

# Regulation of Tight Junction Permeability by Intestinal Bacteria and Dietary Components<sup>1,2</sup>

Dulantha Ulluwishewa,<sup>3,5</sup> Rachel C. Anderson,<sup>3</sup> Warren C. McNabb,<sup>4,5</sup> Paul J. Moughan,<sup>5</sup> Jerry M. Wells,<sup>6</sup> and Nicole C. Roy<sup>3,5\*</sup>

<sup>3</sup>Food Nutrition Genomics Team, Agri-Foods and Health Section, <sup>4</sup>Food and Textiles Group, AgResearch Grasslands, Palmerston North 4442, New Zealand; <sup>5</sup>Riddet Institute, Massey University, Palmerston North 4442, New Zealand; and <sup>6</sup>Host-Microbe Interactomics, ASG, Wageningen University, 6700AH Wageningen, The Netherlands

## Abstract

The human intestinal epithelium is formed by a single layer of epithelial cells that separates the intestinal lumen from the underlying lamina propria. The space between these cells is sealed by tight junctions (TJ), which regulate the permeability of the intestinal barrier. TJ are complex protein structures comprised of transmembrane proteins, which interact with the actin cytoskeleton via plaque proteins. Signaling pathways involved in the assembly, disassembly, and maintenance of TJ are controlled by a number of signaling molecules, such as protein kinase C, mitogen-activated protein kinases, myosin light chain kinase, and Rho GTPases. The intestinal barrier is a complex environment exposed to many dietary components and many commensal bacteria. Studies have shown that the intestinal bacteria target various intracellular pathways, change the expression and distribution of TJ proteins, and thereby regulate intestinal barrier function. The presence of some commensal and probiotic strains leads to an increase in TJ proteins at the cell boundaries and in some cases prevents or reverses the adverse effects of pathogens. Various dietary components are also known to regulate epithelial permeability by modifying expression and localization of TJ proteins. J. Nutr. doi: 10.3945/jn.110.135657.

## Introduction

The human intestine allows the absorption of nutrients while also functioning as a barrier, which prevents antigens and pathogens entering the mucosal tissues and potentially causing disease. The intestinal tract is inhabited by  $10^{14}$  microbes (1), and it is becoming increasingly evident that they are involved in molecular crosstalk with the intestinal epithelium and affect intestinal barrier function (2). Increased intestinal permeability is implicated in autoimmune, inflammatory, and atopic diseases, which can manifest both locally (within the intestinal mucosa) and systemically.

Chronic inflammatory diseases of the intestine, such as inflammatory bowel disease (3) and celiac disease (4), are characterized by a leaky intestinal barrier. In type I diabetes, an autoimmune disease, patients have increased small intestinal permeability (5). The incidence of diabetes can be reduced in diabetes-prone rats by preventing an increase in epithelial permeability (6). Breakdown of the intestinal barrier is also implicated in immune reactions that target organs outside the digestive tract, leading to diseases such as IgA nephropathy (7), nonalcoholic hepatic steatohepatitis (8), and multiple sclerosis in

the brain (9). Furthermore, entry of unwanted antigens can lead to systemic inflammatory response syndrome, characterized by a whole body inflammatory state, and multiple organ failure (10).

The intestinal barrier can also be compromised in individuals with no predisposing conditions. For example, transepithelial permeability of the colon has been shown to increase with age in rats (11), suggesting that that intestinal barrier integrity may decrease in healthy individuals as they age. Physiological conditions such as stress have also been shown to increase epithelial permeability (12).

An important component of the intestinal barrier is the intercellular junctional complex, crucial for the maintenance of barrier integrity. Tight junctions (TJ)<sup>7</sup> are a multifunctional complex that forms a seal between adjacent epithelial cells near the apical surface (13). They seal the paracellular space between epithelial cells, thus preventing paracellular diffusion of microorganisms and other antigens across the epithelium. TJ are not static barriers but highly dynamic structures that are constantly being remodeled due to interactions with external stimuli, such as food residues and pathogenic and commensal bacteria. They can regulate the entry of nutrients, ions, and water while

<sup>1</sup> Supported by the Riddet CoRE. D.U. is funded by a Riddet CoRE PhD Scholarship. R.C.A. is funded by a New Zealand Foundation of Research, Science and Technology Bridge to Employment Fellowship (AGR0902).

<sup>2</sup> Author disclosures: D. Ulluwishewa, R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. Roy, no conflicts of interest.

\* To whom correspondence should be addressed. E-mail: nicole.roy@agresearch.co.nz.

<sup>7</sup> Abbreviations used: EHEC, enterohemorrhagic *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ERK, extracellular signal regulated kinase; JAM, junctional adhesion molecule; MAPK, mitogen-activated protein kinase; MLC, myosin II regulatory light chain; MLCK, myosin light chain kinase; PDZ, PSD95-DlgA-ZO-1 homology; PKC, protein kinase C; ROCK, Rho kinase; TEER, transepithelial electrical resistance; TJ, tight junction; TLR2, Toll-like receptor 2; ZO, zonula occludens.

restricting pathogen entry and thus regulating the barrier function of the epithelium. This review focuses on the molecular crosstalk between intestinal epithelial cells and commensal microbiota, including the mechanisms and signaling pathways used by several species of bacteria to regulate TJ structure and assembly, as well as the effects of dietary components on TJ regulation.

### The Intestinal epithelial barrier

The intestinal epithelium is a single layer of columnar epithelial cells that separates the intestinal lumen from the underlying lamina propria. The intestinal epithelial cells are mainly absorptive enterocytes (over 80%) but also include enteroendocrine, goblet, and Paneth cells (14). These epithelial cells are tightly bound together by intercellular junctional complexes that regulate the paracellular permeability and are crucial for the integrity of the epithelial barrier.

The junctional complexes consist of the TJ, gap junctions, adherens junctions, and desmosomes (13). Adherens junctions are located beneath the TJ and are involved in cell-cell adhesion and intracellular signaling (15). Both TJ and adherens junctions (together known as the apical junctional complex) are associated to the actin cytoskeleton (15,16). Desmosomes and gap junctions are involved in cell-cell adhesion (17) and intracellular communication (18), respectively.

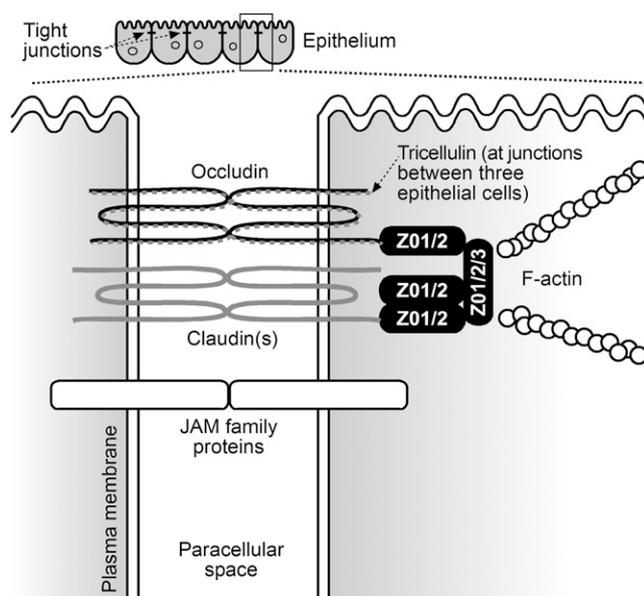
The cytoskeleton is an intricate structure of protein filaments that extends throughout the cytosol that is essential for maintaining the structure of all eukaryotic cells. Disruption of the cytoskeleton is linked to the loss of intestinal barrier integrity (19).

### TJ structure and regulation of intestinal permeability

TJ are complex structures comprising over 50 proteins. They include a series of transmembrane proteins, which form fibrils that cross the plasma membrane and interact with proteins in the adjoining cells (20). The transmembrane proteins interact with the actin cytoskeleton within the cell through plaque proteins, which act as cytoplasmic adaptors (21). Plaque proteins are also involved in the clustering and stabilization of transmembrane proteins. TJ, along with the adherens junctions, are intimately linked to the perijunctional acto-myosin ring, a belt like structure formed by actin and myosin II that encircles the apical pole of epithelial cells (16). This belt projects actin filaments that interface with the TJ, and thus circumferential contractions of the perijunctional actomyosin ring regulate TJ structure and paracellular permeability (Fig. 1). Comprehensive reviews on the complex molecular structure of TJ are available [see, e.g., (22)].

Transmembrane proteins mediate cell to cell adhesion and seal the paracellular space between epithelial cells. They can be divided into tetra-span and single-span proteins. The tetra-span proteins are occludin, the claudin family of proteins, and tricellulin. Tetra-span proteins contain 4 transmembrane domains and 2 extracellular loops, with the N and C terminals in the cytoplasm (23–25). Single span transmembrane proteins are mostly junctional adhesion molecules (JAM) (26).

The claudin proteins are considered to be the structural backbone of TJ (27). In the intestine, claudin-1, -3, -4, -5, and -8 tighten TJ (decrease paracellular permeability), whereas claudin-2 forms charge-selective paracellular pores [for review, see (28)]. The functions of claudins-7, -12, and -15 are unclear, because their effects on intestinal barrier function vary depending on the model system studied (28). Occludin has also been linked to the regulation of intermembrane diffusion and paracellular diffusion



**FIGURE 1** Structure of TJ. Transmembrane proteins such as occludin and claudin (tetraspan) and JAM (single span) seal the paracellular space between adjacent epithelial cells. Plaque proteins, such as the ZO family proteins, act as adaptors that connect transmembrane proteins to the perijunctional actomyosin ring.

of small molecules (29). Compared with cultures of Madin Darby canine kidney cells expressing wild-type occludin, cultures expressing truncated occludin mutants exhibit increased paracellular flux of small molecule markers, whereas transepithelial electrical resistance (TEER), a measure of paracellular ion permeability, is unaffected (29). While claudin seals the space between 2 adjacent cells, the barrier at the junctions between 3 epithelial cells is reinforced by tricellulin. Suppression of tricellulin gene expression impairs epithelial barrier integrity (25).

The JAM family consists of a transmembrane domain and a C-terminal cytoplasmic domain (30). JAM-A and coxsackie and adenovirus receptor, 2 examples of JAM proteins, have been shown to regulate epithelial barrier function, because expression of either protein leads to a reduction in paracellular permeability (31,32). These proteins are also implicated in TJ assembly. JAM-A and coxsackie and adenovirus receptor recruit specific TJ proteins and promote their localization at cell boundaries (32,33). Inhibition of JAM-A also prevents TJ reassembly and recovery of TEER after disruption by calcium depletion in cultured T84 epithelial monolayers (34).

Plaque proteins are characterized by PSD95-DlgA-ZO-1 homology (PDZ) domains. The PDZ domains of plaque proteins bind to PDZ domains of other proteins, forming a scaffold, or interact with specific C-terminal sequences of transmembrane proteins to anchor them to the cytoplasm (35). Several PDZ proteins have been identified, including the zonula occludens (ZO) proteins, ZO-1, ZO-2, and ZO-3, which all possess 3 PDZ domains. The first PDZ domain of ZO-1 interacts with the claudin proteins (36), the second domain of ZO-1 interacts with other ZO proteins to form dimers (37), and the 3rd PDZ domain of ZO-1 interacts with JAM-A (38). Plaque proteins potentially play a central role in TJ regulation, because they can cause reorganization of the cytoskeleton. For example, ZO-1 binds directly to F-actin (39), while AF-6 (another binding partner of JAM-A) is an effector of the Rho-family of small GTPases, signaling proteins involved in TJ regulation (40).

## Regulation of TJ

Regulation of the assembly, disassembly, and maintenance of TJ structure is influenced by various physiological and pathological stimuli. Signaling pathways involved in TJ regulation, and interactions between transmembrane proteins and the actomyosin ring are controlled by several signaling proteins, including protein kinase C (PKC), mitogen-activated protein kinases (MAPK), myosin light chain kinase (MLCK), and the Rho family of small GTPases. Phosphorylation of TJ proteins has also been shown to affect epithelial barrier function. For example, in Madin Darby canine kidney epithelial cell monolayers with a high TEER, ZO-1 is less phosphorylated than in monolayers with a low TEER (41). Phosphorylation of claudin proteins has been linked to both increased and decreased TJ permeability [for review, see (42)]. Also, the association of occludin with TJ is thought to require its phosphorylation (43).

Intestinal epithelial cells express a range of PKC isoforms involved in various signal transduction pathways. Comprehensive reviews describing the role of the various isoforms are available [e.g. (44)]. Specific PKC isoforms have also been shown to increase or decrease TEER in intestinal epithelial cell monolayers (45). PKC has also been implicated in the Toll-like receptor 2 (TLR2) pathway, which plays a key role in microbial recognition and immune modulation (46). Stimulation of the TLR2 pathway leads to activation of PKC $\alpha$  and PKC $\delta$ , which in turn leads to an increase in TEER and a redistribution of ZO-1. MAPK responds to stimuli such as growth factors and various stresses (47). For example, epithelial growth factor, which prevents TJ disruption caused by hydrogen peroxide, does so via a MAPK pathway (48). Extracellular signal regulated kinases (ERK), a group of MAPK, have been shown to interact directly with the C-terminal region of occludin to prevent hydrogen peroxide-induced disruption to TJ (48).

Contractions in the actomyosin ring are largely regulated by phosphorylation of myosin II regulatory light chain (MLC) by MLCK. In Caco-2 cells, initiation of Na<sup>+</sup>-glucose cotransport is followed by increased phosphorylation of MLC (49). Inhibition of MLCK prevents increases in TJ permeability. MLCK-mediated regulation of TJ permeability is also a crucial intermediate step in a variety of pathways employed by other extracellular stimuli, such as cytokines and bacteria, to regulate the TJ barrier (50).

MLC phosphorylation is also implicated in the assembly and regulation of TJ. In a recent study, a Caco-2 cell line expressing a constitutively active MLCK mutant under the control of an inducible promoter was produced (51). Inducing MLCK in fully differentiated monolayers led to a reduction in TEER and redistribution of ZO-1 and occludin.

The Rho family of small GTPases, RhoA, Rac, and Cdc42, are implicated in the regulation of TJ structure and function and the perijunctional actomyosin ring (52). Downstream effectors of Rho, known as Rho kinases (ROCK), phosphorylate MLC and induce contraction of the actomyosin ring (53). Further demonstrating its importance in TJ regulation, inhibition of ROCK prevents proper localization of TJ proteins during TJ assembly in cultured T84 monolayers (54).

Rho GTPase-mediated regulation of TJ is complex, because there are multiple interactions between the different Rho proteins. For example, inactivation of Rho leads to redistribution of ZO-1 and occludin away from the cell membrane and reorganization of perijunctional F-actin, which leads to reduced TEER and increased paracellular flux (52). Increased activation of Rho, however, can also lead to increased TJ disassembly via contraction of the actomyosin ring induced by increased Rho/ROCK signaling and increased MLC phosphorylation (55).

## Barrier enhancement by commensals and probiotic bacteria

Improvements in barrier integrity are associated with changes in TJ structure via changes in TJ protein expression and distribution. Commensal bacteria and probiotics have been shown to promote intestinal barrier integrity both in vitro and in vivo. Probiotics preserve the intestinal barrier in mouse models of colitis (56) and reduce intestinal permeability in human patients with Crohn's Disease (57). Probiotic treatment also reduces epithelial barrier dysfunction following psychological stress in rats (58). Treatment of epithelial cells with *Escherichia coli* Nissle 1917, a human fecal isolate and widely used probiotic, leads to increased expression of ZO-2 protein and redistribution of ZO-2 from the cytosol to cell boundaries in vitro (59). A similar effect is observed in intestinal epithelial cells isolated from germ-free mice treated with *E. coli* Nissle 1917 (60). Similarly, treating T84 cell monolayers with metabolites secreted by *Bifidobacterium infantis* Y1 from the probiotic product VSL#3 leads to an increase in ZO-1 (61). This also leads to an increase in occludin protein expression while reducing claudin-2, thus demonstrating the ability of bacteria and bacterial products to modify TEER and ion selectivity of TJ (61). Furthermore, treatment of Caco-2 cells with the probiotic *Lactobacillus plantarum* MB452 (also from the probiotic product VSL#3) results in increased transcription of occludin and cingulin genes, suggesting that bacteria-induced improvements to intestinal barrier integrity may also be regulated at the gene expression level (62).

Recently, *L. plantarum* was shown to regulate human epithelial TJ proteins in vivo and to confer protective effects against chemically induced disruption of the epithelial barrier (63). Administration of *L. plantarum* into the duodenum of healthy human volunteers was shown to significantly increase ZO-1 and occludin in the vicinity of TJ structures (63). Pretreatment of Caco-2 monolayers with *L. plantarum* significantly attenuated the effects of phorbol ester-induced dislocation of ZO-1 and occludin and the associated increase in epithelial permeability (63). This protection was also seen with an agonist of TLR2. This supports previous studies showing that oral treatment of colitis with the TLR2 ligand PCSK significantly suppressed mucosal inflammation in vivo and provides a plausible mechanism for the use of probiotics in colitis prevention and reduction (64).

Some probiotics and commensals have also been shown to prevent, and even reverse, the adverse effects of pathogens on intestinal barrier function. When cultured simultaneously with enteroinvasive *E. coli* (EIEC) strain O124:NM, *L. plantarum* strain CGMCC No.1258 maintains TEER and molecule permeability in cultured Caco-2 monolayers by preventing EIEC-induced loss of expression and redistribution of TJ-associated proteins (65). EIEC infection leads to the disruption and disorganization of the actin cytoskeleton, but these effects can then be reversed by incubating the epithelial cells with *L. plantarum*, which leads to a high density of actin filaments at the perijunctional regions and TJ proteins being more closely associated with the cytoskeleton. Coculture of Caco-2 cells with *L. plantarum* DSM 2648 has also been shown to prevent enteropathogenic *E. coli* (EPEC)-induced reduction in TEER, possibly because *L. plantarum* reduces EPEC adherence to Caco-2 cells (66).

Pretreatment with metabolites from probiotic bacteria may also be protective against pathogen-induced changes in intestinal barrier function. Treating Caco-2 cells with the cell-free supernatant of *Bifidobacterium lactis* 420 before adding the superna-

tant of enterohemorrhagic *E. coli* (EHEC) strain O157:H7 increased TEER, whereas adding the supernatant of EHEC alone decreased TEER (67). The increase in TEER was not seen, however, if the supernatant was added with or after EHEC treatment; only pretreatment with the bacterial metabolites was effective.

Live probiotic bacteria and their cell-free supernatants, therefore, differ in their ability to protect against pathogen-induced changes to barrier function. This may be attributed to the fact that live probiotic bacteria are able to compete with pathogens for nutrients for growth and adhesion, whereas metabolites secreted by probiotic bacteria may strengthen intestinal TJ via a cell signaling pathway that needs to be initiated before treatment with metabolites secreted by pathogenic bacteria. It is conceivable that promotion of TJ integrity prevents pathogenic bacteria and their effectors from entering via the paracellular pathway to cause further damage.

Some specific bacterial effectors have been shown to improve the integrity of the intestinal barrier; AvrA, secreted by *Salmonella enterica* serovar Typhimurium, is an example (68). Whereas infection with *Salmonella* lacking AvrA leads to the disruption of TJ (by reduced expression of TJ proteins and disorganized expression), *Salmonella* strains expressing AvrA stabilize TJ despite the presence of effectors known to disrupt TJ (69). AvrA seems to target the expression of ZO-1 and occludin, but not claudin, because claudin abundance is reduced and its localization limited to the cytosol, even if AvrA is present. The mechanisms through which ZO-1 and occludin expression and distribution are altered has yet to be elucidated.

Bacteria also utilize epithelial cell signaling proteins involved in TJ regulation, including Rho family GTPases, PKC, and MAPK, to enhance barrier integrity. For example, the ability of the probiotics *Streptococcus thermophilus* and *Lactobacillus acidophilus* to preserve phosphorylation of occludin in EIEC-infected cells can be reduced by treating the cells with ROCK inhibitors (70), suggesting these bacteria employ Rho family GTPases to protect against EIEC-induced TJ disruption.

*E. coli* Nissle 1917 uses a PKC $\zeta$ -dependent signaling pathway to reduce epithelial barrier disruption caused by EPEC (59). PKC $\zeta$  is the only PKC isotype located in the TJ complex and activation of PKC $\zeta$  leads to phosphorylation of ZO-2, resulting in its removal from the TJ and cytoskeleton. *E. coli* Nissle 1917 is thought to reduce the PKC phosphorylation caused by EPEC and redistribute PKC $\zeta$  away from the cell boundaries to the cytosol, reducing ZO-2-PKC $\zeta$  colocalization and thus allowing proper formation of TJ and association of ZO-2 with the cytoskeleton.

Metabolites secreted by *B. infantis* Y1, which increase the TEER in cultured epithelial monolayers, have been shown to promote MAPK-dependent pathways. *B. infantis*-induced TEER increases can be prevented by inhibiting ERK, and treating epithelial cells with *B. infantis* metabolites leads to a transient phosphorylation (hence activation) of ERK1/2 and a decrease in phosphorylation of p38 (61). However, it has also been shown that the ability of *S. thermophilus* and *L. acidophilus* to protect against EIEC infection, which is reduced by ROCK inhibitors, does not seem to be affected by inhibition of ERK1/2 or p38 (70). This suggests that different species of bacteria may use multiple pathways to modulate TJ integrity.

Commensals and probiotics are also known to decrease intestinal barrier dysfunction caused by cytokines. Treatment of cell monolayers with the cytokines TNF $\alpha$  and IFN $\gamma$  leads to a decrease in TEER and an increase in epithelial permeability (71). This TEER decrease can be prevented by preincubating with the

probiotics *S. thermophilus* ATCC19258 and *L. acidophilus* ATCC4356 or the commensal *Bacteroides thetaiotaomicron* ATCC29184 (71). DNA from the commensal bacteria *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* SP 07/3 have also been shown to induce a signal transduction cascade via an epithelial cell surface receptor, which reduces TNF $\alpha$ -induced p38 phosphorylation (72).

### Effects of dietary components on TJ integrity

In addition to bacteria, TJ are also regulated by dietary components. In celiac disease, pathogenesis is induced by gliadin, a glycoprotein present in wheat. When IEC6 and Caco-2 cells are exposed to gliadin in vitro, interaction between occludin and ZO-1 is compromised and the cytoskeleton is rearranged, leading to increased monolayer permeability (73). The mechanism for this has been linked to zonulin, the human homolog of the zonular occludens toxin from *Vibrio cholera* that is known to modulate TJ (74). Gliadin induces zonulin release, leading to PKC-mediated cytoskeletal reorganization (75). Ex vivo human intestinal samples from celiac patients in remission also showed zonulin release when exposed to gliadin, causing cytoskeletal rearrangement and ZO-1 reorganization, leading to increased permeability (73). Gliadin causes zonulin release by binding to the CXCR3 receptor in intestinal cells (76).

Most food components have not been studied in this way, however. In a screening of vegetable extracts, an extract of sweet pepper was found to decrease TEER in Caco-2 monolayers after a 10-min incubation period (77). In another study, *Solanaceae* spices, such as cayenne pepper (*Capsicum frutescens*) and paprika (*Capsicum anuum*), were found to cause an immediate decrease in TEER in vitro in the ileocecal adenocarcinoma cell line HCT-8 (78). In the case of paprika, this was accompanied by an increase in small molecule permeability and aberrant staining of ZO-1. Conversely, black pepper (*Piper nigrum*), green pepper, nutmeg, and bay leaf extracts caused an increase in TEER, although small molecule permeability and ZO-1 organization were not affected. The active compound in sweet pepper was identified as capsiainoside, and this was shown to reorganize actin filaments and decrease TEER (79). The increase in TEER caused by black and green pepper can be attributed to piperine (78). Although the authors speculate that the decrease in ion permeability was caused by cell swelling, the possible involvement of TJ was not investigated.

In a more recent screening of over 300 food extracts, galangal (*Alpinia officinarum*), marigold (*Tagetes erecta*), *Acer nikoense*, and hops (*Humulus lupulus*) were found to decrease TEER and increase paracellular flux of Lucifer yellow across Caco-2 monolayers, without having any cytotoxic effect on the cells (80). Extracts of linden (*Tilia vulgaris*), star anise (*Illicium anisatum*), *Arenga engleri*, and black tea (*Camellia sinensis*), on the other hand, were found to decrease paracellular flux and increase TEER.

Surfactants are known to affect TJ permeability. The food-grade surfactant sucrose monoester fatty acid causes a decrease in TEER and an increase in the permeability of the egg white allergen ovomucoid across Caco-2 monolayers (81). Furthermore, the perijunctional rings of the surfactant-treated cells were partially disbanded when examined under fluorescence microscopy. Similarly, when Caco-2 monolayers are exposed to *Quillaja* saponin at nontoxic levels, TEER decreases and paracellular flux increases (82).

Some proteins and amino acids alone modulate intestinal permeability. For example, protamine (an arginine-rich protein) decreases paracellular flow of lactulose in vivo in rat small

intestines (83). In contrast, TJ permeability is shown to increase following L-alanine perfusion in rats (84). The casein peptide Asn-Pro-Trp-Asp-Gln increases TEER in Caco-2 cells in a dose-dependent manner, which correlates with increased levels of occludin gene and protein expression (85). Feeding diabetes-prone rats hydrolyzed casein decreased intestinal permeability as demonstrated by reduced lactulose uptake (86). This correlated with an increased level of ileal claudin-1 gene expression and increased TEER in ex vivo ileal samples.  $\beta$ -Lactoglobulin (from skim milk) increases TEER across Caco-2 monolayers when the TJ are destabilized by culturing in serum free media (87). The putative mechanisms of action involve PKC-mediated signal transduction pathways, because treating the Caco-2 monolayer with a PKC inhibitor before adding  $\beta$ -lactoglobulin reduces the TEER increase. The authors also concluded that  $\beta$ -lactoglobulin-induced increases in TEER may be caused by modifications to the cytoskeletal structure, because treating the cells with cytochalasin D (known to disrupt the cytoskeleton) also inhibits  $\beta$ -lactoglobulin-induced increases in TEER. This could be further verified by immunostaining cytoskeletal structures of Caco-2 cells both untreated and treated with  $\beta$ -lactoglobulin.

At supraphysiologic levels, tryptophan disrupts TJ in hamster small intestinal epithelia, shown by visible perturbations in TJ (transmission electron microscopy), decreased TEER and increased insulin flux (88). In contrast, glutamine can restore stress-induced loss of barrier integrity (89). With Caco-2 monolayers where maturation was achieved by treatment with sodium butyrate (compared with spontaneously matured Caco-2 monolayers), exposure of cells to the atmosphere during media change leads to a temporary decrease in TEER. The speed of TEER recovery is improved if the cells are exposed to glutamine before the stress. Furthermore, when Caco-2 cells are deprived of glutamine via inhibition of glutamine synthetase, occludin, claudin-1, and ZO-1 protein expression is decreased (90). Studies have shown that treatment with glutamine leads to activation of the MAPK, ERK, and JNK (91); thus, glutamine could potentially modulate TJ via a MAPK-dependent signal transduction pathway.

As well as having nutritional value, trace elements such as zinc may also assist with the maintenance of intestinal barrier integrity. Caco-2 cells grown in zinc-deficient media have reduced TEER and altered expression of ZO-1 and occludin (localized away from the cell boundaries, less homogenous) compared with Caco-2 cells grown in zinc-replete media (92). This is accompanied by disorganization of F-actin filaments.

Other dietary components such as fatty acids, polysaccharides, and flavonoids are also known to alter TJ. The medium-chain fatty acids capric acid and lauric acid increase paracellular flux and cause a rapid decrease in TEER in Caco-2 cells (93). DHA,  $\gamma$ -linolenic acid, and EPA have also been shown to decrease TEER and increase paracellular permeability of fluorescein sulfonic acid in a concentration-dependent manner (94,95). Caco-2 cells exposed to sodium caprate had irregular expression of ZO-1 and occludin at the cell boundaries. Whereas the decrease in paracellular permeability was observed within 3 min of capric acid exposure, reorganization of TJ proteins took at least 60 min. Sodium caprate is known to increase TJ permeability in rat ileum ex vivo, reducing TEER, increasing paracellular flux, and inducing dilations in TJ visible by transmission electron microscopy (96). Conjugated linoleic acids have also been shown to modulate paracellular permeability in epithelial cells (97). Caco-2 cells grown in media supplemented with the *trans*-10 isomer of conjugated linoleic acids have a slower rate of TEER increase, increased paracellular flux, and

altered distribution of occludin and ZO-1. Chitosan, a polysaccharide widely used in the food industry, is also known for its absorption-enhancing properties (98). Caco-2 cells treated with chitosan have altered distribution of ZO-1 and F-actin leading to increased paracellular permeability (99). Quercetin, the most common flavonoid in nature, increases TEER (100,101) and reduces paracellular flux of Lucifer yellow (101) across Caco-2 monolayers in a dose-dependent manner. This was accompanied by an increase in claudin-4 (100,101). Although the overall expression of claudin-1, occludin, and ZO-2 was not affected (100,101), these proteins were redistributed and associated with the actin cytoskeleton (101). Furthermore, there was greater localization of claudin-1 and -4 at TJ in Caco-2 cells treated with quercetin (100,101). Quercetin is also shown to inhibit activity of PKC $\delta$ ; TJ regulation by quercetin is likely PKC $\delta$  dependent (101).

Although dietary components may regulate TJ permeability by directly targeting signal transduction pathways involved in TJ regulation, certain dietary components have been identified that influence cytokine signaling, thereby modifying TJ permeability. For example, epigallocatechin gallate, the predominant polyphenol in green tea, when incubated with T84 monolayers does not affect epithelial permeability. When treated concomitantly with IFN $\gamma$ , however, epigallocatechin gallate prevents the IFN $\gamma$ -induced decrease in TEER and increase in paracellular flux (102). Soy milk fermented by *L. plantarum*, *Lactobacillus fermentum*, and *L. rhamnosus* is also shown to prevent IFN $\gamma$ -induced decrease in TEER in the Caco-2/TC7 cell line (103). This effect, however, cannot be seen with nonfermented soy milk. The protective effect of soy milk is attributed to isoflavone aglycones synthesized in the fermented milk, thus demonstrating the importance of food-bacteria interactions in barrier function regulation. Similarly, the isoflavonoid genistein prevents TNF $\alpha$ -induced decreases in TEER in the colonic cell line HT-29/B6, but does not affect TEER itself (104).

## Conclusion

Many of the studies on the beneficial effects of bacteria on TJ integrity and the underlying mechanisms have focused on probiotic strains. This may reflect a greater interest in understanding the beneficial effects of probiotics due to their commercial applications, but it may also be due to difficulties in culturing commensal bacteria, the vast majority of which are obligate anaerobes. Some probiotics were isolated from humans, such as *E. coli* Nissle 1917, a human fecal isolate. Not all commensal bacteria may be able to modulate epithelial barrier functions, as shown in a study comparing commensal and probiotic strains (71). Using a reductionist in vitro model, which allows the co-culture of obligate anaerobic bacteria with intestinal epithelial cell lines, would facilitate comparison of mechanisms employed by probiotics and commensals.

However, the intestinal barrier is a complex environment and regulation of barrier function cannot be elucidated by in vitro models alone. Interactions between the dietary components and the microbiota are also crucial in the regulation of barrier integrity. The intestinal microbiota in the large intestine ferments substances that cannot be digested in the small intestine (such as digestion resistant starches, cellulose, pectins, and some oligosaccharides), allowing for the recovery of metabolic energy and absorbable substrates for the host. Intestinal microbiota can also indirectly affect intestinal barrier function via fermentation of undigested carbohydrates in the intestine. An example of this is the production of butyrate by colonic bacteria, which enhances the intestinal barrier by facilitating TJ assembly (105). Certain

dietary carbohydrates, on the other hand, are able to shift the commensal community toward a more advantageous structure by selectively stimulating the growth and/or activity of one, or a limited number of bacteria within the gastrointestinal system, which in turn can affect TJ integrity (106). Thus, it is important to consider the interactions between the different components of the intestinal barrier when developing strategies for enhancing barrier integrity using food and/or bacteria.

### Acknowledgments

We thank Pauline Hunt for drawing Figure 1 and Bruce Sinclair for proofreading the manuscript. All authors designed the approach for the review; and D.U. conducted the literature search and review and wrote the manuscript. All authors edited and commented on the manuscript, are responsible for the final content, and read and approved the final version of the paper.

### Literature Cited

- Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. 2006;124:837–48.
- Wells JM, Rossi O, Meijerink M, van Baaren P. Microbes and Health Sackler Colloquium: epithelial crosstalk at the microbiota-mucosal interface. *Proc Natl Acad Sci USA*. Epub 2010 Sep 8.
- Suenaert P, Bulteel V, Lemmens L, Noman M, Geypens B, Van Assche G, Geboes K, Ceuppens JL, Rutgeerts P. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *Am J Gastroenterol*. 2002;97:2000–4.
- Vogelsang H, Schwarzenhofer M, Oberhuber G. Changes in gastrointestinal permeability in celiac disease. *Dig Dis*. 1998;16:333–6.
- Damci T, Nuhoglu I, Devranoglu G, Osar Z, Demir M, Ilkova H. Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients. *Eur J Clin Invest*. 2003;33:397–401.
- Watts T, Berti I, Sapone A, Gerarduzzi T, Not T, Zielke R, Fasano A. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. *Proc Natl Acad Sci USA*. 2005;102:2916–21.
- Davin JC, Forget P, Mahieu PR. Increased intestinal permeability to (51 Cr) EDTA is correlated with IgA immune complex-plasma levels in children with IgA-associated nephropathies. *Acta Paediatr Scand*. 1988;77:118–24.
- Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut*. 2001;48:206–11.
- Yacyszyn B, Meddings J, Sadowski D, Bowen-Yacyszyn MB. Multiple sclerosis patients have peripheral blood CD45RO+ B cells and increased intestinal permeability. *Dig Dis Sci*. 1996;41:2493–8.
- Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr*. 2005;94:386–93.
- Mullin JM, Valenzano MC, Verrecchio JJ, Kothari R. Age- and diet-related increase in transepithelial colon permeability of Fischer 344 rats. *Dig Dis Sci*. 2002;47:2262–70.
- Saunders PR, Kosecka U, McKay DM, Perdue MH. Acute stressors stimulate ion secretion and increase epithelial permeability in rat intestine. *Am J Physiol*. 1994;267:G794–9.
- Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol*. 1963;17:375–412.
- van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol*. 2009;71:241–60.
- Perez-Moreno M, Fuchs E. Catenins: keeping cells from getting their signals crossed. *Dev Cell*. 2006;11:601–12.
- Madara JL. Intestinal absorptive cell tight junctions are linked to cytoskeleton. *Am J Physiol*. 1987;253:C171–5.
- Garrod D, Chidgey M. Desmosome structure, composition and function. *Biochim Biophys Acta*. 2008;1778:572–87.
- Sosinsky GE, Nicholson BJ. Structural organization of gap junction channels. *Biochim Biophys Acta*. 2005;1711:99–125.
- Banan A, Choudhary S, Zhang Y, Fields JZ, Keshavarzian A. Ethanol-induced barrier dysfunction and its prevention by growth factors in human intestinal monolayers: evidence for oxidative and cytoskeletal mechanisms. *J Pharmacol Exp Ther*. 1999;291:1075–85.
- Chiba H, Osanai M, Murata M, Kojima T, Sawada N. Transmembrane proteins of tight junctions. *Biochim Biophys Acta*. 2008;1778:588–600.
- Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem*. 1998;273:29745–53.
- Furuse M. Molecular basis of the core structure of tight junctions. *Cold Spring Harb Perspect Biol*. 2010;2:a002907.
- Furuse M, Fujita K, Hiiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol*. 1998;141:1539–50.
- Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol*. 1993;123:1777–88.
- Ikenouchi J, Furuse M, Furuse K, Sasaki H, Tsukita S, Tsukita S. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol*. 2005;171:939–45.
- Balda MS, Matter K. Transmembrane proteins of tight junctions. *Semin Cell Dev Biol*. 2000;11:281–9.
- Furuse M, Sasaki H, Fujimoto K, Tsukita S. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J Cell Biol*. 1998;143:391–401.
- Bücker R, Schumann M, Amasheh S, Schulzke J-D. Claudins in intestinal function and disease. In: Yu ASL, editor. *Current topics in membranes*. Burlington (MA): Academic Press; 2010. p. 195–227.
- Balda MS, Whitney JA, Flores C, González S, Cerejido M, Matter K. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol*. 1996;134:1031–49.
- Bazzoni G. The JAM family of junctional adhesion molecules. *Curr Opin Cell Biol*. 2003;15:525–30.
- Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol*. 1998;142:117–27.
- Cohen CJ, Shieh JT, Pickles RJ, Okegawa T, Hsieh JT, Bergelson JM. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci USA*. 2001;98:15191–6.
- Bazzoni G, Martinez-Estrada OM, Orsenigo F, Cordenonsi M, Citi S, Dejana E. Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. *J Biol Chem*. 2000;275:20520–6.
- Liu Y, Nusrat A, Schnell FJ, Reaves TA, Walsh S, Pochet M, Parkos CA. Human junction adhesion molecule regulates tight junction resealing in epithelia. *J Cell Sci*. 2000;113:2363–74.
- Fanning AS, Anderson JM. PDZ domains: fundamental building blocks in the organization of protein complexes at the plasma membrane. *J Clin Invest*. 1999;103:767–72.
- Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol*. 1999;147:1351–63.
- Utepbergenov DI, Fanning AS, Anderson JM. Dimerization of the scaffolding protein ZO-1 through the second PDZ domain. *J Biol Chem*. 2006;281:24671–7.
- Itoh M, Sasaki H, Furuse M, Ozaki H, Kita T, Tsukita S. Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. *J Cell Biol*. 2001;154:491–7.
- Fanning AS, Ma TY, Anderson JM. Isolation and functional characterization of the actin binding region in the tight junction protein ZO-1. *FASEB J*. 2002;16:1835–7.
- Linnemann T, Geyer M, Jaitner BK, Block C, Kalbitzer HR, Wittinghofer A, Herrmann C. Thermodynamic and kinetic characterization of the interaction between the Ras binding domain of AF6 and members of the Ras subfamily. *J Biol Chem*. 1999;274:13556–62.

41. Stevenson BR, Anderson JM, Braun ID, Mooseker MS. Phosphorylation of the tight-junction protein ZO-1 in two strains of Madin-Darby canine kidney cells which differ in transepithelial resistance. *Biochem J*. 1989;263:597-9.
42. Findley MK, Koval M. Regulation and roles for claudin-family tight junction proteins. *IUBMB Life*. 2009;61:431-7.
43. Wong V. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. *Am J Physiol*. 1997;273:C1859-67.
44. Farhadi A, Keshavarzian A, Ranjbaran Z, Fields JZ, Banan A. The role of protein kinase C isoforms in modulating injury and repair of the intestinal barrier. *J Pharmacol Exp Ther*. 2006;316:1-7.
45. Song JC, Hanson CM, Tsai V, Farokhzad OC, Lotz M, Matthews JB. Regulation of epithelial transport and barrier function by distinct protein kinase C isoforms. *Am J Physiol Cell Physiol*. 2001;281:C649-61.
46. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology*. 2004;127:224-38.
47. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim Biophys Acta*. Epub 2010 Dec 16.
48. Basuroy S, Seth A, Elias B, Naren AP, Rao R. MAPK interacts with occludin and mediates EGF-induced prevention of tight junction disruption by hydrogen peroxide. *Biochem J*. 2006;393:69-77.
49. Turner JR, Rill BK, Carlson SL, Carnes D, Kerner R, Mrsny RJ, Madara JL. Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *Am J Physiol*. 1997;273:C1378-85.
50. Scott KG, Meddings JB, Kirk DR, Lees-Miller SP, Buret AG. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology*. 2002;123:1179-90.
51. Shen L, Black ED, Witkowski ED, Lencer WI, Guerriero V, Schneeberger EE, Turner JR. Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. *J Cell Sci*. 2006;119:2095-106.
52. Nusrat A, Giry M, Turner JR, Colgan SP, Parkos CA, Carnes D, Lemichez E, Boquet P, Madara JL. Rho protein regulates tight junctions and perijunctional actin organization in polarized epithelia. *Proc Natl Acad Sci USA*. 1995;92:10629-33.
53. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science*. 1996;273:245-8.
54. Walsh SV, Hopkins AM, Chen J, Narumiya S, Parkos CA, Nusrat A. Rho kinase regulates tight junction function and is necessary for tight junction assembly in polarized intestinal epithelia. *Gastroenterology*. 2001;121:566-79.
55. Bruewer M, Hopkins AM, Hobert ME, Nusrat A, Madara JL, Rho A. Rac1, and Cdc42 exert distinct effects on epithelial barrier via selective structural and biochemical modulation of junctional proteins and F-actin. *Am J Physiol Cell Physiol*. 2004;287:C327-35.
56. Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology*. 2001;121:580-91.
57. Gupta P, Andrew H, Kirschner BS, Guandalini S. Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. *J Pediatr Gastroenterol Nutr*. 2000;31:453-7.
58. Zareie M, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, Soderholm JD, Perdue MH, Sherman PM. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut*. 2006;55:1553-60.
59. Zyrek AA, Cichon C, Helms S, Enders C, Sonnenborn U, Schmidt MA. Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol*. 2007;9:804-16.
60. Ukena SN, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, Bleich A, Bruder D, Franzke A, et al. Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. *PLoS ONE*. 2007;2:e1308.
61. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, Looijer-van Langen M, Madsen KL. Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. *Am J Physiol Gastrointest Liver Physiol*. 2008;295:G1025-34.
62. Anderson RC, Cookson AL, McNabb WC, Park Z, McCann MJ, Kelly WJ, Roy NC. *Lactobacillus plantarum* MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. *BMC Microbiol*. 2010;10:316.
63. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, Wells JM. Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum* *in vivo* and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol*. 2010;298:G851-9.
64. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. *Gastroenterology*. 2007;132:1359-74.
65. Qin H, Zhang Z, Hang X, Jiang Y. *L. plantarum* prevents enteroinvasive *Escherichia coli*-induced tight junction proteins changes in intestinal epithelial cells. *BMC Microbiol*. 2009;9:63.
66. Anderson RC, Cookson AL, McNabb WC, Kelly WJ, Roy NC. *Lactobacillus plantarum* DSM 2648 is a potential probiotic that enhances intestinal barrier function. *FEMS Microbiol Lett*. 2010;309:184-92.
67. Putaala H, Salusjarvi T, Nordstrom M, Saarinen M, Ouwehand AC, Bech Hansen E, Rautonen N. Effect of four probiotic strains and *Escherichia coli* O157:H7 on tight junction integrity and cyclooxygenase expression. *Res Microbiol*. 2008;159:692-8.
68. Hardt WD, Galan JE. A secreted *Salmonella* protein with homology to an avirulence determinant of plant pathogenic bacteria. *Proc Natl Acad Sci USA*. 1997;94:9887-92.
69. Liao AP, Petrof EO, Kuppireddi S, Zhao Y, Xia Y, Claud EC, Sun J. *Salmonella* type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. *PLoS ONE*. 2008;3:e2369.
70. Trivedi K, Barrett KE, Resta-Lenert SC. Probiotic inhibition of the entry of enteroinvasive *E. coli* into human intestinal epithelial cells involves both Rho-dependent and independent pathways. *Gastroenterology*. 2003;124:A106.
71. Resta-Lenert S, Barrett KE. Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. *Gastroenterology*. 2006;130:731-46.
72. Ghadimi D, Vrese MD, Heller KJ, Schrezenmeir J. Effect of natural commensal-origin DNA on toll-like receptor 9 (TLR9) signaling cascade, chemokine IL-8 expression, and barrier integrity of polarized intestinal epithelial cells. *Inflamm Bowel Dis*. 2010;16:410-27.
73. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, Thakar M, Iacono G, Carroccio A, et al. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol*. 2006;41:408-19.
74. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci*. 2000;113:4435-40.
75. Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR, Drago S, Congia M, Fasano A. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. *Gut*. 2003;52:218-23.
76. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, Rallabhandi P, Shea-Donohue T, Tamiz A, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroenterology*. 2008;135:194-204 e3.
77. Hashimoto K, Matsunaga N, Shimizu M. Effect of vegetable extracts on the transepithelial permeability of the human intestinal Caco-2 cell monolayer. *Biosci Biotechnol Biochem*. 1994;58:1345-6.
78. Jensen-Jarolim E, Gajdzik L, Haberl I, Kraft D, Scheiner O, Graf J. Hot spices influence permeability of human intestinal epithelial monolayers. *J Nutr*. 1998;128:577-81.
79. Hashimoto K, Kawagishi H, Nakayama T, Shimizu M. Effect of capsaicin, a diterpene glycoside, on tight-junctional permeability. *Biochim Biophys Acta*. 1997;1323:281-90.
80. Konishi Y. Modulations of food-derived substances on intestinal permeability in Caco-2 cell monolayers. *Biosci Biotechnol Biochem*. 2003;67:2297-9.
81. Mine Y, Zhang JW. Surfactants enhance the tight-junction permeability of food allergens in human intestinal epithelial Caco-2 cells. *Int Arch Allergy Immunol*. 2003;130:135-42.

82. Narai A, Arai S, Shimizu M. Rapid decrease in transepithelial electrical resistance of human intestinal Caco-2 cell monolayers by cytotoxic membrane perturbants. *Toxicol In Vitro*. 1997;11:347–54.
83. Shi X, Gisolfi CV. Paracellular transport of water and carbohydrates during intestinal perfusion of protamine in the rat. *Am J Med Sci*. 1996;311:107–12.
84. Sadowski DC, Meddings JB. Luminal nutrients alter tight-junction permeability in the rat jejunum: an in vivo perfusion model. *Can J Physiol Pharmacol*. 1993;71:835–9.
85. Yasumatsu H, Tanabe S. The casein peptide Asn-Pro-Trp-Asp-Gln enforces the intestinal tight junction partly by increasing occludin expression in Caco-2 cells. *Br J Nutr*. 2010;104:951–6.
86. Visser JT, Lammers K, Hoogendijk A, Boer MW, Brugman S, Beijer-Liefers S, Zandvoort A, Harmsen H, Welling G, et al. Restoration of impaired intestinal barrier function by the hydrolysed casein diet contributes to the prevention of type 1 diabetes in the diabetes-prone BioBreeding rat. *Diabetologia*. 2010;53:2621–8.
87. Hashimoto K, Nakayama T, Shimizu M. Effects of beta-lactoglobulin on the tight-junctional stability of Caco-2-SF monolayer. *Biosci Biotechnol Biochem*. 1998;62:1819–21.
88. Madara JL, Carlson S. Supraphysiologic L-tryptophan elicits cytoskeletal and macromolecular permeability alterations in hamster small intestinal epithelium in vitro. *J Clin Invest*. 1991;87:454–62.
89. Li N, DeMarco VG, West CM, Neu J. Glutamine supports recovery from loss of transepithelial resistance and increase of permeability induced by media change in Caco-2 cells. *J Nutr Biochem*. 2003;14:401–8.
90. Li N, Lewis P, Samuelson D, Liboni K, Neu J. Glutamine regulates Caco-2 cell tight junction proteins. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G726–33.
91. Rhoads JM, Argenzio RA, Chen W, Rippe RA, Westwick JK, Cox AD, Berschneider HM, Brenner DA. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. *Am J Physiol*. 1997;272:G943–53.
92. Finamore A, Massimi M, Conti Devirgiliis L, Mengheri E. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. *J Nutr*. 2008;138:1664–70.
93. Lindmark T, Kimura Y, Artursson P. Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *J Pharmacol Exp Ther*. 1998;284:362–9.
94. Usami M, Komurasaki T, Hanada A, Kinoshita K, Ohata A. Effect of gamma-linolenic acid or docosahexaenoic acid on tight junction permeability in intestinal monolayer cells and their mechanism by protein kinase C activation and/or eicosanoid formation. *Nutrition*. 2003;19:150–6.
95. Usami M, Muraki K, Iwamoto M, Ohata A, Matsushita E, Miki A. Effect of eicosapentaenoic acid (EPA) on tight junction permeability in intestinal monolayer cells. *Clin Nutr*. 2001;20:351–9.
96. Soderholm JD, Oman H, Blomquist L, Veen J, Lindmark T, Olaison G. Reversible increase in tight junction permeability to macromolecules in rat ileal mucosa in vitro by sodium caprate, a constituent of milk fat. *Dig Dis Sci*. 1998;43:1547–52.
97. Roche HM, Terres AM, Black IB, Gibney MJ, Kelleher D. Fatty acids and epithelial permeability: effect of conjugated linoleic acid in Caco-2 cells. *Gut*. 2001;48:797–802.
98. Thanou M, Verhoef JC, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. *Adv Drug Deliv Rev*. 2001;52:117–26.
99. Schipper NG, Olsson S, Hoogstraate JA, deBoer AG, Varum KM, Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement. *Pharm Res*. 1997;14:923–9.
100. Amasheh M, Schlichter S, Amasheh S, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. *J Nutr*. 2008;138:1067–73.
101. Suzuki T, Hara H. Quercetin enhances intestinal barrier function through the assembly of zonula occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. *J Nutr*. 2009;139:965–74.
102. Watson JL, Ansari S, Cameron H, Wang A, Akhtar M, McKay DM. Green tea polyphenol (-)-epigallocatechin gallate blocks epithelial barrier dysfunction provoked by IFN-gamma but not by IL-4. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G954–61.
103. Di Cagno R, Mazzacane F, Rizzello CG, Vincentini O, Silano M, Giuliani G, De Angelis M, Gobbetti M. Synthesis of isoflavone aglycones and equol in soy milks fermented by food-related lactic acid bacteria and their effect on human intestinal Caco-2 cells. *J Agric Food Chem*. 2010;58:10338–46.
104. Schmitz H, Fromm M, Bentzel CJ, Scholz P, Detjen K, Mankertz J, Bode H, Epple HJ, Riecken EO, et al. Tumor necrosis factor-alpha (TNFalpha) regulates the epithelial barrier in the human intestinal cell line HT-29/B6. *J Cell Sci*. 1999;112:137–46.
105. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr*. 2009;139:1619–25.
106. Gibson GR. Fibre and effects on probiotics (the prebiotic concept). *Clin Nutr Suppl*. 2004;1:25–31.