) (Figure 3).

TJs are dynamically regulated by various stimuli, selectively allowing the passage of water, ions, and nutrients through intercellular pores. The expression of their proteins is regulated at the transcriptional and posttranscriptional level. In addition, TJs are regulated by protein redistribution. For example, ZO-1 may switch from TJs to cytoplasm in an energy dependent manner, while occludin passively diffuses between the apical and lateral membranes (Moonwiriyakit et al. 2023). Phosphorylation is a major mechanism to regulate TJ protein distribution and turnover. In this regard, occludin phosphorylation has been widely studied (Farshori and Kachar 1999). Occludin contains a C terminal tail with multiple phosphorylation sites for kinases such as protein kinase C, casein kinase 2, and c-SRC. Phosphatases are also known to regulate occludin phosphorylation, including protein phosphatase 2A (PP2A), PP1, and protein tyrosine phosphatase

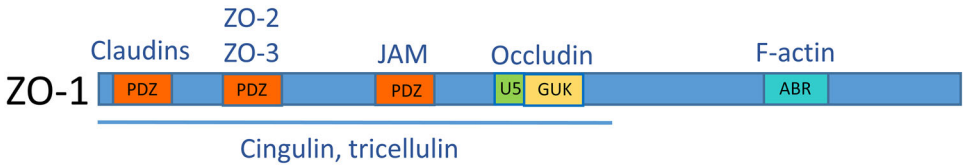

FIGURE 3 | Zonulae occludens 1 (ZO-1) domains and TJ protein interaction. ABR: actin-binding region; Guk: guanylate kinase homology domain; JAM: junction adhesion molecules; U5: unique 5.

1B (Nunbhakdi-Craig et al. 2002; Raleigh et al. 2011; Rao 2009; Seth et al. 2007; Srivastava et al. 2022). Inhibition of casein kinase 2 and the consequent dephosphorylation of amino acid S408 triggers the assembly of ZO-1 with occludin and claudins, so that claudin-2 channels become inactivated (Raleigh et al. 2011). In turn, phosphorylation of S408 allows the homodimerization of occludin and the consequent trans-homodimerization of claudin-2 (the head- to-head binding of claudin-2 between adjacent cells), to form a paracellular pore.

Multiple signal transduction pathways are known to regulate TJs. Among them myosin light chain kinase (MLCK), a Ca^{2+}/calmodulin-dependent serine/threonine kinase, stands out as a primary player (Markovich et al. 2024). MLC phosphorylation increases paracellular permeability in response to nutrients and is linked to Na^+-nutrient transport (H. Zhang et al. 2024). Phosphorylation triggers a contractile force that increases physical tension in TJs and subsequently intestinal permeability to small, nutrient-sized molecules (Turner et al. 2000; Turner et al. 1997). Other important regulators are Toll-like receptors (TLRs), which are involved in the coordination of the intestinal response to bacteria and other luminal contents, including nutrients like oligosaccharides and fatty acids (Capitan-Canadas et al. 2014; Fessler et al. 2009; Ortega-Gonzalez et al. 2014). Activation of TLR4 induces inflammation-related signal transduction pathways like those mediated by NF-κB or mitogen-activated activated protein kinases (MAPK), resulting in enhanced intestinal permeability through a mechanism that involves upregulation of MLCK (M. Nighot et al. 2019).

cAMP-dependent protein kinase (AMPK) acts as a nutrient sensor and is pivotal in maintaining energy homeostasis. It is activated by elevated adenosine monophosphate, along with decreased levels of glucose. Activation of AMPK results in maintenance of intestinal barrier homeostasis by increasing transepithelial resistance and decreasing paracellular permeability (Olivier et al. 2019; Sun et al. 2017). In fact, it has been shown that deletion of intestinal epithelial AMPK alters distal colon permeability (although changes in TJ protein expression were not found). The mechanism involves increased CDX2 expression, an effect that is partially mediated by histone modifications in the *CDX2* promoter, and the phosphorylation of TJ proteins (claudins-1 and 4 and cingulin, among others) (Gao et al. 2023; Olivier et al. 2019; Sun et al. 2017).

The maintenance of apical junction complexes is an energy-intensive process in which nucleotides are primarily required for the synthesis of ribosomal and messenger RNA. In addition, energy is required mainly in the form of Guanosine Triphosphate (GTP), for protein synthesis, and in the form of ATP for protein translocation and folding, for their posttranslational modifications and for their trafficking. The very regulation of TJs requires energy in the form of ATP, since they are dynamically associated with actin and myosin filaments. While the actomyosin ring around the cells provides stability and intercellular tension, regulating the flow through the TJs is an ATP-dependent mechanism, as myosin filaments require ATP for extension and contraction and actin also for polymerization (Miyoshi and Takai 2008; Quiros and Nusrat 2014).

Transcellular transport is very selective, generally needing apical and basolateral transmembrane transporters. In turn, paracellular transport is somewhat less selective and involves the movement of molecules via two distinct mechanisms: the pore pathway and the leak pathway (Pácha 2000; Wijtten et al. 2011). The pore pathway is defined as a high conductance, size- and charge-selective route that facilitates the movement of small ions and solutes with a diameter of <0.6 nm. It depends on the formation of pores by claudin 2 and 15. The leak pathway is not charge sensitive and mediates the movement of large solutes, up to 12.5 nm. This latter pathway remains less understood, but is regulated by occludin, tricellulin, ZO-1, claudin 4, and perijunctional actomyosin (Horowitz et al. 2023; Lingaraju et al. 2015). The 4-kDa fluorescein isothiocyanate dextran is a widely used marker of leak pathway permeability (Horowitz et al. 2023) (Figure 4). Both the pore and the leak pathways are essential for nutrient absorption (Figure 5). Regulation of TJ by Na^+-glucose cotransport, an acute and reversible response to luminal nutrients, illustrates the importance of this mechanism. Glucose transcellular transport is mediated chiefly by SGLT1 (a cotransporter of glucose and Na^+) and GLUT2 (a facilitated-diffusion glucose transporter), located at the apical and basal sides of the epithelium, respectively (L. Chen et al. 2016). The driving force for apical glucose uptake is provided by the electrochemical gradient, which is generated mainly by basolateral Na^+-K^+ ATPase and K^+ channels, and therefore by apical uptake of luminal Na^+. However, the latter would promptly become limiting as it is depleted in the process. The pore paracellular pathway allows transport of Na^+ back to the intestinal lumen, so that the absorptive process can continue unimpeded. On the other hand, Na^+ and nutrient absorption drive passive paracellular fluid absorption via the leak pathway, activated through MLCK and the contraction of actomyosin. Fluid is absorbed from the unstirred layer of the lumen, close to the epithelium. Due to the action of brush border enzymes, this layer contains high concentrations of nutrient monomers. Fluid absorption therefore carries nutrients by the mechanism of solvent drag (He et al. 2020; Horowitz et al. 2023).

In addition to the regulation of transport mechanisms, when the intestinal epithelium is damaged, permeability may be heavily altered, allowing unrestricted passage of molecules (Romero-Calvo et al. 2011).

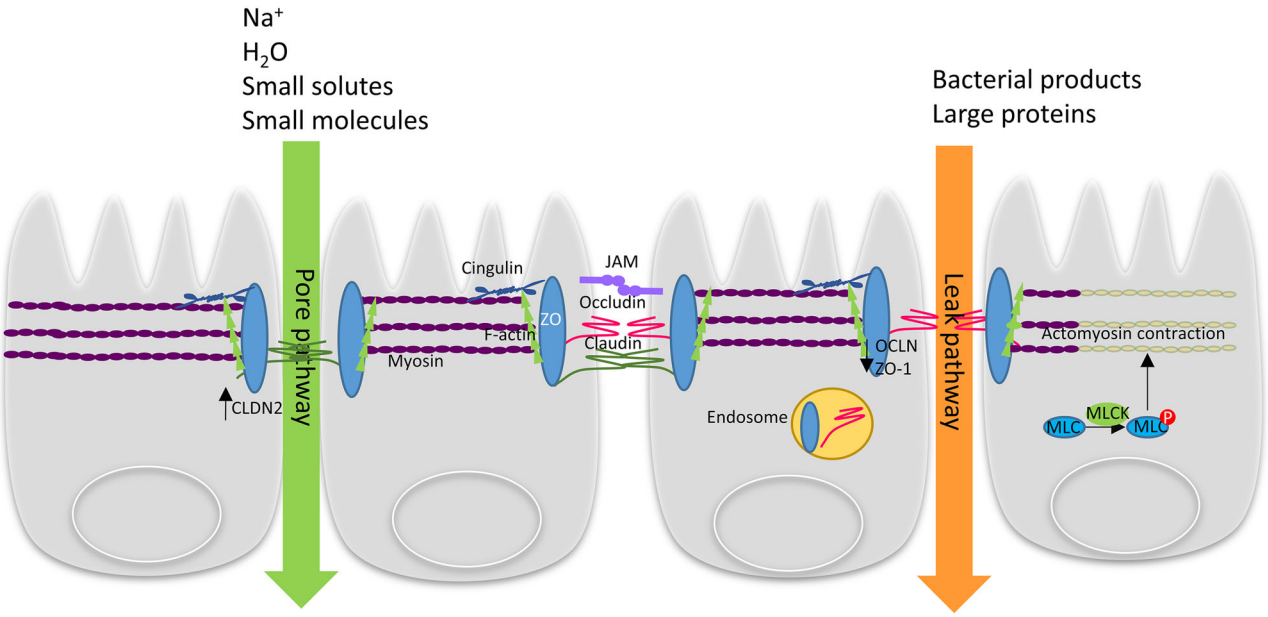

FIGURE 4 | Pore and leak pathways. CLDN: claudin, JAM: junction adhesion molecules; MLC: myosin light chain; MLCK: myosin light chain kinase; OCLN: occludin; ZO: zonulae occludens.

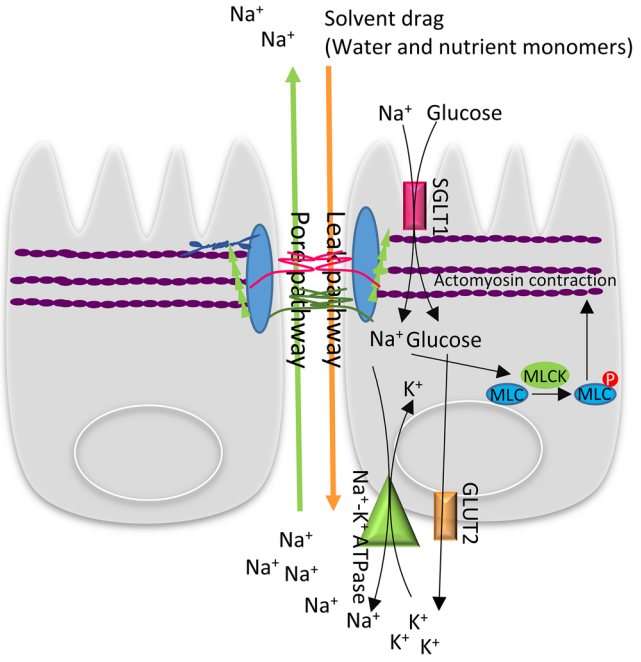

FIGURE 5 | Regulation of pore and leaky pathways by Na^+ and nutrients.

Macronutrients and the lack of nutrients play a key role in the regulation of intestinal permeability (Choi et al. 2023; Seethaler et al. 2022).

3 | Regulation of the Intestinal Permeability by Proteins, Peptides, and Amino Acids

Protein restriction has been shown to alter TJs and to decrease sIgA (Amaral et al. 2006; Menezes et al. 2003; Y. Zhu et al. 2018), while a thinner mucus layer has been observed in high-protein diets because of the presence of mucus degrading bacteria (Chen et al. 2021). However, the effects of protein restriction are complex. Thus, in a study in which rats were fed diets with different protein content for 2, 4, and 10 weeks, TJ modifications were shown but the outcomes were not easy to interpret; feeding low-protein diets (12% wt/wt) for 2 weeks decreased jejunal ZO-1 and occludin expression compared with rats fed a control protein diet (19.3% wt/wt). On the other hand, higher ZO-1 and occludin levels were observed after 4 weeks, as well as occludin levels after 10 weeks, in the jejunum of rats fed the low-protein diet for 2 week and switched to the control diet, while levels remained unchanged in normal protein diets. The latter may represent a compensatory response fueled by higher availability of luminal nutrients. Further studies are needed to better describe the effects of protein deprivation in TJs and mucus (Y. Zhu et al. 2018). Dietary protein is essential for adequate development of the mucosal immune system. For instance, if weanling mice are fed an amino acid diet for ~5 weeks a decrease in sIgA as well as in serum IgA and IgG is observed (Menezes et al. 2003). This defect is reversible upon administration of a casein diet, starting at 5% casein supplementation but requiring at least 10% casein for total recovery (Amaral et al. 2006). Antigen availability has been proposed to account for these effects.

A mechanism involved in the regulation of sIgA by proteins has been recently proposed. The IgA response in the intestinal tract can develop by T-cell-dependent and independent mechanisms. In response to infection or immunization, antigen-activated B cells, including those producing sIgA, undergo antigen-driven B-cell receptor affinity maturation process in a T-cell dependent manner, a process that is undertaken in permanent germinal centers located in gut-associated lymphoid structures such as Peyer's patches. Nevertheless, the presence of mucosal intestinal IgA in the absence of T cells and microbiota indicates the existence of T-cell-independent mechanisms. IgA class switch

independently of T cells has been documented mainly in the intestinal lamina propria, driven by dendritic cells or innate lymphoid cells. Recently, a novel mechanism of sIgA induction has been unveiled in mice fed a high-protein diet. These mice, and not those receiving high-carbohydrate or high-fat diets, produce high concentrations of succinate that, in turn, increase the generation of reactive oxygen species and consequently the production of significantly higher quantities of extracellular vesicles. These vesicles activate TLR4 and subsequently the epithelial expression of the IgA-inducing cytokine, APRIL, the B cell chemokine, CCL28, and the IgA transporter, PIGR (the receptor that facilitates sIgA transcytosis) (Tan et al. 2022).

Some specific proteins and peptides derived from enzymatic digestion reportedly enhance IBF. Digestion-derived bioactive peptides are fragments of proteins, typically containing 2–20 amino acid residues, which are biologically inactive as part of the native molecule, but which may become bioactive upon release. Tables 1 and 2 summarize the main in vitro and in vivo studies on the effect of dietary proteins, peptides, and amino acids on intestinal permeability. Effects on sIgA levels are dependent on the specific protein. For instance, in weanling mice fed, a soy protein isolate for 21 days a decrease in sIgA levels (and mucins) was observed when compared to casein fed mice (considered a high-quality protein) (Zeng et al. 2020). This may be related to reduced IL-4, IL-13, and TGFb levels. The exact mechanism has not been identified, but a lower presence of branched amino acids, different miRNAs and changes in the microbiota have been proposed to be involved. sIgA modulation by specific proteins has been mainly shown in models in which the intestine is not mature and in those in which the intestinal immunity is compromised. Ovotransferrin (a glycoprotein from avian egg) has been shown to exert immunostimulatory effects, with regulation of the imbalanced of Th1:Th2 ratio and sIgA overexpression, in a cyclophosphamide-induced immunosuppression mouse model (G. Zhu et al. 2018). As cyclophosphamide treatment induced the MAPK pathway to cause intestinal damage, blockage of this pathway by ovotransferrin was indicated as a possible mechanism of action. Bovine lactoferrin is also known for its immune-modulating and anti-inflammatory effects and has been shown to induce sIgA in models of short bowel syndrome and acute stress. In fact, in this model in rats, orally administered lactoferrin (0.5 g/kg per d) was shown to increase sIgA in the ileum, an effect that was not observed in the "sham" group (bowel transection and reanastomosis) or in the control group fed lactoferrin (Wu et al. 2014). Lactoferrin increases total and sIgA in the distal small intestine (Godínez-Victoria et al. 2017). Stress compromises IBF, and sIgA is modulated by stress. Pretreatment with bovine lactoferrrin for 7 days prior to acute stress immobilization for 1 h triggered higher levels of sIgA and pIgR in the proximal intestine as well as those of the α/J-chain and pIgR in the distal intestine (Peña-Juárez et al. 2016). However, in a model of chronic stress achieved by immobilization for 1 h, 7 days, lactoferrin limited the positive effects of stress at this level (Cruz-Hernández et al. 2021).

IgA secretion is enhanced by a number of bioactive peptides obtained from egg, milk, plants, and fish proteins in healthy animals (Duarte Villas Mishima et al. 2024; J. Li et al. 2018; Q. Li et al. 2019; Martínez-Augustin et al. 2014). This is typically associated with an increase in the number of IgA^+ cells in the small intestine and colonic mucosa and with an isotype switch from IgM to IgA (Q. Li et al. 2019; Martínez-Augustin et al. 2014). In addition, protective effects in disease models have been documented. For instance, the peptidic fraction of a *Lactobacillus helveticus* fermented milk showed protection against *Escherichia coli* O157:H7, associated with increased numbers of IgA^+ B cells and higher intestinal and serum IgA (Leblanc et al. 2004). A fraction of milk fermented by *Lactobacillus helveticus* R389 was similarly protective against *Salmonella enteriditis* serovar Typhimurium (Vinderola et al. 2007). In some cases, specific peptides have been isolated. For instance, in a study focused on prevention of fatigue in mice, a pea protein hydrolysate, but not the intact protein, was found to exert a positive effect, which was linked to improved gut immunity, including increased phagocytosis and sIgA production (Feng et al. 2021). While the three most abundant peptides (QLEELSK, KGDFELVGQ, and FFELTPEKNQ) were identified, they were not characterized in terms of bioactivity. Similarly, a protein hydrolysate from Alaska pollock was found to protect against cyclophosphamide induced immunosuppression in mice, including higher sIgA and enhanced IgA+ B cell differentiation. Two main peptides with immunomodulatory activity were identified (GVIK and ACNGR) but the mechanism was not characterized (Q. Li et al. 2019).

We will focus next on other elements of the intestinal barrier, such as TJ, epithelial proliferation, or mucus secretion. Most studies feature milk proteins, hydrolysates, and peptides. In general, available studies suggest that milk proteins and their hydrolysates can modify cell permeability, induce mucus release and sIgA secretion (please see above), and enhance mucus and TJ protein expression. For instance, bovine lactoferrin and its peptides stimulate the proliferation and differentiation of IECs in vitro (Buccigrossi et al. 2007; Hagiwara et al. 1995; Jiang et al. 2014), as well as in vivo in piglets (Reznikov et al. 2014), in addition to the effects described above. The mechanism is only partially characterized but seems to involve MAPK and cyclooxygenase 2. Lactoferrin actions are aided by resistance to digestion. In this sense, oral supplementation of bovine lactoferrin in piglets induced intestinal maturation, assessed by an increase in the area, depth, and width of crypts in the jejunum, and the upregulation of expression of the TJ protein occludin (Wells et al. 2022). Casein, a milk-derived protein, and particularly its hydrolysates, appear to be more effective than other peptides or proteins from milk or other origins in restoring intestinal permeability. Thus, studies in long-term starved rats showed a better recovery of intestinal permeability when fed casein hydrolysates than intact casein, whey protein, or whey protein hydrolysates (J. Boza et al. 1996, 1995). Further evidence for the therapeutic potential of casein was obtained by Paparo et al. (2020). In this study, the authors compared five different formulas based on protein hydrolysates from whey, casein, soy, and rice, and an amino acid free formula. Reconstituted formulas were subjected to an in vitro infant gut simulated digestion (sequential gastric and duodenal static model), and the protein fraction subsequently tested. Casein was found to be the most effective IBF modulator, including effects on mucin 5AC, occludin, and ZO-1 in human enterocytes (Caco 2 and NCM460 cells). Effects of milk whey proteins have been shown. A bioactive bovine whey protein extract was shown to reduce intestinal permeability (in Caco 2 cells both in basal and TNF-stimulated conditions. Nevertheless, this same extract failed to alleviate exercise-induced gut permeability in healthy adults (Ulluwishewa et al. 2024). In addition, in patients suffering from

TABLE 1 | Main in vitro studies on the effect of dietary proteins, peptides, and amino acids on intestinal permeability.

Nutrient	Concentration	Length intervention	Cell type	Outcomes	Ref
Lactoferrin	0.1–1000 μg/mL	48 h	Caco-2	Lactoferrin ↑ growth and proliferation	Buccigrossi et al. 2007
Bovine lactoferrin	1.9–150 μg/mL	4-24 h	IEC-18	Lactoferrin ↑ proliferation	Hagiwara et al. 1995
Human and bovine lactoferrin	2.5–800 μg/mL	25 days	Caco-2, C3A	↑ proliferation and differentiation	Jiang et al. 2014
Whey protein hydrolysate or bovine β-lactorphin	0.1, 1% w/v (hydrolysate) 0.05–0.5 mM (β-lactorphin)	2-24 h	HT29-MTX	Amidated β-lactorphin ↑ mucin expression and secretion	Martínez-Maqueda D, et al. 2013
Extensively hydrolyzed whey formula (EHWF), extensively hydrolyzed casein formula (EHCF), hydrolyzed rice formula (HRF), soy formula (SF), and amino acid-based formula (AAF)	25 μg/mL	48 h	Caco-2 NCM460	EHCF: ↑MUC5AC, occludin, ZO-1 SF: ↑ occludin EHWF and HRF: ↑TSLP SF and HRF: ↑IL33 release EHCF: ↑IL10 and IFNγ and activate Treg	Paparo et al. 2020
Casein, casein hydrolysate, lactalbumin hydrolysate, egg albumin, egg albumin hydroysate, meat hydrolysate, β-casomorphin-7 and a mix of aminoacids.	0.5–5% w/v casein or lactalbumin hydrolysates 12–120 μM β-casomorphin-7 4% w/v aminoacids 5% w/v other products	30 min	Isolated vascularly perfused rat jejunum	Casein and lactalbumin hydrolysates, and β-casomorphin-7 ↑ mucin release No effect of casein, chicken egg albumin or hydrolysate, meat hydrolysate or a mix of aminoacids	Claustre et al. 2002
Pepsin-trypsin digested gliadin	1 mg/mL	3 h	Caco-2	↑ IL-6 ↑ redistribution of zonulin and occludin	Giorgi et al. 2020
Pepsin-trypsin digested gliadin	1 mg/mL	90 min	Intestinal tissues of wild-type C57BL/6	↑ zonulin release, permeability Not in CXCR3$^{-/-}$ mouse	Lammers et al. 2008
Pepsin-trypsin digested gliadin	1 mg/mL	24 h, 4h	Human macrophages and intestinal organoids	↓ oxidative phosphorylation ↓ defensin α	Miyagawa et al. 2024
β-Casomorphin-7 and its derived peptides	1120 μM		Isolated perfused rat jejunum	↑ mucin production (depending on the route of administration)	Trompette et al. 2003
β-casomorphin-7	1–100 μM	24 h	DHE HT29-MTX	↑ *Muc2*, *Muc3* (DHE) ↑ MUC5AC (HT29/MTX)	Zoghbi et al. 2006
α_{s1}-casein fragments: ^{143}AYFYPEL^{149}, ^{144}YFYPEL^{149}, ^{144}YFYPE^{148}, ^{144}YFYP^{147} and ^{144}YFY^{146,} β-casein fragment: ^{60}YPFPGPI^{66}	61 nM–10 μM (guinea pig ileum) 550 nM–10 μM (mouse vas deferens)		Guinea pig ileum, mouse vas deferens HT29-MTX	Various α_{s1}-casein fragments showed ileal opioid activity ^{144}YFYPEL^{149} ↑ mucin production	Fernández-Tomé et al. 2016

(Continues)

TABLE 1 | (Continued)

Nutrient	Concentration	Length intervention	Cell type	Outcomes	Ref
Casein hydrolysate and casein fragments 90–94 (RYLGY), 143–149 (AYFYPEL), and 144–149 (YFYPEL)	0.1, 1% w/v (casein hydrolysate) 0.05, 0.1, 0.5 mM (peptides)	2-24h	HT29-MTX cell line	Casein hydrolysate, AYFYPEL, YFYPEL ↑ mucin, *MUC5AC*	Martínez-Maqueda D, et al. 2013
GPA peptide (isolated from fish skin gelatin hydrolysate)	0.1–2 mM	6 h	MODE-K	↓ proinflammatory cytokine, cytotoxicity, reactive oxygen species, malondialdehyde ↑ autophagy	Deng et al. 2020
Egg white protein Ovotransferrin-derived IRW	50, 100 μM	24 h	Caco-2	IRW opposes LPS ↓ TEER	Bao and Wu 2022
MBCP peptide obtained from *Bubalus bubalis milk, after digestion of* Mozzarella di Bufala Campana DOP	0.07 μM	21 d	Caco-2	↑ differentiation ↓ TNF-induced permeability	Tenore et al. 2019
Arg	3–10 mM	48 h	IEC6	↑ proliferation ↓ apoptosis	Xia Z, et al. 2019
Cystine (Cys2)	0.1–1 mM	24 h	Caco-2	↓ H_2O_2-induced TJ permeability ↑ GSH ↓ proinflammatory cytokines	Hasegawa et al. 2021
Gln and glutamine synthase inhibitor L-S-(3-amino-3-carboxypropyl)-S-Methylsulfoximin	0.2 and 0.6 mM	2 h	Caco-2	Low Gln is sufficient to support IBF Gln deprivation ↓ IBF	DeMarco et al. 2003
Gln	25 and 50 mM	24 h	Caco-2	Gln ↓ TEER, Permeability unchanged	Huang et al. 2022
Gln	0.1–4 mM	5 days	Caco-2	Gln deprivation ↓ TJ	Li et al. 2004
Gln	0.5 and 2mM	48 h	IPEC-1	Gln ↑ CaMKK2-AMPK → ↑ TJ, IBF	Wang et al. 2016
Gln	0.6–30 mM	180 min	Jejunum of Wistar rats	Gln ↑ ion pump activity, paracellular permeability	Yang et al. 1999

Abbreviations: AAF, amino acid-based formula; EHCF, extensively hydrolyzed casein formula; EHWF, extensively hydrolyzed whey formula; GSH, reduced glutathione; HRF, hydrolyzed rice formula; IBF, intestinal barrier function; LPS, lipopolysaccharide; SF, soy formula; TEER, transepithelial electrical resistance; TNF, tumor necrosis factor; TJ, tight junction.

Crohn's disease, intestinal permeability and morphology were improved after receiving either glutamine or whey protein at 0.5 g/kg ideal body weight/day for 2 months (equivalent to 1/3 of protein requirement) (Benjamin et al. 2012).

The effect of caseins on mucin production and release has been widely studied. For example, casein has been shown to increase intestinal mucin secretion in parallel with the upregulation of *Muc2* and *Muc4* in ileum and colon tissues when administered as a hydrolysate (Fernández-Tomé et al. 2017). In another study, when compared to casein, a casein hydrolysate was also found to better stimulate mucin secretion in perfused rat jejunum (maximal response at 417% of controls). In this study, a lactalbumin hydrolysate was less effective than casein, and a mixture of amino acids had no effect. In turn, chicken egg albumin and its hydrolysate, or a meat hydrolysate, did not modify mucin release (Claustre et al. 2002). In contrast, compared with animals fed a casein-based diet, a diet prepared with soy protein isolate was shown to attenuate intestinal mucin production in weaning mice, as demonstrated by the reduced number of intestinal goblet cells and the reduced relative expression levels of mucin 1 (*Muc1*) and mucin 2 (*Muc2*) (Zeng et al. 2020). In a study

TABLE 2 | Main in vivo studies on the effect of dietary proteins, peptides, and amino acids on intestinal permeability.

Treatment	Dosage	Control	Age/Weight	Species	Length intervention	Outcomes	Ref
Protein deprived diet	Ad libitum	15% w/w casein-containing diet	Weaning	C57BL/6J mice	10 weeks	Poorly developed gut-associated lymphoid and ↓ sIgA	Menezes et al. 2003
Low protein diet	Ad libitum	Normal protein diet	21 days	Sprague-Dawley rats	14, 28, 70 days	Normal diet improves jejunal morphology and gene expression	Zhu et al. 2018
High-casein diet, high whey protein diet, or high soy protein diet in a colitis model induced by DSS	Ad libitum	Standard diet	3 weeks	Female BALB/c mice	2, 4, 10 weeks	High-protein diets changed microbial composition and thickness of colonic mucus layer → worsening DSS colitis	Chen et al. 2021
High-protein, high-carbohydrate or high-fat diets	Ad libitum	–	6 weeks	Male C57BL/6 mice	6 weeks	Association between high protein diet and ↑ sIgA. Microbiota of high protein fed mice produces ↑succinate → ↑ extracellular vesicles → TLR4 activation → ↑ IgA	Tan et al. 2022
Casein and casein hydrolysate	225 g of casein /kg of diet 229 g of casein hydrolysate/kg of diet	Normally fed rats	21 days	Male Wistar rats	3 day starvation 4 day recovery	Both diets restore nutritional status and intestinal function in starved rats. Permeability normalized with hydrolysate, not with full protein	Boza et al. 1995
Bioactive bovine whey protein extract after exercise	200 mg/day	–	18–50 year	Adult humans	14 days	No effect on exercise-induced gut permeability	Ulluwishewa et al. 2024
Soybean, chicken, and pork proteins either with low fat (12% kcal) or high fat (60% kcal)	184.47, 189.56, 184.70 g/kg, respectively	–	7 weeks	C57BL/6J mice	12 weeks	Meat protein ↑ claudin 1 in low=fat but not high-fat diet	Hussain et al. 2019
Soy protein isolate	200 g/kg	Casein diet	3 weeks	C57BL/6 mice	21 days	Soy protein vs casein ↓ IBF: ↓ sIgA and numbers of goblet cells. ↓ Muc1, Muc2, Tff3	Zeng et al. 2020

(Continues)

TABLE 2 | (Continued)

Treatment	Dosage	Control	Age/Weight	Species	Length intervention	Outcomes	Ref
Isolated whey protein and whey protein hydrolysate	22.5% diet	Normally fed rats (no starved)	Weaning	Male Wistar rats	4 day fasting + 4 day recovery	Not complete recovery of intestinal permeability after starvation with either isolated whey protein or hydrolysate. Both have similar effects	Boza J, et al. 1996
Pea (Pisum sativum L.) peptides and pea intact protein	100–400 mg/kg BW	Water		Kunming mice	30 day	Pea peptides but not intact protein ↑ immunity, phagocytosis, sIgA	Feng et al. 2021
Bubalus bubalis milk-derived products in experimental colitis	Oral gavage 10–100 mg/kg BW	DNBS	20–25 g	ICR mice	3 day after inflammatory insult	Restores impaired permeability and ameliorates colitis	Tenore et al. 2019
Peptidic fraction released in milk fermented by *Lactobacillus helveticus R389* in a model of infection with *Escherichia coli O157:H7*	Oral gavage 50 µg	Sterile water	6–8 week	BALB/c mice	2, 5, or 7 days preinfection. 2, 5, 7, 10 day postinfection	↑ IgA-secreting B lymphocytes. ↑ intestinal and serum IgA	Leblanc et al. 2004
Milk fermented by *Lactobacillus helveticus R389* and its nonbacterial fraction obtained by milk fermentation, in a model of infection by Salmonella enteritidis serovar Typhimurium	Bacterial fermented: instead of drinking water Nonbacterial fraction: 100 µg/d	Water	6–8 week	BALB/c mice	2, 5, or 7 days	↑ sIgA with bacterial fermented product	Vinderola et al. 2007
Bovine lactoferrin before acute stress immobilization	50–5000 µg/d	Saline solution	8 weeks	Male BALB/c mice	7 day pretreatment	↑ sIgA and pIgR	Peña-Juárez et al. 2016

(Continues)

Treatment	Dosage	Control	Age/Weight	Species	Length intervention	Outcomes	Ref
Bovine lactoferrin in chronic stress	50–5000 μg /d	Saline solution	8 weeks	Male BALB/c mice	7 days	Lactoferrin ↓ stress induced IgA and IgG response, protein α-chain and pIgR	Cruz-Hernández TR, et al. 2021
Bovine lactoferrin	1.0, 3.6 g/L formula	Standard formula	0 days	Piglets	7 or 14 days	↑ crypt cell proliferation	Reznikov et al. 2014
Bovine lactoferrin	50–5000 μg/d	–	8 weeks	Male BALB/c mice	2, 5, or 7 days	↑ total and specific IgA and protein expression, α-chain and pIgR	Godínez-Victoria et al. 2017
Bovine lactoferrin in a model or intestinal resection	0.5 g/kg/d	Water	4 weeks	Male Sprague–Dawley rats	21 days	↑ sIgA and TJ protein expression	Wu et al. 2014
Albumin, glutelin, and globin (from walnut protein hydrolyzates)	200–800 mg/kg	Sterile saline solution Positive control group: thymopeptide	Adults	Female BALB/c mice	35 days	↑ sIgA	Li et al. 2018
Ovotransferrin in immunosuppression	2–200 mg/kg	Physiological saline (of cyclophosphamide)	4–6 weeks	Female Kunming (KM) mice	7 days	↑ intestinal immune response, including IgA and sIgA expression	Zhu et al. 2018
Sequence [94-123] of β-casein (β-casofensin) genetic variants A1, A2, A3, and B	10 μL (at 0.1 μM) /g of body weight•d	Water	10 days	Wistar rat	10 days	Effect depends on variants: A1 ↑ intestinal permeability A2 and B ↑ goblet cells A2 ↑ MUC2 A1, A3, B ↓ ZO-1 and occludin	Bruno et al. 2017
Alaska Pollock peptide in cyclophosphamide immunodepression	50-500 mg/kg/d	Saline solution	7–8 weeks	Male BALB/c mice	27 days	↑ small intestinal mucosal immunity ↑ intestinal sIgA and IgA	Li et al. 2019
GPA peptide from fish skin gelatin hydrolysate in a model of colitis induced by DSS	100 mg/kg/d	Regular drinking water	5 weeks	Male C57BL/6 mice	7 day pretreatment + 7 day after DSS	↓ inflammation and oxidative stress, ↑ autophagy, ZO-1, Occludin and TEER	Deng et al. 2020
Aminoacid diet + Casein diet	Ad libitum 15% w/w diets. Recovery with 5%, 10%, 15% w/w casein diet	15% w/w casein or aminoacid diet for 9 weeks	3 weeks	C57BL/6J mice	5 week aminoacid or proteino diet+ 4 week casein diet recovery	↓ intestinal sIgA in aminoacid fed vs. casein fed mice, reversible with casein diet (5% w/w being enough)	Amaral et al. 2006

(Continues)

TABLE 2 | (Continued)

Treatment	Dosage	Control	Age/Weight	Species	Length intervention	Outcomes	Ref
Arg in a model of heat stress	250 mg/kg/d	Control diet groups: ± heat stress	200 ± 20 g	Male Sprague–Dawley rats	7 day pretreatment + 3 heat stress	↓ intestinal injury, intestinal permeability ↑ mRNA levesl of ZO-1 and claudin-1 and villus/crypt ration Arg vs heat stress group. Involvement of AMPK signaling	Xia Z, et al. 2019
Ala-Gln ad Gln	Ala-Gln diet (0.5% w/w Ala-Gln), Gln diet (0.34% w/w Gln and 0.21% w/w Ala)	0.62% Ala	10.01±0.03 Kg	Duroc×Large White×Landrace piglets	28 days	Gln ↑ goblet cell number in dudoenum Ala-Gln ↑ jejunum mRNA expression and protein of ocludin and ZO-1, also IgAs and IgG. It also ↑ villi height in jejunum and goblet cell number in duodenum and ileum	Xing et al. 2017
Gln	400 mg/kg/d	Glucose 400 mg/kg/day	3 days	Low-birth-weight infants	14 days	↓ return-to-birth-weight time and faster weight gain velocity ↑ fecal sIgA	Sampurna et al. 2018
Gln in a model of mucositis induced by MTX	2% w/v oral Gln in the drinking water	Gln without MTX MTX without Gln	250–300 g	Male Sprague-Dawley rats	2 day before MTX+ 3 day after MTX	↑ TLR4, MyD88 ↓ mucosal injury	Sukhotnik I, et al. 2014
Gln in a model of short bowel syndrome	4% diet	3 Control diet groups: rats with bowel transection (TX/CON), and rats with bowel resection with (RX/ABX) or without (RX/CON) an oral antibiotic cocktail	6 weeks	Male Sprague-Dawley rats	20 day after operation	RX/Gln vs. RX/CON ↓ bacterial translocation to MLN, ↓ serum anti-LPS IgG levels, - stool and jejunal mucosal sIgA and IgA^+ cells - stool anti-LPS-specific IgA in antibiotic and Gln treated groups vs. C groups	Tian et al. 2009

(Continues)

TABLE 2 | (Continued)

Treatment	Dosage	Control	Age/Weight	Species	Length intervention	Outcomes	Ref
Gly	0.5–2% w/w in diet		21 days	Yorkshire × Landrace piglets	7 days	2% glycine ↑ mucins in jejunum and ileon, β-defensins 2 and 3 and SigA in jejunum, CD4+CD8+ Tcells in jejunuma and fatty acid production, ↓ claudin-2 in jejunum, and colonic abundance of pathogenic bacteria.	Ji et al. 2022
Leu	15–40.0 g/kg diet	Leucine 10 g/kg diet	23.19 ± 0.20 g	Catfish	8 weeks	↑ mRNA for ZO-1 and ocludin, antioxidative genes and IgM. Improved int4estinal barrier function	Zhao et al. 2023
Met	0.12% w/w supplemented	Basal diet, without supplementation	28–30 days	Duroc × Landrace × Yorkshire piglets	14 days	Improved intestinal architecture, ↑ TEER	Chen Y, et al. 2014
Methionine + cysteine (M+C) in a model of *Eimeria* challenge	Corn- and soybean meal-based diet, isonitrogenous and isocaloric + 0.6–1.0% w/w Standardized ileal digestible M+C	0.6% (w/w) M+C	1 days	Male broilers	3, 10 days	≥0.8% M+C ↑ jejunum sIgA and anti-*Emeria* IgA vs. 0.6%	Ren et al. 2020

Abbreviations: AMPK, adenosine monophosphate kinase; BW, body weight; DSS, dextran sulfate sodium; LPS, lipopolysaccharide; MTX, methotrexate; RX/ABX, rats with bowel resection with oral antibiotic cocktail; RX/CON, rats with bowel resection without oral antibiotic cocktail; TEER, transepithelial electrical resistance; TLR4, toll-like receptor 4; TX/CON, rats with bowel transection.

to evaluate the effect of protein sources and fat content on intestinal TJ and mucus, soybean, chicken, and pork proteins either with low fat (12% kcal) or high fat (60% kcal) were given to C57BL/6J mice for a period of 12 weeks. In mice fed with low-fat diets, pork protein increased mucosal thickness and goblet cell number. However, these parameters were preserved only with chicken protein in high-fat fed mice, which also showed the highest MUC2 expression. Both meat protein diets promoted epithelial proliferation (Ki67) in either condition. Interestingly, soybean protein fed mice exhibited major changes in TJ protein expression by high-fat intake (increased ZO-1 and lower claudin 1 and occludin). In turn, meat protein diets appear to upregulate claudin 1 in low-fat but not high-fat diet (but otherwise exhibited little change in high- vs. low-fat conditions) (Hussain et al. 2019).

Several studies have shown that the stimulation of mucus secretion by enzymatic hydrolysates of casein depends on the activation of the opioid receptor, which is exerted by bioactive peptides (S. Fernández-Tomé et al. 2016). This is the case of β-casomorphins (β-casomorphin-4, -5, -6, and -7 peptides derived from the digestion of the milk protein casein). Casomorphin-7, the best characterized of these peptides, is very efficient in inducing mucus secretion and the expression of mucins in goblet cells (Trompette et al. 2003). Luminal and intra-arterial administration of this peptide resulted in enhanced mucus secretion in the jejunum of perfused rats (Trompette et al. 2003), an effect that has also been observed in vitro in the DHE and HT29-MTX cell lines, widely used to study mucin production. β-casomorphin-7 enhanced the expression of *Muc2* and *Muc3* in DHE cells, and additionally of MUC5AC (mRNA and protein) in HT29/MTX cells (Zoghbi et al. 2006). Interestingly, the effect on mucus secretion was inhibited by mu-opioid blockade, demonstrating the role of opioid receptor in casein-mediated effects. Another casein peptide, β-casofensin, a fragment (94–123) of bovine β-casein, has been shown to contribute to this effect. In fact, administration of this peptide to rat pups (10–18 postnatal days) led to an increase in the number of goblet cells along the small intestine, and induced the expression of *Muc2* and *Muc4* in the small intestine (Bruno et al. 2017; Plaisancié et al. 2013). β-casomorphin-7 also reportedly reduced defensin a in IEC6 cells, while also suppressing oxidative phosphorylation (Miyagawa et al. 2024). The same effect was assessed in vivo in colitic mice, so that this peptide may be harmful in IBD.

α-Casein is also a source of peptides that enhance mucin production by a similar mechanism. α_{S1}-Casein (f143–149) (AYFYPEL) and α_{S1}-casein (f144-149) (YFYPEL) showed agonistic opioid receptor activity and stimulated *MUC5AC* expression in HT29/MTX cells (S. Fernández-Tomé et al. 2016; Martínez-Maqueda et al. 2013). The effect on the opioid receptors is not exclusive to casein protein and its derivatives. For example, bovine β-lactorphin (β-lactoglobulin f, another milk-derived peptide, induced mucus release in HT-29/MTX cells, and also upregulated mucin gene expression when the amidated form (a reportedly more potent opioid) was used (Martínez-Maqueda et al. 2013).

Besides the above-mentioned peptides derived from caseins, other specific food-derived bioactive peptides have shown intestinal barrier enhancing effects. This is the case of MBCP, a peptide isolated after in vitro digestion of *Mozzarella di Bufala Campana DOP* that inhibited lipopolysaccharide or/and tumor necrosis factor-induced permeability defect and inflammatory response (e.g., NFκB phosphorylation) in Caco 2 cells. In addition, *Mozzarella di Bufala Campana* treated animals showed reduced permeability and milder inflammation in the dinitrobenzenesulfonic acid model of acute colitis (Tenore et al. 2019). It also protected TJ from tumor necrosis factor "loosening" modulation. These effects have been related to reduced NFkB activation. Tripeptide Isoleucine-Arginine-Tryptophan (IRW), a peptide derived from egg ovotransferrin, and Gly-Pro-Ala (GPA) peptide, isolated from fish skin gelatin hydrolysate (Bao and Wu 2022; Deng et al. 2020), have also protective effects against lipopolysaccharide-evoked IBF dysfunction in vitro. GPA in addition exerts intestinal anti-inflammatory effects in vivo, involving induction of autophagy by enhancing the interaction of Nur77 with TRAF6 and p62 (Deng et al. 2020).

There is an interest nowadays on alternative protein and peptide sources, including marine products, such as algae, and industrial waste products. Although studies on barrier function are limited, some of these have shown promising effects on intestinal immune function. These new protein sources may be important as sources of functional foods and as low-cost products to support agro-industry (although this is not always the case), and they have been advocated as an alternative to reduce dependence on conventional sources for environmental reasons (Cian et al. 2015; Lamminpää et al. 2024).

On the other hand, peptides derived from gliadin digestion reportedly alter TJ, increasing intestinal permeability (da Silva et al. 2023; Giorgi et al. 2020; Lammers et al. 2008). Furthermore, gliadin-derived species have been shown to induce the activation of immune cells, leading to an inflammatory response. These mechanisms may contribute to gluten-related disorders (Herrera and Dodero 2021; Serena et al. 2019).

In general, single amino acids including arginine, cysteine, glutamine, leucine, methionine, and tryptophan improve IBF (Y. Chen et al. 2014; Hasegawa et al. 2021; G. Liu et al. 2022; Xia et al. 2019; Zhao et al. 2023). Gln is a major energetic substrate for the small intestinal epithelium, particularly in the postabsorptive state. Enterocytes are highly dependent on exogenous glutamine, due to very low mucosal glutamine synthetase activity and scarce cellular glutamine content (H. Yang et al. 1999). Glutamine is an intermediary of energy metabolism and is the substrate for the synthesis of different substances such as peptides, nitrogenous bases (which form part of nucleotides), glutathione, and neurotransmitters. It also contributes to ammonium detoxification and acid–base balance. It is frequently considered a conditionally essential nutrient at this level, in conditions such as sepsis or trauma. Thus, several studies have focused on the effects of glutamine supplementation on the intestine.

Clinical studies have shown that glutamine is able to modulate intestinal permeability in healthy individuals and in patients with COVID-related malnutrition, Crohn's disease or irritable bowel syndrome (Achamrah et al. 2017; Mohajeri et al. 2021). Glutamine improves the ATP content of the mucosa of starved rats and attenuates the permeability disturbance in segments of the jejunum of rats starved for 48 h (H. Yang et al. 1999). Nevertheless, a recent systematic review failed to detect a positive

effect of glutamine in IBD patients (Severo et al. 2021), perhaps reflecting differences in rodents and humans.

In an interesting study carried out in piglets, dietary supplementation with Ala-Gln (vs. Ala), a Gln precursor with improved stability and solubility, resulted in increased height of duodenal and jejunal villi, augmented expression of TJ proteins occludin and ZO-1 in jejunal mucosa, and improved concentrations of sIgA and IgG in the jejunal mucosa (Xing et al. 2017). Supplementation with Gln had more limited effects, including increased jejunal villus height and number of goblet cells in duodenal epithelium.

in vitro studies with IEC lines indicate that glutamine deficiency increases intestinal permeability to several macromolecules, including ^{14}C-mannitol and fluorescein isothiocyanate dextran (DeMarco et al. 2003). Glutamine supplementation results in the opposite effect (C. Y. Huang et al. 2022). In addition, glutamine deprivation reduces claudin 1, occludin, and ZO-1 expression and induces the disappearance of perijunctional claudin 1 and, partially, of occludin (N. Li et al. 2004). At the molecular level, several pathways are involved in the effects of glutamine. Among them, glutamine deprivation in Caco 2 cells has been shown to activate phosphatidylinositol 3 kinase/AKT, a mechanism responsible for the changes in transepithelial resistance, intestinal permeability and downregulation of claudin 1. Another relevant pathway is the calcium/calmodulin-dependent kinase 2/AMPK pathway. This is activated in glutamine-supplemented enterocytes, leading to an increase in TJ protein expression and modulation of their intracellular distribution (B. Wang et al. 2016).

The protective effect of glutamine has also been tested in cells in nonbasal conditions. For example, glutamine prevents the deleterious effect induced by methotrexate (Sukhotnik et al. 2014). Methotrexate is used in cell lines and animal models to mimic the mucositis associated with chemotherapy effects (Beutheu Youmba et al. 2012). This cancer-derived complication is characterized by modifications in TJ distribution and expression.

Amino acids glutamine, glycine and L-tryptophan, plus a mixture of methionine and cysteine, among others, have been shown to induce sIgA secretion. Several factors and processes are involved in this effect, including the intestinal microbiota, intestinal antigen sampling and presentation, modulation of sIgA production by plasma cells (both T-dependent and T-independent), and even sIgA transport (Ji et al. 2022; Liang et al. 2019; W. Ren et al. 2016; Z. Ren et al. 2020; Sampurna et al. 2018; Spaeth et al. 1994; Tian et al. 2009).

Thus, the available evidence shows that both protein quantity and quality are relevant to IBF regulation. Milk-derived proteins and peptides have received the most attention. Although effects appear to be beneficial for the most part, detrimental actions are also reported, for instance, in the case of gliadin peptides or in the more general case of protein allergy (Kalach et al. 2013). Information about protein/peptide mechanism and structure–activity relationship is slowly growing, making it possible to fine tune the bioactivity and use of these products in functional foods. However, it should be noted that most available studies have been carried out in in vitro and laboratory animal models, and in basal conditions for the most part. Hence, additional studies in farm animals and humans are required, both in basal conditions and in the context of disease, in order to facilitate translation to industry.

4 | Regulation of Intestinal Permeability by Carbohydrates and Sugars

In addition to its fundamental role in carbohydrate absorption, the small intestine is a secondary gluconeogenic organ. Intestinal gluconeogenesis is considered metabolically protective.

Dietary carbohydrates regulate intestinal permeability in a structure- and composition-dependent fashion. While the monosaccharides fructose and glucose and the disaccharide sucrose increase intestinal permeability, nondigestible oligosaccharides (NDOs), the soluble fraction of dietary fiber, decrease it. Regulation of the microbiota and the immune system have been related to these effects. Tables 3 and 4 summarize main in vitro and in vivo studies on the effects of different carbohydrates and sugars on intestinal permeability.

Fructose has been shown to increase intestinal permeability, a condition often referred to as "leaky gut," on the basis of evidence mostly derived from animal and in vitro studies. Thus, in a piglet model, high fructose intake disrupted the expression of TJ proteins occludin and claudin 1, without any further impact on growth. In line with these observations, the effects of fructose have been related to increased endotoxemia in models of mouse and rat, with decreased expression of ZO-1, occludin, and claudins 1, 2, 4, and 5 (Binienda et al. 2020; Guo et al. 2021). This disruption leads to increased intestinal permeability, allowing endotoxins like lipopolysaccharide to enter the bloodstream and contribute to systemic inflammation (Guney et al. 2023). Fructose is believed to increase oxidative stress and inflammation within the intestinal epithelium, further compromising the barrier function. A study has shown that fructose intake contributes to steatohepatitis with liver fibrosis by inducing P450-2E1 (CYP2E1) and inducible nitric oxide synthase both in the small intestine and the liver of rodents (Cho et al. 2021). Additionally, high fructose intake has been associated with alterations in the gut microbiota composition, which can exacerbate intestinal permeability by promoting the growth of pathogenic bacteria and reducing beneficial microbial populations (Montrose et al. 2021). Thus, in a mouse model of colitis and restraint-stress, the administration of fructose induced alterations in IBF that ultimately produced intestinal inflammation. Changes in the restraint stress model were associated with disturbance of the microbiota composition that led to an increase in the abundance of histamine and a decrease in the abundance of taurine (Montrose et al. 2021; Yu et al. 2023). As a result, the expression of TJ proteins and MUC2 was inhibited, the function of NLRP6 attenuated, and autophagy level reduced. High fructose intake has been linked to a decrease in Firmicutes/Bacteroidetes ratio, associated with reduced short chain fatty acids (SCFAs) levels and elevation in Gram negative bacteria population, resulting in increased endotoxin levels. In turn, high endotoxin levels may weaken IBF through the activation of the innate immune response that may lead to bacterial translocation by disruption of TJ.

Comparisons among mono- and disaccharides indicate that both high fructose and glucose diets induce dysbiosis and inflam-

TABLE 3 | Main in vitro studies on the effects of different carbohydrates and sugars on intestinal permeability.

Nutrient	Concentration	Length intervention	Cell type	Outcomes	Reference
Fructose glucose	25 mM	5 h	Caco-2BBe	Fructose marginally counteracts cytokine-evoked ↓ TEER	Zhang et al. 2021
Fructose	0, 2.5, and 5 mM	24 h	T84	↓ ZO-1, ↓ TEER, ↑ apoptosis, ↑ permeability to 4 kD tracer, via CYP2E1 and iNOS	Cho et al. 2021
FOS	0.001–10 mg/mL	24 h	T84	↑ TJ via prebiotic-independent, AMPK-dependent mechanisms	Wongkrasant et al. 2020
GOS, FOS	1.4% w/v GOS 2% w/v FOS	24 h	Caco–2	↑ TEER, unrelated to changes in TJ gene expression	Lafontaine et al. 2020
Difructose anhydride III and IV, FOS, raffinose	100 mM	2 h	Caco–2	All ↑ intracellular Ca^{2+} absorption by the paracellular route and ↑ intracellular Ca^{2+} levels, possibly leading to TJ opening	Suzuki and Hara 2004
Xylobiose	5, 10, 20 mg/mL	3–24 h	Caco–2	↓ CLDN2, ↑ HSP27	Rini et al. 2024
HMOs (2′-fucosyllactose (2′-FL), 3-fucosyllactose (3-FL), lacto-N-triaose II (LNT2), GOS	10 mg/mL	6–72 h	LS174T	3-FL, LNT, and GOS ↑ MUC2 and differentially modulate other goblet cell genes	Cheng et al. 2020
HMOs	0.3–60 mg/mL	48 h	Caco–2/HT29-MTX co-culture	Some HMO blends (including 2′FL) protect against TNF and IFNγ IBF alterations	Natividad et al. 2020
HMOs	20 mg/mL	24 h	Caco-2Bbe LS174T	↑ chaperones, ↑ TEER, ↑ MUC2, ↑ TFF3 (LS174T only)	Wu et al. 2019
HMOs	20 mg/mL	24 h	Neonatal human intestinal organoids	↑ crypt budding, ↑ MUC2	Wu et al. 2019
2′-Fucosyllactose, 3′-sialyllactose (3′-SL), GOS and lactose	2.5 mg/mL	72 h	LS174T	2'-FL ↑ MUC2, TFF3, CHST5 in TNF stimulated cells. GOS ↑ MUC2, TFF3. 2'-FL ↓ TLR4, MyD88, NF-κB No effect of 3′-SL, lactose	Yao et al. 2023

Abbreviations: 2′-FL, 2′-fucosyllactose; 3-FL, 3-fucosyllactose; FOS, fructooligosaccharides; GOS, galactooligosaccharides; HMO, human milk oligosaccharides; LNT2, lacto-N-triaose II; LPS, Lipopolysaccharide; TEER, transepithelial electrical resistance; TNF, tumor necrosis factor.

mation, the effect of fructose being higher. However, it should be noted that there are conflicting studies in this regard (Do et al. 2018; Ondee et al. 2023; X. Zhang et al. 2021). Of note, substitution of artificial sweeteners seems a poor strategy, since artificial sweeteners may also alter intestinal permeability (M and Vellapandian 2024). For example, sucralose, aspartame, and saccharin have been shown to exert detrimental effects on intestinal berrier function (Shil et al. 2020).

On the other hand, NDOs behave as prebiotics, being fermented by intestinal bacteria. Induction of the growth of health beneficial inhibits the growth of harmful bacteria and increases the abundance of bacterial components and SCFA that positively regulate TJs. In this regard, NDOs are selectively fermented by specific bacterial populations in the colon producing SCFA, mainly acetate, butyrate, and propionate. SCFAs enhance the expression and activity of TJ proteins, thereby strengthening the intestinal barrier. Butyrate, in particular, has been shown to promote the expression of occludin and ZO-1 in IPEC-J2 cells and of claudin-1 in rat cdx2-IEC cells, reducing intestinal permeability and increasing intestinal villus height (H. B. Wang et al. 2012). Similarly, Na^+ butyrate improves intestinal morphology, increases jejunal transepithelial electrical resistance, and ameliorates intestinal damage caused by weaning stress in piglets (C. Huang et al. 2015; C. C. Wang et al. 2018). Propionic acid also upregulates the expression of ZO-1, occludin, and cadherin, promoting intestinal function and enhancing transmembrane resistance through the AMPK pathway (Tong et al. 2016; Voltolini et al. 2012).

SCFA are well-known regulators of AMPs in the distal gut (ileum and colon). Thus, inulin restored a defensin expression in the ileum of mice exposed to a Western-style diet, and additionally augmented β-defensin 1 and TJ genes in the colon, enhancing

TABLE 4 | Main in vivo studies on the effects of different carbohydrates and sugars on intestinal permeability.

Treatment	Dosage	Control	Age	Species	Length intervention	Outcomes	Reference
Fructose	30% (w/v) in drinking water ad libitum	Tap water	7 weeks	Fischer 344 rats	8 weeks	↑ endotoxemia, ↑ oxidative stress, ↑ Bacteroidetes, and Proteobacteria	Cho et al. 2021
Fructose	30% (w/v) in drinking water ad libitum	Tap water	6–8 weeks	Wild type and Cyp2e1-null mice	8 weeks	↑ endotoxemia, ↑ inflammation in small intestine and colon, ↓ TJ protein (WT mice only), via nitrosative stress	Cho et al. 2021
Fructose	0.2% in diet	Normal diet	Weaned	Piglet	35 days	↓ TJ gene expression, changes in ileal/colonic microbiota No change in inflammation	Guo et al. 2021
Fructos in experimental DSS colitis	Fructose as sole carbohydrate 15% kcal fructose diet	Control diet, high glucose diet	ns	Germ free mice, $Il10^{-/-}$ GF mice, mice infected with *C. rodentium*	1–2 weeks	Worsened DSS colitis via structural changes in colonic mucus and altered microbial population	Montrose et al. 2021
Fructose	15% in drinking water	Water	7 weeks	C57BL/6 mice	9 weeks	↑ systemic IL-6, ↑ gut inflammation No change in permeability ↑ cecum Il13, Ifnγ, Tnf mRNA, MLCK protein Changes in microbiota	Zhang et al. 2021
Fructose in restraint stress	20% (w/v) in drinking water	Tap water	5-6 week old	Mice	14 days	Aggravates effects of stress on microbiome, ↓ MUC2, ↓ TJ, ↑ damage to mucosal immune barrier	Yu et al. 2023
Fructose or glucose ± *L. plantarum* dfa1	24.8% fructose or glucose	Normal diet	8 weeks	C57BL/6 mice	12 weeks	Both ↑ obesity, ↑ prediabetes Fructose>glucose ↑ steatohepatitis Glucose>fructose ↑ permeability and inflammatory markers *L. plantarum* dfa1 attenuates alterations	Ondee et al. 2023
Fructose/glucose	55.25% of total calories in diet	Normal diet	6 weeks	C57BL/6J mice	12 weeks	↑ glycemia, dyslipemia, ↑ fat mass, ↑ hepatic inflammation, ↑ permeability, TJ alteration, ↑ endotoxemia, ↓ microbiota diversity	Do et al. 2018

(Continues)

TABLE 4 | (Continued)

Treatment	Dosage	Control	Age	Species	Length intervention	Outcomes	Reference
Glucose	15% glucose in drinking water	Water	7 weeks	C57BL/6 mice	9 weeks	↑ weight gain ↑ jejunal and cecal permeability, ↑ inflammation, ↑ glucose intolerance, ↑ adiposity ↑ cecum Il13, Ifnγ, Tnf mRNA, MLCK protein Changes in microbiota	Zhang et al. 2021
FOS	2.5%–7.5% in diet	Normal diet	6 weeks	BALB/c mice	6 weeks	↑ fecal and Peyer's patch IgA, ↑ Th1 and Th2 cytokine responses, ↓ serum IgG_1 (Th2-related response)	Hosono et al. 2003
FOS	5% in diet	Control diet	10 weeks	BALB/c mice	7 days	↑ fecal SIgA, modulates microbiota, ↑ SCFA	Jangid et al. 2022
FOS	5% in diet	Control diet	Newborns	BALB/c mice	44 days	↑ intestinal IgA, ↑ pIgR ↑ B cell isotype switching to IgA	Nakamura et al. 2004
Inulin-type Fructans	5% in diet	Normal diet	3 weeks	NOD mice	24 weeks	Long but not short fructans ↑ IBF (but ↑ claudin 2), modulated microbiota, ↓ diabetes incidence, ↑ regulatory T cells	Chen et al. 2017
Inulin ± probiotics	10% inulin (oligofructose enriched) ± *L. rhamnosus* GG and *B. lactis* Bb12	High-fat diet	12-13 weeks	F344 rats	4 weeks	Inulin ↑ cecal sIgA, ↑ IL-10 in Peyer's patches Combination ↑ ileal sIgA, ↓ oxidative burst activity in neutrophils	Roller et al. 2004
Inulin	10% inulin or 5% sodium butyrate in diet	Western-style diet ± fructose Normal diet	6–8 weeks	C57BL/6 mice	12 weeks	Both ↓ steatohepatitis ↑ IBF (defensins), ↓ endotoxemia, ↑ TJ gene expression (inulin ↑ claudin 2)	Beisner et al. 2021
Agaro-Oligosaccharides in deoxynivalenol mycotoxin injury	200 mg/kg BW by gavage	Control diet	5 weeks	C57BL/6J mice	28 days	↓ inflammation, ↑ IBF, ↑ SCFA	Wang et al. 2024

(Continues)

TABLE 4 | (Continued)

Treatment	Dosage	Control	Age	Species	Length intervention	Outcomes	Reference
2'-FL in experimental colitis	400 mg/kg BW orally	Phosphat buffered saline	6 weeks	C57BL/6J mice	14 day pretreatment + 7 days	↓ colitis, ↑ MUC2	Yao et al. 2023
HMOs mix	1.5 or 2.5 g/L blend of 5 HMOs	Standard infant formula	7-21 days	Human infants	6 months	↑ *Bifidobacterium longum* subsp infantis ↓ *Clostridioides difficile*, ↑ sIgA, ↓ alpha-1-antitrypsin	Bosheva et al. 2022
HMO blend in necrotizing enterocolitis model	2-3 mg/g/d	Lactose	5 days	C57BL/6 neonatal mice	5 days	Protection against necrotizing enterocolitis ↑ goblet cell number, ↑ MUC2, ↓ permeability to 4 kD tracer	Wu et al. 2019
HMO 2'-FL + 3'-SL	0.625% 2'FL ± 0.625% 3'SL	Normal diet	3 weeks	Sprague Dawley rats	8 weeks	HMO ↓ body weight in males HMO ↓ permeability in females Sex-dependent TJ modulation HMO modify microbiota	Chleilat et al. 2020
Wheat Germ	10% in diet	Control diet or high-fat, high-sucrose diet	6 weeks	C57BL/6 mice	12 weeks	Improved microbiota balance, ↑ SCFA (control diet only), ↓ inflammation, ↑ ileal IL-10, ↑ AMP	Ojo et al. 2019
Pinto Beans	10% in diet	Control diet or high-fat, high-sucrose diet	6 weeks	C57BL/6J mice	30 days	Modulated gut microbiome ↑ SCFA, improved glucose homeostasis	Ojo et al. 2021

Abbreviations: AMP, antimicrobial peptide; BW, body weight; 2′-FL, 2′-fucosyllactose; 3-FL, 3-fucosyllactose; FOS, fructooligosaccharides; GOS, galactooligosaccharides; HMO, human milk oligosaccharides; IBF, intestinal barrier function; LNT2, lacto-N-triaose II; LPS, lipopolysaccharide; MLCK, myosin light chain kinase; SCFA, short-chain fatty acids; 3'-SL, 3'-Sialyllactose; TEER, transepithelial electrical resistance; TJ, tight junction; TNF, tumor necrosis factor.

IBF. Mechanistically, ileal effects were linked with SCFA production (Beisner et al. 2021). A similar IBF strengthening action dependent on SCFA has been described for pinto beans, involving increased *Reg3b* and *Reg3g* (Ojo et al. 2021), and for wheat germ (Ojo et al. 2019). Ingested SCFA have similar effects on AMP (Campbell et al. 2012). Differences in NDO have been documented. In the nonobese diabetes model, long- rather than short-chain inulin-type fructans exert protective effects, involving prebiotic actions, augmented occludin and claudin 2, and increased AMP β-defensin-1 and cathelicidin-related AMP (K. Chen et al. 2017). The difference may hinge on preferential colonic rather than small intestinal fermentation and stronger TLR2 ligation. In addition to their prebiotic effect, NDOs directly interact with epithelial cell receptors, and involvement of TLR/protein kinase C/AMPK associated pathways has been shown. In this sense, fructooligosaccharides, important NDOs widely studied and used in the food industry, are known ligands of TLR2 that induce barrier protective effects regulating TJ. Activation of protein kinase C α/δ in T84 and Caco-2 cell lines underlie these effects. These protein kinase C isoforms interact with the phosphatidylinositol 3 kinase/AKT pathway via MyD88 and enhance TJ sealing in a ZO-1 mediated manner (Cario 2008; Cario et al. 2007). MAPK have been shown to be induced downstream protein kinase C δ stimulation by fructooligosaccharides. Nevertheless, in intestinal organoids, the regulation of TJ by fructooligosaccharides after the stimulation of TLR/protein kinase C δ pathways has been shown to be independent of MAPK, indicating different stimulatory pathways depending on the tested model. Fructooligosaccharides can also enhance TJ by reversing lipopolysaccharide suppression of AMPK activity through a calcium sensing receptor (CaSR)-phospholipase C—Ca^{2+}/calmodulin-dependent protein kinase kinase-β (CaMKKβ) pathway (Lafontaine et al. 2020; Wongkrasant et al. 2020). Although most studies indicate a protective role of fructooligosaccharides on TJ integrity, some have shown fructooligosaccharides-induced increased paracellular permeability. Thus, in Caco 2 cells, fructooligosaccharides have been shown to regulate calcium-sensing receptors, resulting in increased paracellular Ca^{2+} transport and elevation of intracellular Ca^{2+} levels, with the consequent activation of MLCK, that, in turn, leads to the condensation of actin microfilaments and the physiological opening of TJ pores (Suzuki and Hara 2004).

Other oligosaccharides like xylobiose, obtained from plant cell walls, or agaro-oligosaccharides (AgaroS) extracted from red algae, also reportedly enhance the intestinal barrier. The former has been shown to suppress claudin 2 and to increase HSP27 expression in intestinal Caco 2 cells via posttranscriptional regulation, potentially strengthening intestinal barrier integrity (Rini et al. 2024). Selective inhibition of phosphatidylinositol 3 kinase abolished the effect of xylobiose on claudin 2 expression, whereas modulation of HSP27 was sensitive to the inhibition of PI3K, mitogen-activated protein kinase and SRC. AgaroS has shown protective effects in a mouse model of intestinal dysfunction induced by deoxynivalenol, a mycotoxin commonly found as a food contaminant. Pretreatment with AgaroS resulted in increased protein expression levels of key intestinal barrier markers such as claudin 1, occludin, Ki67, and mucin 2 in the ileum and colon (Q. Wang et al. 2024). These effects were associated with changes in the microbiota and antioxidative actions.

Studies with infants suggest that supplementation with prebiotics positively affects postnatal immune development and increases fecal secretory IgA (Bakker-Zierikzee et al. 2006; Bosheva et al. 2022), in contrast to data from healthy adult human subjects, in which only minor effects have been observed (Seifert and Watzl 2007). Animal studies confirm that the most frequently used oligosaccharides (i.e., fructooligosaccharides and inulin, or mixtures) induce sIgA (Nakamura et al. 2004; Seifert and Watzl 2007). In a recent systematic review on the effect of fructooligosaccharides on inflammation, immunomodulation, oxidative stress, and gut immune response, the authors stated that the most frequently reported feature in the studies included was the increase in IgA secretion in the serum and cecum of both healthy and nonhealthy animals (Costa et al. 2022). In animal studies, oligosaccharides dosage is usually 5–10%. They reportedly increase fecal IgA and IgA secretion, the secretory component, and IgA+ plasma cell numbers in Peyer's patch cells in a dose-dependent fashion (Costa et al. 2022; Hosono et al. 2003; Nakamura et al. 2004; Roller et al. 2004; Seifert and Watzl 2007). Relations between alterations in intestinal microbiota by oligosaccharides with the consequent induction in the production of SCFA have been proposed as the mechanism of action of prebiotics (Jangid et al. 2022).

It is worth mentioning that, according to a recent systematic review, there is a "moderate grade of evidence" supporting chicory inulin and probiotics reduce intestinal barrier permeability in humans, and only "very low grade" evidence of fructose impairing IBF (Nascimento et al. 2024). Therefore, there is a need for further studies mainly in humans.

Among oligosaccharides, those present in human milk are currently being extensively studied. HMOs reinforce barrier function as determined by transepithelial electrical resistance and the reduction of permeability to macromolecules (Chleilat et al. 2020; Natividad et al. 2020; Wu et al. 2019). They contribute to intestinal maturation as shown in 3-week-old Sprague Dawley rat pups fed 2'-*O*-fucosyllactose (2'FL)- and 3'sialyllactose (3'SL)-fortified diets alone or in combination at "physiological" doses for 8 weeks (R. Y. Wu et al. 2019). Interestingly, in this article, only females showed a significant reduction in intestinal permeability in all HMOs fortified groups when compared to controls. Not only in physiological conditions, but also in colitis models, HMOS have been shown to preserve intestinal permeability. As expected, prebiotic effects of HMOs have been described and related to these actions, but also direct effects. Restoration of goblet cells and induction of MUC2 was observed in a murine model of necrotizing enterocolitis when a pool of HMOS was administered (R. Y. Wu et al. 2019). In vitro induction of MUC2 by HMOs, together with a lower dextran permeability and increased transepithelial resistance, was also observed in this study in both LS174T goblet cells and Caco-2 cells. These protective effects were also shown in vitro in the HT29 methotrexate cell line, which produces high amounts of mucins. 2'FL, difucosyllactose, lacto-N-tetraose, lacto-N-neotetraose, 3'-SL, and 6'-SL were studied alone or in combinations of three, five, and six HMOs, and effects on permeability to fluorescein-isothiocyanate-labelled dextran 4 KDa translocation and/or transepithelial resistance were characterized in basal conditions and under proinflammatory conditions (tumor necrosis factor and interferon γ). Authors reported that the six HMO blend (HMO6) augmented basal transepithelial

resistance and reduced cytokine effects on permeability and transepithelial resistance in a concentration-dependent fashion. The three and five HMO blends exerted similar protective effects, with a prominent effect of 2'FL, one of the most abundant but most quantitatively variable oligosaccharides in human milk (Natividad et al. 2020).

The modulatory effects of 2'-FL, 3-FL, lacto-N-triaose II (LNT2), and galacto-oligosaccharides on the expression of goblet cell secretory related genes were determined in LS174T colonic cells by real-time quantitative Real-time Polymerase Chain Reaction (RT-PCR) (Cheng et al. 2020). 3-FL, LNT2, and galactooligosaccharides modulated LS174T gene expression profile consistent with IBF reinforcement in a concentration- and time-dependent manner. In the presence of tumor necrosis factor, 3-FL and LNT2 enhanced MUC2 and TFF3 gene expression. Similarly, after an IL-13 challenge, MUC2 was upregulated by 2'-FL, 3-FL, and LNT2, and TFF3 by the latter two. LNT2 significantly reversed tunicamycin-induced downregulation of TFF3, RETNLB, and CHST5. These results indicated differential effects of several HMOs depending on culture conditions. In a study to compare the effects of 2′-FL, 3′-sialyllactose (3′-SL), galactooligosaccharides, and lactose on goblet cell function in vitro, using the same LS174T cell line, 2'-FL improved mucin secretion under inflammatory conditions (tumor necrosis factor) compared to galactooligosaccharides, while no effects of 3'-SL or lactose were observed. The induction of mucin production was associated with NLRP6. In addition, and although the relation with MUC2 expression was not studied, the authors found that 2′-FL could exert anti-inflammatory actions through the inhibition of the TLR4/MyD88/NFκB pathway (Yao et al. 2023).

Effects of HMOS on sIgA have not been widely studied. Nevertheless, in a multicenter study in which healthy 7–21 days old infants received a standard cow's milk-based infant formula alone or with five HMOs (2'-fucosyllactose, 2',3-di-fucosyllactose, lacto-N-tetraose, 3'-sialyllactose, and 6'-sialyllactose, 2.5 g/L) up to age 3 and 6 months, induction of sIgA was found in HMOs fed children at both check points (Bosheva et al. 2022).

Availability has until recently limited the studies and use of HMOS. Nevertheless, many of them have recently been made available in large quantities thanks to biotechnological synthesis, so that an increase in the studies on their effects on intestinal permeability in humans and in livestock is foreseen. In fact, some of them are currently used in infant formulas, and they are expected to have a wide applicability in both health and industrial levels.

5 | Regulation of the Intestinal Permeability by FAts

An excessive intake of fat enhances the capacity of fat handling by the enterocyte, with augmented absorption and esterification of lipids and export in chylomicrons. In addition, enterocytes activate β-oxidation and ketogenesis and attenuate glucose uptake and utilization. In mice, at least this is protective against obesity and hyperglycemia. This response can be modeled by epithelial overexpression of sirtuin 3. Alterations in mitochondrial function and oxidative stress may ensue, and they have been documented in colonocytes after several weeks of feeding a high-fat diet (HFD). This may play a role in the changes observed in IBF in these conditions. Thus, it has been widely observed that HFDs induce intestinal paracellular permeability in rodent models by altering TJ proteins, the mucus layer, or cytokine levels. Elevated plasma levels of 4 kD fluorescent-dextran and lipopolysaccharide have been documented in mice on an HFD. These permeability markers were strongly and negatively correlated with the expression of TJ proteins, such as ZO-1 and occludin (Cani et al. 2008). Higher urinary excretion levels of intestinal permeability markers (phenolsulfonphthalein and ^{51}Cr-EDTA) were observed in rats fed an HFD, associated with reduced TJ protein expression in the small intestine. Notably, this study indicates that the alterations in TJ protein levels are a consequence of dietary fat intake rather than obesity, as no differences were observed in genetically predisposed obese rats (Suzuki and Hara 2010).

Dietary fat also influences the mucus layer, thereby affecting intestinal permeability. Kawano et al. observed a reduction in the number of goblet cells in mice fed an HFD for 4 weeks (Kawano et al. 2016). Likewise, another study demonstrated that mice on an HFD displayed a compromised mucus layer with decreased MUC2 expression. This finding was correlated with reduced expression of TJ proteins, the presence of lipopolysaccharide in plasma, and signs of inflammation (Gulhane et al. 2016). These effects are associated with increased oxidative and endoplasmic reticulum stress in IECs.

HFDs reduce AMP production (Pan et al. 2023). In particular, they disrupt the circadian rhythm of intestinal expression of *Reg3g*, which is involved in energy homeostasis and host-microbial crosstalk (Frazier et al. 2022). Compared to HFD, diets enriched in extra virgin olive oil or flaxseed oil upregulated *Reg3g* (Qiu et al. 2021). High-fat/high-sucrose diet led to elevated ileal expression of liver-expressed antimicrobial peptide 2 (LEAP2), another AMP regulated by the microbiota that also has metabolic implications, as it acts as a ghrelin receptor antagonist (Shen et al. 2021). Another example is lipocalin 2, which is induced by feeding an HFD and protects against inflammation and dysbiosis (Qiu et al. 2021). HFD also has an IBF weakening effect in dextran sulfate sodium colitis in mice, blocking cathelin-related AMP expression, with increased endotoxemia and hepatic steatosis (Gabele et al. 2011). Reduced adenosine monophosphate favors microbial colonization of ileal mucosa in HFD feeding conditions in mice (Tomas et al. 2016). Recently, it has been shown that the proinflammatory effects of a high-fat/high-sugar diet in mice involve intestinal taste receptor-type 1 member 3 (TAS1R3), a receptor expressed by enteroendocrine cells. This downregulates PPARg and their TJ and AMP target genes, resulting in changes in the microbiota, producing less SCFA (Shon et al. 2023).

The impact of dietary fats on intestinal permeability depends not only on their concentration but also on their quality. Tables 4–6 summarize the main studies on the effect of facts on intestinal permeability. As a rule, saturated fatty acids (SFAs) augment intestinal permeability. This effect has been related to changes in TJ protein expression and/or distribution, but in some cases, other mechanisms have been described, namely, uncoupling oxidative phosphorylation, oxidative and endoplasmic reticulum stress, and other metabolic-related actions, as mentioned above (Ghezzal et al. 2020; Gori et al. 2020; Guerbette et al. 2024;

TABLE 5 | Main in vitro studies on the effects of different fats on intestinal permeability.

Nutrient	Concentration	Control	Length intervention	Cell type	Outcomes	References
C16:0	1 mM	Vehicle (methanol)	1.5–24 h	Caco-2	↑ permeability to 4 kD tracer, altered the expression of TJ and adherens junction proteins	Gori et al. 2020
C12:0, C14:0, C16:0 and C18:0	250 μM	0.6% dimethyl sulfoxide	3 days	IPEC-J2	C16:0 and C18:0 ↑ permeability independently of TJ, induced proton leak, mitochondrial remodeling, and ↑ reactive oxygen species	Guerbette et al. 2024
C16:0	5 mM	Phosphate buffered saline 0.1% bovine serum albumin	24–48 h	LS17AT Human organoids	↑ endoplasmic reticulum and oxidative stress ↓ KLF4, ↓ MUC1, disrupted MUC2 glycosylation, secretion and folding, ↓ TEER	Gulhane et al. 2016
C10:0 and C12:0	5, 13 mM 0.375, 0.75 mM	Medium	2–60 min	Caco-2	Both ↑ permeability to 4 kD tracer, ↓ cellular dehydrogenase activity and ATP level Only C10:0 altered ZO-1 and occludin	Lindmark et al. 1998
C6:0, C8:0 C10:0 and C12:0	C6:0, C8:0 30–120 mM, C10:0 10 mM and C12:0 0.06-5 mM	Medium	20–60 min	Caco-2	C8:0, C10:0 and C12:0 ↑ [^{14}C] mannitol permeability C10:0 acts via TJ and cytoskeletal modulation	Lindmark et al. 1995
C16:0	400 μM	Medium	24 h	Caco-2	↓ TEER, ↑ permeability to 4 kD tracer, altered TJ levels, ↑ pro-inflammatory cytokines, ↑ TLR4/NF-KB signaling	Ouyang et al. 2022
C18:3n-3, C20:5n-3, C22:6n-3, C18:2n-6, C20:4n-6 and C22:5n-6	30 μM 30–150 μM (C22:6 n-3)	Vehicle (ethanol)	7 days	Caco-2	C18:2n-6, C20:4n-6 and C22:5n-6 ↓ occludin C22:6n-3 ↓ ZO-1 at 150 μM No PUFA affected TEER in basal conditions C22:6n-3 counteracts inflammatory downregulation of IBF	Beguin et al. 2013
C13:3n-6 and C22:6n-3	10, 50, 100 μM		24 h	Caco-2	↑ permeability, ↓ TEER via protein kinase C modulation	Usami et al. 2003

Abbreviations: IBF: Intestinal barrier function; PUFA: Polyunsaturated fatty acids; TEER: Transepithelial electrical resistance; TJ: Tight junction; TNF: Tumor necrosis factor.

Gulhane et al. 2016; Lindmark et al. 1998; Lindmark et al. 1995; Ouyang et al. 2022). Palmitic acid appears to be fatty acid with the most prominent effects in this regard. Another proposed mechanism is TLR4 activation; however, this effect would be dependent on some sort of intermediary molecule, since binding of fatty acids to this receptor has not been shown (Velloso et al. 2015). A candidate for this role is fetuin A, a glycoprotein induced by fatty acids secondary to endoplasmic reticulum stress. Polyunsaturated fats appear to have the opposite effect. In vivo, SFA increase postprandial lipopolysaccharide concentrations, while after consumption of polyunsaturated fatty acids (PUFAs), lower concentrations of lipopolysaccharide have been found (Cândido et al. 2020). Several aspects may contribute to the observed increased permeability to lipopolysaccharide, including alkaline phosphatase expression (which dephosphorylates lipopolysaccharide), lipopolysaccharide clearance, and bile acid metabolism. However, differences disappear over time, indicating any effect on endotoxemia is short-lived.

In turn, in rodent models of alcohol-liver disease saturated fats appear to be protective, while unsaturated fat diets are harmful, due to significant downregulation of TJ expression compared to

TABLE 6 | Main in vivo on the effects of different fats on intestinal permeability.

Treatment	Dosage	Control	Age	Species	Length intervention	Outcomes	Reference
HFD	72% fat, 28% protein, and 1% carbohydrate	Normal diet	12 weeks	C57bl6/J mice	4 week	↑ permeability, ↓ ZO-1, ↓ occludin ↑ endotoxemia (reduced by antibiotic treatment or CD14 KO)	Cani et al. 2008
HFD	7% soybean oil and 23% lard by weight	Normal diet	4 weeks	OLETF and LETO rats	16 weeks	↑ permeability, ↓ claudin-1, ↓ claudin-3, ↓ occludin, ↓ ZO-1 ↑ plasma bile acids	Suzuki and Hara 2010
HFD	60% fat	Normal diet	4 week/24 week 4 week 8 week 24 weeks	C57Bl6/J mice Macrophage-specific chemokine (C-C Motif) receptor 2 (Ccr2) knockout (M-Ccr2KO) and intestinal epithelial cell-specific tamoxifen-inducible Ccl2 knockout (Vil-Ccl2KO)	24 week/4 week 20 week 12 week	↓ colon length, ↓ cecal weight ↓ Paneth and goblet cells via impaired intestinal stem cell function ↑ mucosal macrophages KO models ↓ inflammation, ↑ IBF, ameliorate metabolic disturbances	Kawano et al. 2016
HFD	46% fat	Normal diet	6–8 weeks	C57BL/6 and Winnie mice	3 weeks	↑ endoplasmic reticulum stress, ↓ mucus layer, ↑ cytokines, ↓ claudin 1, ↑ endotoxemia, aggravates colitis (Winnie mice)	Gulhane et al. 2016
HFD	30%fat	Normal diet	8 weeks	C57BL/6J mice	17 weeks	↑ macrophages, proinflammatory cytokines, ↓ IL-22, ↓ IL-23, ↓ mucus ↓ Reg3γ, ↓ ZO-1/occludin, ↑ endotoxemia (reversed by dimethyl itaconate)	Pan et al. 2023
HFD	37.4%	Normal diet	8-10 weeks	WT, Reg3$\gamma^{+/-}$, Reg3$\gamma^{-/-}$ C57Bl/6J male mice	4 weeks	Diet and REG3γ rhythmically modulate microbiota	Frazier et al. 2022
HFD	60%	Normal diet	8 weeks	Wild-type (WT) and Lcn2$^{-/-}$ (lipocalin 2) C57BL/6J mice	2, 4, 8, 12, 16 weeks	↑ lipocalin 2 early, tends to counter dysbiosis ↓ lipocalin 2 long-term → dysbiosis, metabolic dysregulation	Qiu et al. 2021

(Continues)

TABLE 6 | (Continued)

Treatment	Dosage	Control	Age	Species	Length intervention	Outcomes	Reference
HFD in experimental colitis	60%	Normal diet	ns	Male C57bl/6 mice	12 weeks	↑ endotoxemia, blunts ↑ antimicrobial peptide Cramp ↑ hepatic inflammation and fibrosis	Gabele et al. 2011
HFD	40%	Normal diet	8 weeks	Male C57BL/6JRj mice	4 and 5 weeks	Dysregulation of PPAR-γ and AMP, modulation of microbiota, uncontrolled bacterial colonization, ↑ permeability, ↓ NKCC1/CFTR	Tomas et al. 2016
High-fat, high-sucrose diet	45% fat and 17% sucrose	10% fat and 7% sucrose	6 weeks	C57BL/6J male mice	0, 3, 10, 21, 56 days	3 d ↑ liver-expressed antimicrobial peptide 2 (LEAP2) → amelioration of glucose intolerance by ghrelin receptor antagonism	Shen et al. 2021
Western diet	60% fat + 23% sucrose by gavage	Normal diet	5–6weeks	C57BL/6 and Tas1r3$^{-/-}$ (Taste receptor type 1 member 3) mice	10 weeks	↑ butyrate-producing microbiota, ↑ TJ protein, ↑ AMP in KO mice	Shon et al. 2023
Palm oil	0.2 mL by gavage	Water	3 months	Male C57BL/6J mice	1–5 days	↑ permeability to 4 kD tracer, ↓ occludin, TJ disarray, ↑ proinflammatory cytokines changed microbiota	Ghezzal et al. 2020
Lieber-DeCarli liquid diet with ethanol (35% of total calories), supplemented with either unsaturated or saturated fat	Unsaturated: corn oil, 55–60% linoleic acid, plus soybean oil Saturated: medium-chain triglyceride:beef tallow, 82:18 ratio, plus soybean oil	Saturated or unsaturated fat with control liquid maltose-dextrin diet	8 weeks	C57BL/6N mice	8 weeks	Unsaturated fat ↑ liver injury, steatosis, inflammation, macrophage infiltration, oxidative stress, TLR levels, ↑ endotoxemia in response to chronic alcohol Unsaturated fat ↓ ileal TJ mRNA levels	Kirpich et al. 2012

Abbreviations: AMP, antimicrobial peptide; HFD, high-fat diet; IBF, intestinal barrier function; LEAP2, liver-expressed antimicrobial peptide 2; TJ, tight junction; TLR, toll-like receptor.

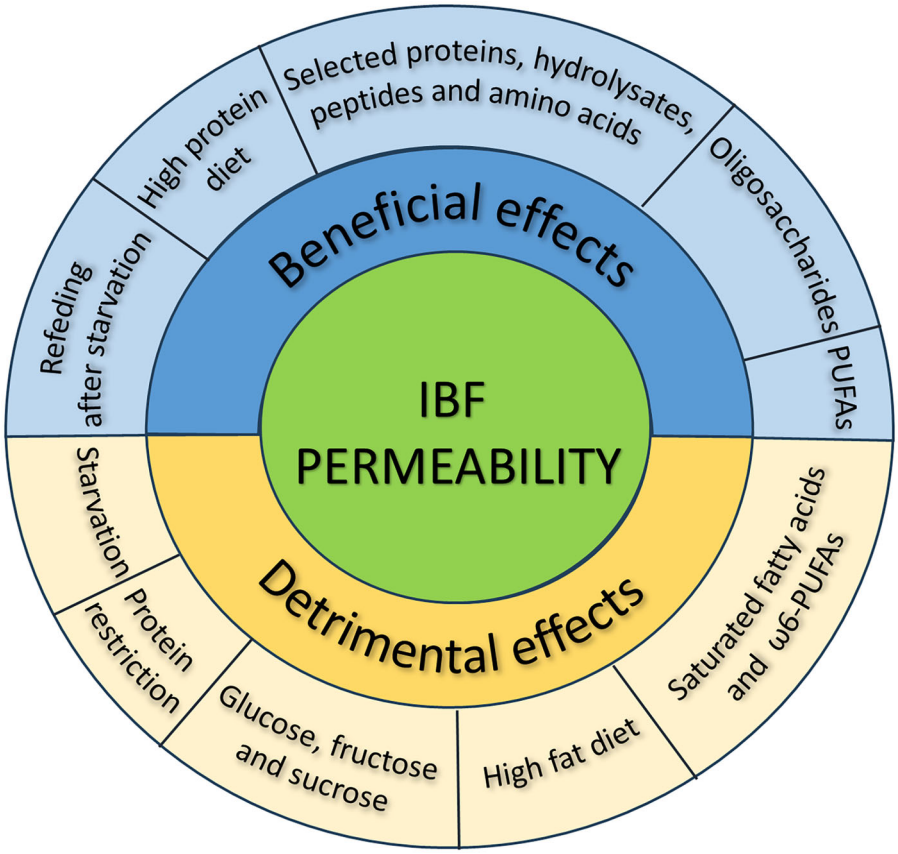

FIGURE 6 | Summary of the effects of starvation and macronutrients on intestinal permeability.

those on a saturated fat diet (Kirpich et al. 2012). This has been related to augmented oxidative stress in the former. However, no discernible differences were observed between diets in ileum permeability measured by 4-kDa fluorescein isothiocyanate dextran *ex vivo* in this study. The effect of dietary fat on permeability depends on the degree and position of fatty acids unsaturation. Among PUFA, studies in Caco 2 cells have shown that n-3 PUFA do not affect the presence of occludin in TJs, while n-6 PUFAs decrease it (at 30 μM in both cases), albeit without altering permeability (Beguin et al. 2013). Interestingly, higher concentrations of n-3 PUFA, specifically docosahexaenoic acid, slightly diminished ZO-1 immunofluorescence intensity, without alterations in Caco 2 barrier function or occludin levels (Beguin et al. 2013). Indeed, studies demonstrated that concentrations of 100 μM of n-3 and n-6 PUFAs (g-linoleic acid and docosahexaenoic acid, respectively) altered Caco-2 cell permeability, suggesting the effect of PUFAs at this level is concentration-dependent (Usami et al. 2003). A corn-oil-based diet, which is enriched in n-6 PUFA, weakened IBF in rats compared with a saturated fat diet (Kirpich et al. 2012).

6 | Starvation and intestinal permeability

Different studies have addressed the impact of starvation on the intestinal barrier from several approaches and experimental models (Figure 6). Details are provided in Tables 7–9. Effects of long-term starvation on the intestinal barrier in humans are poorly studied, but intestinal atrophy and edema have been documented in extreme cases, and diarrhea is a common, although not universal, occurrence (Kelly 2021). Evidence indicates an increase in intestinal permeability to macromolecules in malnourished patients receiving parenteral nutrition (Maxton et al. 1989; Piena et al. 1998). Studies in healthy patients after starvation are scarce. In British volunteers who fasted for just 5 days, mannitol uptake was substantially (47%) reduced, but intestinal permeability (measured by ^{51}Cr-EDTA and lactulose) was unaffected (Elia et al. 1987). No differences in permeability were found after 36 h starvation in healthy subjects (Maxton et al. 1989). On the contrary, in human duodenal biopsies of patients who fasted overnight either because they were not allowed or were unable to receive enteral nutrition, decreased villous height and increased lactulose/mannitol permeability ratio have been documented (Van Der Hulst et al. 1998). Duodenal mucosal atrophy has been also described in patients undergoing endoscopic gastrostomy (Nunes et al. 2023).

In fasted rats, the intestine undergoes reversible atrophy, whereby villus height is reduced, proliferation slows down, and the number of amplifying cells is reduced, whereas stem cells and Paneth cells are increased. When feeding resumes, this enriched population facilitates recovery. Intestinal permeability has been described to be enhanced in rats and pigs, where starvation (3 days in rats and during pregnancy in pigs) also induces a hypersecretory state and decreased mucin and AMP production, plus epithelial atrophy (J. Boza et al. 1995; J. J. Boza et al. 1999; Y. Chen et al. 2017). In the same line, Wirén et al. found that 48 h starvation in rats increased permeability to ^{51}Cr-EDTA in both jejunum and ileum, and reduced jejunal villous height.

TABLE 7 | Main in vitro studies on the effects of starvation on intestinal permeability.

Model	Intervention	Control	Length intervention	Cell type	Outcomes	Reference
Autophagy induction	Rapamycin SMER28 Resveratrol Metformin Calcitriol	Not indicated	24 and 48 h	Caco-2	Autophagy (and starvation) ↑ IBF by ↑ claudin 2 degradation via clathrin adaptor protein AP2M1 subunit	Ganapathy et al. 2022
Serum free medium, autophagy induction	Rapamycin SMER28 Resveratrol Metformin	DMEM + 10% FBS	12, 24, 48 h	Caco-2, MDCK-II, T84	Starvation and autophagy ↑ TJ by ↑ occludin via ERK1/2	Saha et al. 2023
Starvation	Krebs-Ringer bicarbonate	DMEM/F12 5% FBS, supplemented	3, 6, 12 h	IPEC-J2	↑ lysosomal degradation of claudins 1, 3 and 4 short term. Claudins 3/4 dynamin-dependent endocytosis. Recovered expression long term, proteasome dependent. Occludin, ZO-1 unchanged	Li and Ajuwon 2021
Autophagy induction, starvation	Rapamycin PP242 Earle's balanced salt solution	DMEM 10% FBS	24, 48, 72, 96 h	Caco-2, MDCK-I, MDCK-II	Autophagy ↑ TEER Starvation/autophagy ↓ claudin-2, ↑ cytoplasmic and lysosomal localization Starvation ↓ permeability to small solutes	Nighot et al. 2015

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; TEER, transepithelial electrical resistance; TJ, tight junction.

Moreover, jejunal permeability was enhanced by resection (which had no effect by itself). As expected, a correlation between mucosal permeability and changes in transepithelial resistance was noted (M. Wirén 1999). Another study aimed to elucidate how the intestinal epithelium of rainbow trout acclimates to short-term nutrient deprivation reported a significant increase in intestinal permeability (Baumgarner et al. 2013). Conversely, the surface area of microvilli and D-glucose transport have been found to be augmented during progressive starvation (up to 8 days) in rats, while refeeding restored this parameter (Gupta and Waheed 1992). Thus, starvation seems to generally debilitate IBF. Because most of the evidence on fasting and refeeding is derived from rodent models, whose feeding pattern differs significantly from that of humans, results should be interpreted with caution.

Of particular interest is the role of autophagy in maintaining intestinal epithelium integrity. Autophagy is induced during starvation, high-energy demand, or growth factor deficiency to generate energy and support metabolic processes. Therefore, autophagy is an important mechanism to maintain IBF, including antimicrobial defense and mucosal immune response (Maxton et al. 1989). Importantly, autophagy contributes to IBF by modulation of tight junctions and protection from cell death (Foerster et al. 2022). Autophagy leads to heightened claudin 2 degradation and ERK1/2-dependent augmented occludin TJ content (Ganapathy et al. 2022; P. K. Nighot et al. 2015; Saha et al. 2023), both resulting in enhanced intestinal epithelial TJ barrier function. A study conducted in Caco 2 IECs showed that nutrient starvation-induced autophagy enhanced TJ barrier function, increased transepithelial electrical resistance, and reduced the Na^+/Cl^- chloride paracellular permeability ratio. Starvation shifted claudin 2 membrane:cytoplasm distribution ratio toward the cytoplasmic location (P. K. Nighot et al. 2015). In fact, in a model of starvation in Caco 2 cells, increased binding of claudin 2 with clathrin and adaptor protein AP2 (AP2A1 and AP2M1 subunits) and to LC3 (microtubule-associated *protein* 1A/1B-light chain 3, a marker of autolysosomes) was found via co-immunoprecipitation assays. These data indicate the role of clathrin-mediated endocytosis in autophagy-induced claudin 2 degradation (Ganapathy et al. 2022). In addition, autophagy induction in starvation prevents the activation of NLRP3 inflammasome and its product IL-1 that induces IL-17E/25 secretion, thus preserving TJ barrier function (Saha et al. 2023). Time-dependent changes have been reported. Thus, short-term starvation (6 h) of IPTEC-J2 cells, an intestinal porcine enterocyte cell line, using a Krebs-Ringer bicarbonate buffer, resulted in downregulation of claudins (1, 3, and 4) in a lysosome-dependent fashion involving dynamin but not clathrin or caveolae. However, longer starvation resulted in increased claudin levels (E. Li and Ajuwon 2021).

Autophagy also influences Paneth cell function. In response to autophagy in a mouse model of starvation (48 h), decreased mRNA and protein expression of Paneth cell antimicrobial proteins lysozyme, cryptdin, and RegIIIγ, as well as increased intestinal permeability, was observed. Food deprivation also induced cell dysfunction, and caused an increased bacterial translocation and aberrant granule morphological characteristics (Hodin et al. 2011).

TABLE 8 | Main studies in animal models on the effects of starvation on intestinal permeability.

Intervention	Control	Age/weight	Species	Outcomes	Reference
Intravenous or intragastric parenteral nutrition, 16 days	Regular chow-fed rats	100–150 g (9–11 weeks)	Female Fisher rats	Intravenous feeding ↓ sIgA levels	Alverdy et al. 1985
Starvation, 4 weeks	Feeding to apparent satiation	140 g	Rainbow trout	40 mucosal proteins differentially expressed, 9 related to innate immunity Starvation ↓ α-1 proteinase inhibitor, ↑ p-glycoprotein, ↑ type II keratin E2 Starvation ↓ capacity to inhibit enzymatic stress, ↑ xenobiotic resistance, ↓ paracellular permeability	Baumgarner et al. 2013
Starvation 3 day + 4 day refeeding with casein or casein hydrolysate diet	Normal feeding	21 days	Male Wistar rats	Both diets led to the recovery of the severely starved rats, except for liver glutamate dehydrogenase activity. ↑ permeability to ovalbumin not recovered with casein diet.	Boza et al. 1995a
Starvation 3 day + 3 day refeeding	Normal feeding with soy-based diet	21 days	Male Wistar rats	Starvation ↓ muscle glutamine, ↓ intestinal mucosal protein, ↑ oxidized glutathione, ↑ ovalbumin permeability, ↑ apoptosis Refeeding resulted in rapid repair of gut atrophy	Boza et al. 1999
Fasting 1–8 d + 1–12 h refeeding	Food and water ad libitum	90–95 days	Male Wistar rats	↑ D-glucose transport in starvation via ↑ surface area of microvilli and ↑ fluidity of brush border membranes. Reversed by refeeding.	Gupta and Waheed 1992
Starvation 48 h	Unfasted animals	12 weeks	Male C57BL/6 mice	Paneth cell abnormalities → ↓ IBF, ↑ translocation	Hodin et al. 2011
Starvation 36 h	Regular chow-fed mice	4–5 weeks	Male BALB/c mice	↓ B cells in Peyer's patches, ↓ antigen-specific IgA, partly recovered after refeeding Naive B cells migrate from Peyer's patches to bone marrow and return upon refeeding	Nagai et al. 2019
Starvation 48 h ± jejunal resection Anesthesia	Regular chow-fed rats	200–300 g	Male Wistar rats	Starvation ↑ permeability (enhanced by resection), ↓ jejunal villous height IBF not affected by resection or anesthesia	Wirén et al. 1999

Abbreviation: IBF, intestinal barrier function.

Fasting and refeeding regulate sIgA. During fasting IgA+ B, cells at the germinal center of Peyer's patches are lost via apoptosis, while naïve B cells migrate from Peyer's patches to the bone marrow. Upon refeeding, naïve cells are recruited back to Peyer's patches. sIgA production is compromised as a result (Nagai et al. 2019). The intraluminal presence of foodstuffs is essential for the maintenance of sIgA, as shown by a study in which rats that were fed via a gastrectomy displayed higher levels of sIgA than those fed intravenously (Alverdy et al. 1985).

7 | Future Perspectives for IBF Management With Macronutrients

The evidence discussed so far strongly supports the concept that IBF is relevant for gut and systemic health, and that it is modulated by macronutrients. Thus, macronutrient intake is relevant for IBF-related disease prevention and possibly even treatment. There is substantial evidence that casein hydrolysates and peptides favor IBF strength compared to other protein sources.

TABLE 9 | Main studies in humans on the effects of starvation on intestinal permeability.

Intervention	Test	Number of subjects	Age	Sex	Outcomes	Reference
Total starvation 4–5 d Low-calorie diet followed by 5 days total starvation	Oral permeability test	5 lean/4 obese/4 obese	20–54 years	Both	Starvation ↓ absorption and excretion of mannitol, attributed to small intestinal changes Low calorie diets may prevent small intestinal alterations	Elia et al. 1987
Effect of sunitinib (inhibitor of starvation-induced AP2M1)	Measurement of paracellular permeability and trans-epithelial electrical resistance in Ussing chambers	15	Not indicated	Not specified	↑ urea transport, ↓ TEER, ↑ claudin 2 via inhibition of AP2M1	Ganapathy et al. 2022
Severe acute malnutrition	Clinical observations and intestinal histopathological analysis	Not indicated	Adults	Both	Most subjects exhibited intestinal atrophy, edema, and, in some cases, diarrhea	Kelly 2021
Malnourished patients on long-term parenteral nutrition	5-hour urinary lactulose/rhamnose ratio d-xylose/3-O-methyl-d-glucose absorption	9	24–73 years	Both	↑ lactulose/rhamnose ratio ↓ absorption	Maxton et al. 1989
36 h starvation	5-hour urinary lactulose/rhamnose ratio	6	Not indicated	Not indicated	↓ absorption Permeability not increased	Maxton et al. 1989
Malnourished/ underfed patients under endoscopic gastrostomy feeding	Duodenal mucosal histology assessment	30	38–87 years	Both	Duodenal villi shortening and ultrastructural alterations at baseline, mostly reversed by feeding	Nunes et al. 2023
Enteral nutrition during extracorporeal membrane oxygenation	4-hour urinary lactulose/rhamnose ratio, D-xylose, 3-O-methyl-d-glucose	16	Neonates (36–43 gestational week)	Not indicated	↑ lactulose/rhamnose ratio in 13 patients Normal D-xylose excretion in 11 patients ↓ 3-O-methyl-d-glucose in 15 patients No effect of enteral nutrition	Piena et al. 1998
Overnight fasting	6-hour urinary lactulose/mannitol ratio Duodenal biopsies	26 12 healthy	18–80 years	Both	↑ lactulose/mannitol ratio vs. healthy controls ↓ duodenal villous height	Van Der Hulst et al. 1998

Abbreviation: TEER, transepithelial electrical resistance.

Glutamine appears to be an especially prominent nutrient for IBF homeostasis, which has a strong base in current knowledge of epithelial biology and biochemistry. Both approaches may be useful and safe in a variety of intestinal and extraintestinal conditions. In fact, although not many clinical studies have been carried out, those currently available seem to corroborate this hypothesis (Abbasi et al. 2024; Laatikainen et al. 2020; Marchbank et al. 2008).

NDOs constitute a particular glucidic fraction for its relevance in regulating IBF. While traditionally considered modulators of the microbiota, direct actions on the epithelium and other cells appear to be relevant as well. From a nutritional standpoint, foods with a high NDO content may be protective against diseases such as inflammatory bowel disease. Enriched NDO may be additionally used in the development of nutraceuticals and functional foods. It should be noted, however, that evidence

for clinical effects is insufficient in many cases. One of the mechanisms described for NDOs is increased SCFA production in the intestinal lumen, leading to mucosal anti-inflammatory and IBF reinforcing effects.

Currently available evidence supports avoiding high-fat diets, fructose and possibly glucose as well for IBF homeostasis. Although not covered in our review, defects in IBF may also result from the ingestion of macronutrients modified in food processing, including Maillard reactions in glucids and proteins, generating advanced glycosylation end products. Importantly, endotoxemia has been described to arise from short-term intake of such derivatives. This may account for the known association between processed foods and disease. However, there are important gaps of knowledge in this regard (Jansen et al. 2023; Phuong-Nguyen et al. 2023).

Despite the cumulating body of evidence in this field, further studies are warranted to decipher the molecular bases of the effect of macronutrients on intestinal permeability. Most of the relevant studies have been carried out either in vitro or in rodents, and in basal conditions. Studies in vitro have mainly used stablished cell models of intestinal epithelium, that is, traditional models in which only one epithelial cell type is present. New models using intestinal organoids (in which all epithelial cell types are present) or more complex systems such as organs-on-a-chip will allow a better understanding of the effects of macronutrients and the underlying molecular basis. Intestine on a chip consists in the coculture of epithelial vasculature (endothelial lining), gut epithelial cells, and migrated immune cells such as monocytes or dendritic cells. In-depth characterization will be essential for application of knowledge to human and livestock.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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