



Review

Modulation of tight junction integrity by food components

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ABSTRACT

The primary function of the human intestine is to absorb nutrients and water. However, equally important is its ability to act as a selective barrier to protect the human system. Intestinal epithelium is formed by a monolayer of epithelial cells. Adjacent cells of the monolayer are sealed together by the formation of tight junctions (TJs)—complex protein systems. The structure of TJ involves transmembrane proteins linked to a cytoplasmic plaque, which is formed by a network of scaffolding and adaptor proteins, signalling components and actin-binding cytoskeleton linkers. TJs regulate paracellular transport of compounds as well as physical barrier function of epithelium, which is linked to pathogenesis of inflammatory bowel disease, ulcerative colitis, Crohn's disease and food allergies. Epithelium is intensively exposed to food components. Various food components can affect the functioning of TJ by modifying expression of TJ protein components and affecting signalling pathways involved in TJ regulation. The targeted usage of food components to modulate TJ permeability is of vital importance for enhancing the absorption of poorly permeable drugs or bioactive compounds and, on the other hand, for sealing the junction in order to limit the risk of intestine pathology.

The aim of this review is to compile and analyse the previous research investigating the possible relevance of food components to TJ regulation, with a special consideration of phenolic compounds.

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1. Introduction

The primary function of human intestine is to absorb nutrients and water. However, equally important is its ability to act as a selective barrier to protect the human system (Groppe, Smith, & Groff, 2009). It serves as an effective defence against permeation of toxins, antigens and pathogens from the luminal environment into the circulatory system. The main constituents of intestinal epithelial cells are absorptive enterocytes (80%), with enteroendocrine, goblet and Paneth cells making up the remaining 20% (Schonhoff, Giel-Moloney, & Leiter, 2004). The surface area of gut epithelium layer is heavily folded in so-called 'valves of Kerckring' and forms millions of finger-like villi. These features increase the surface area of the small intestine to 250 m² allowing absorption of more nutrients than if it were smooth (Madara, 2011). The barrier role of the epithelial cells is dual. Fulfilling a gate function, it forms both a physical and biological obstacle. The physical barrier inhibits transport of microbes and larger molecules such as allergenic compounds, which cannot pass through the paracellular space. The biological barrier serves as a defence against xenobiotics due to expression of detoxification enzymes and efflux transporters. Crucial for barrier integrity is formation of tight junctions (TJs), protein complexes formed near the apical surface of the cells, sealing paracellular space between epithelial cells (Anderson & Van Itallie, 2009; Farquhar & Palade, 1963). In addition to the above described gate function, TJ serves as a fence, which hinders apical proteins from diffusing into the basal region and vice versa. Thus, it enables the maintenance of different compositions of integral membrane proteins as well as lipids in the apical and basolateral membrane domains. The third function of TJs, that has recently been acknowledged, is their role in signalling: signal recognition, transduction and response (González-Mariscal, Tapia, & Chamorro, 2008; Terry, Nie, Matter, & Balda, 2010; Van Itallie & Anderson, 2006).

In recent years, it has been suggested that junctional complex has a highly dynamic structure (Raleigh et al., 2011; Shen, Weber, Raleigh, Yu, & Tumer, 2011; Steed, Balda, & Matter, 2010). The composition of TJ associated proteins is flexible and can be modulated by several factors. In this context, the effect of nutrients on epithelial barrier function has been reported (Hashimoto, Matsunaga, & Shimizu, 1994; Isoda, Han, Tominaga, & Maekawa, 2001; Sadowski & Meddings, 1993; Suzuki, Tanabe, & Hara, 2011; Ulluwishewa et al., 2011; Yasumatsu & Tanabe, 2010). It is of vital importance to highlight that modulation of TJ could have dual implications. The increased permeability of intestinal epithelium has been linked to pathogenesis of inflammatory bowel disease, ulcerative colitis, Crohn's disease and food allergies. On the other hand, transient increase in paracellular transport could improve bioavailability of desirable bioactive compounds, which normally are poorly absorbed.

The purpose of this review is to compile and analyse the previous research investigating the possible relevance of food components to TJ regulation, with a special consideration of phenolic compounds.

2. Intestinal absorption

2.1. Modes of intestinal absorption

Enterocytes simultaneously serve as a protective barrier and facilitate transport of selected nutrients across the epithelium. Subsequently, nutrients diffuse into small blood vessels to provide nourishment to the organism. Molecules cross the intestinal epithelium by four main pathways, illustrated in Fig. 1. Firstly, by passive diffusion across the cell membranes. This transcellular diffusion involves mainly lipophilic compounds. Secondly, by carrier mediated transcellular transport of ions, glucose, amino acids, di- and tri-peptides. This is known as active transport and involves the action of two sets of transport proteins in the plasma membrane. One is present on the apical side of the cells and selectively

transports molecules from the gut into the cell. The other set is located in the basolateral surfaces of the cells and facilitates the same molecules to diffuse into the extracellular fluid. The third mode of transport is a transcytosis of high-molecular-weight substances such as proteins which could be apically taken into the cell by endocytosis, intracellularly transported to the other side of the cell via the transcytotic vesicles and then released to the basolateral space by exocytosis (Shimizu, 1999). Finally, the fourth mode of absorption is passive diffusion between adjacent cells also called paracellular transport, mainly applicable to water-soluble low molecular weight compounds. The paracellular transport of compounds is regulated by the formation of a protein–protein network–epithelial junctional complex. This complex forms a physical barrier by mechanically linking adjacent cells and sealing intracellular space. Therefore, molecules cannot leak across the cell layer. However, the TJs are not sealed completely; they allow flow of some solutes and water. This passive movement through the space between adjacent cells, paracellular transport, is important in the absorption of amino acids, monosaccharides and small hydrophilic compounds, especially after a meal, when they are present in high concentration in the gut lumen (Déprez, Mila, Huneau, Tome, & Scalbert, 2001; Sadowski & Meddings, 1993).

2.2. Permeability of TJ

TJs seal paracellular spaces of epithelium and thus create paracellular barriers that, depending on local transport requirements, differ in electrical conductance, ionic charge preference and the level of permeability for uncharged solutes (Van Itallie et al., 2008). These differences are referred to as permselectivity. The permeability of epithelia varies significantly between tissues; the epithelium of intestine is several orders of magnitude more permeable than the epithelium of skin, urinary bladder and stomach (Ballard, Hunter, & Taylor, 1995; Farquhar & Palade, 1963; Turner, 2000). In the intestine itself, permeability differs in the villus–crypt axis. The apical part of villus has a pore size of 6 Å, basal part of villus of 10 Å and crypts as large as 60 Å (Fihn, Sjöqvist, & Jodal, 2000). Moreover, it can be regulated in response to pharmacological, physiological and pathophysiological status (Ballard et al., 1995; Lowe, Miyai, Steinbach, & Hardison, 1988; Rodgers & Fanning, 2011; Sadowski & Meddings, 1993; Van Itallie, Fanning, Holmes, & Anderson, 2010; Ward, Tippin, & Thakker, 2000). Current information suggests that the paracellular barrier is most usefully explained as having two physiological components: a system of charge-selective small pores of 4 Å in radius called a pore pathway, and a leak pathway lacking charge or size discrimination (leak pathway) (Shen et al., 2011; Van Itallie et al., 2008). The pore pathway is influenced by claudins expression patterns and carries large quantities of small uncharged solutes and specific ions. The leak pathway is likely controlled by different proteins and signals and allows small quantities of large molecules to pass (Anderson & Van Itallie, 2009). The suitability of this model is confirmed by *in vitro* studies carried out in cell lines using polyethylene glycol oligomers of differentiated size. The results clearly showed that permeability is biphasic, consisting of a high-capacity and size-restrictive pore pathway as well as a low capacity and size-independent leak pathway (Linnankoski et al., 2010; Van Itallie, Fanning, Bridges, & Anderson, 2009). Paracellular barrier function is commonly characterised by transepithelial electrical resistance (TEER). It measures ion conductance across the epithelial sheet at a given moment of time, therefore it reflects solely the permeability of small pores pathway. Additionally, the flux assay measuring the movement of large marker molecules, such as mannitol, inulin, Lucifer yellow (LY) or fluorescent dextrans over a period of time was used to measure permeability (Al-Sadi et al., 2011; Grube, Wolfrum, & Langguth, 2008; Noda, Tanabe, & Suzuki, 2012; Van Itallie et al., 2009).

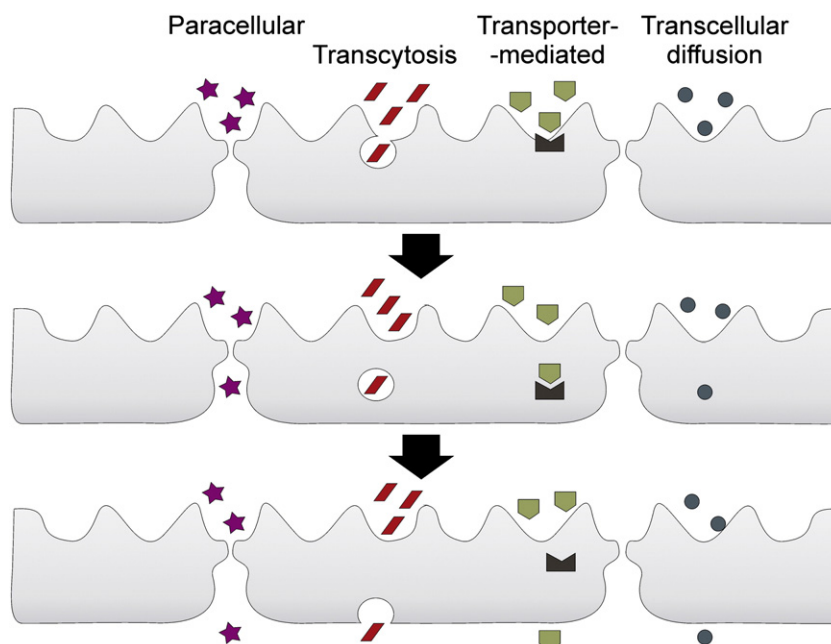


Fig. 1. Schematic diagram of four pathways of intestinal absorption. Paracellular permeability is associated with passive diffusion through the space between neighbouring cells of water soluble low molecular substances (★). Macromolecules (■) can be transported across the cells by transcytosis. Specific transporters located at the apical side of cell membrane mediate the transport of specific nutrients (■). Hydrophobic substances (●) can penetrate cell membrane and might be transported via cells interior by passive diffusion.

2.3. Caco-2 cell model of intestinal epithelium

The idea of developing an *in vitro* assay to study intestinal absorption emerged due to the boom in the drug discovery programmes of the seventies and eighties. Demand for quick and reliable model to evaluate intestinal permeability, more suitable for mimicking physiological conditions than those based on membrane partitioning, generated immense interest in developing cell based models. The attempts to either culture intestinal epithelial cells or to establish cell lines from enterocytes had been unsuccessful (Borchardt, 2011). Therefore, the attention was drawn to human colon adenocarcinoma cell lines. The first human colon carcinoma cell line HT-29 was established by Jorgen Fogh in 1964. A few years later the same author established Caco-2 (colon adenocarcinoma) line from the cells isolated from 72 years old Caucasian male (Fogh, Fogh, & Orfeo, 1977). However, the discovery that those cells under specific culture conditions undergo differentiation into enterocytes was made several years later (Pinto et al., 1983). Caco-2 cells cultured for around 21 days form a polarised monolayer of differentiated enterocytes. During that time at an apical side of cells TJs are formed, as well as regular microvilli typical for a brush border in small intestine. The Caco-2 cells are also able to express a variety of brush border digestive enzymes, transporter proteins, efflux proteins, phase I and phase II enzymes and receptors (Hilgers, Conradi, & Burton, 1990; Sun, Chow, Liu, Du, & Pang, 2008). In order to better resemble the conditions of the intestine, Caco-2 cells are cultured on permeable filter supports placed in two chambers, representing apical and basolateral sides.

The mucus layer covering intestinal epithelium may form an additional barrier affecting the absorption. The lack of mucus production ability of Caco-2 cell line can be considered as its limitation. In order to overcome this problem, co-cultures of Caco-2 cells and HT29-H or HT29-MX were applied (Hilgendorf et al., 2000). Another disadvantage of the cell line is that the tightness of the monolayer resembles that of the colon and not the small intestine and therefore the permeability of paracellularly transported compounds can be underestimated. As Caco-2 cell line is a heterogeneous population of cells, the properties of the cells may change with time in culture (Zweibbaum, Laburthe, Grasset, & Louvard, 2011). This may be an explanation for differences in morphology, expression of enzymes and transporters between

laboratories (Sambuy et al., 2005). Moreover, culturing conditions strongly affect cell performance. Both factors may lead to highly variable results obtained in different laboratories and make the comparison of results very difficult. Thus, it needs to be emphasised that the attempts to develop a novel cell line, which will optimally mimic conditions of human small intestine, should be intensified.

Despite the above described limitations, Caco-2 cell line is still considered to be the best model of intestinal absorption available up to now, showing the gene expression pattern closest to that of small intestinal enterocytes (Christensen et al., 2012). The model has emerged as one of the standard *in vitro* tools to predict *in vivo* intestinal absorption of various substances (Langerholm, Maragkoudakis, Wollgast, Gradisnik, & Cencic, 2011; Xie, Kosińska, Xu, & Andlauer, 2012). Moreover, Caco-2 cell model is widely utilised to identify and elucidate the mechanisms of TJ modulation (Al Sadi & Ma, 2007; Suzuki & Hara, 2004).

3. Structure of TJ

Protein complexes connecting epithelial cells include TJs, gap junctions, adherens junctions (AJ) and desmosomes. TJs are typically located at the apical side, whereas AJs and desmosomes are located within the basolateral membrane (Hartsock & Nelson, 2008). Desmosomes and AJ are involved in cell–cell adhesion (Runswick, O'Hare, Jones, Streuli, & Garrod, 2001), whereas gap junctions take part in intracellular communication (Kumar & Gilula, 1996). The junctional complexes encircle the cells resulting in a continuous belt that interconnects neighbouring cells.

In order to understand the molecular mechanism controlling TJ structure and function, it is important to determine their molecular composition and organisation. The last 30 years of intensive research in this field has allowed characterisation of the main molecular components and has given a new insight into their interactions and mechanisms that regulate TJ functions. At least 40 different proteins are involved in TJ formation (Furuse, 2010). They play many interrelated roles in cell polarity, signalling, transcriptional regulation, cell cycle, vesicle trafficking in addition to formation of the paracellular barrier (Balda & Matter, 2008). The schematic structure of TJ is presented in Fig. 2, whereas the structural features and major functions of TJ associated proteins are compiled in Table 1.

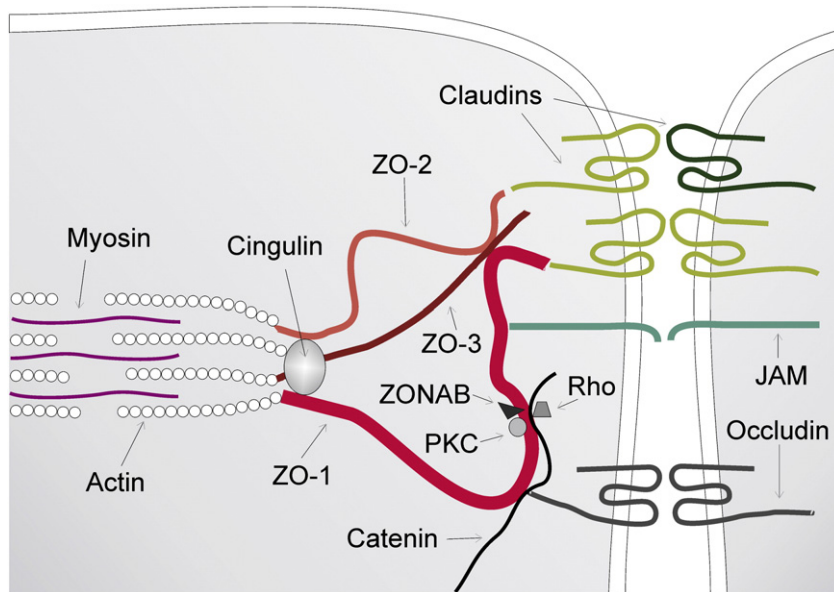


Fig. 2. Structure of tight junction showing interactions between transmembrane, cytosolic plaque and cytoskeletal proteins. ZO, zonula occludens; JAM, junctional adhesion molecule; PKC, protein kinase C; Rho, family of small GTPases; ZONAB, zonula occludens-1 associated nucleic acid binding protein.

3.1. Integral membrane proteins

Integral membrane proteins comprise occludin, claudins, junctional adhesion molecules (JAMs) and tricellulin. These transmembrane proteins are linked to cytoplasmic plaque proteins, among which zonula occludens (ZO) and cingulin are the most important for the integrity of the epithelial barrier since they anchor transmembrane proteins to the perijunctional actin myosin ring (PAMR). Transmembrane proteins mediate cell to cell adhesion and can be divided into tetraspan proteins (containing four transmembrane domains) and single-span proteins. Occludin, claudins and tricellulin are tetraspan proteins, whereas single-span transmembrane proteins are mainly JAMs.

In humans, claudins constitute a family of more than 20 members. It has been recently discovered that claudins are the main proteins responsible for regulation of epithelial paracellular transport (Furuse, 2010; Van Itallie & Anderson, 2006; Yamazaki, Tokumasu, Kimura, & Tsukita, 2011). Claudins (tetraspanning membrane proteins) have two extracellular loops, and N- and C-terminal cytoplasmic domains (Lal-Nag & Morin, 2009). Extracellular loops are critical for homophilic (between identical protein) and/or heterophilic (between nonidentical proteins) interactions and the formation of ion selective channels. The C-terminal cytoplasmic domain contains a PDZ domain binding motif. Claudins interact with the PDZ-domain of cytosolic proteins, including ZO-1, ZO-2, and ZO-3, which anchor claudins to the cytoskeleton. PDZ is an acronym derived from first letters of names of proteins first discovered to share this domain *i.e.* Post synaptic density 95, Disc large and ZO-1 domain. Claudin–claudin interactions between adjacent cells can be either homophilic or heterophilic. The intracellular C-terminal domain of claudin possesses multiple regulatory sites, including serine and threonine phosphorylation sites (Lal-Nag & Morin, 2009). Signaling pathways including protein kinase C (PKC), Rho guanosine triphosphatases (Rho GTPases), mitogen-activated protein kinases (MAPKs) and phosphatases are involved in the regulation of these phosphorylation sites (Van Itallie & Anderson, 2006).

Claudins are key components for the structure and function of TJs. They are responsible for cation- and anion-selective pores formation and ion flux, measurable by TEER. It has been proposed that the extracellular loops of claudins can create selective paracellular pores, that allow passive diffusion of ions (Van Itallie & Anderson, 2006; Van Itallie et al.,

2008). The profile and level of different claudins are critical determinants of permselectivity of TJ (Yu, 2009). The modulation of expression of distinct members of claudin family may change the permeability and charge selectivity of paracellular route in different directions (Vreeburg, van Wezel, Ocana-Calahorra, & Mes, 2012). Claudins can be classified according to the function as sealing and pore forming. Expression of claudin-2 selectively increases the permeability for solutes smaller than 4 Å, whereas claudin-1, -3, -4, -5 and -8 tighten TJ (Colegio, Van Itallie, Mccrea, Rahner, & Anderson, 2002; Schulzke, Gunzel, John, & Fromm, 2012; Van Itallie et al., 2008).

Occludin is a second tetraspan membrane protein with 2 extracellular loops, a short cytoplasmic N-terminus and a long cytoplasmic C-terminus. The C-terminus interacts with the PDZ domain of ZO-1. This connection is essential in linking occludin to the actin cytoskeleton. A role of occludin in the regulation of paracellular permeability has been suggested (Raleigh et al., 2011). Its localisation in TJ complex is regulated by reversible phosphorylation catalysed by kinases and phosphatases (Tsukamoto & Nigam, 1999). Phosphorylation and dephosphorylation of occludin is a crucial post-translational modification since it is connected to assignment of occludin to different subcellular compartments. Kinases and phosphatases involved are expected to be major players in the assembly–disassembly process. Associations between extracellular loops of occludin and claudin are important for barrier formation. It has been suggested that occludin plays a role in the regulation of flux of large macromolecules, presumably through the non-restrictive leak pathway (Al-Sadi et al., 2011). Nevertheless, occludin phosphorylation also modifies permeability of claudin based pores (Raleigh et al., 2011).

Tricellulin is an important component at a meeting point of three cells. Like claudin and occludin, tricellulin is also a tetraspan protein with two extracellular loops. It is localised at the TJ strand of tricellular contacts of epithelial cells and exhibits a partial homology with occludin in the region responsible for its binding to ZO-1 (Mariano, Sasaki, Brites, & Brito, 2011).

The last class of integral membrane proteins comprises members of immunoglobulin superfamily proteins and includes JAM and coxsackie and adenoviral serotype 2/5 receptor (CAR) (Martin-Padura et al., 1998). JAM has one N-terminal extracellular region (containing two extracellular Ig-like domains), one transmembrane region and one C-terminal cytoplasmic tail with a PDZ-binding motif. The JAM–PDZ interaction anchors

Table 1
Tight junction (TJ) associated proteins.

Name	Structural features	Major functions
<i>Transmembrane proteins</i>		
Claudins	Four transmembrane domains, two extracellular loops, two cytoplasmic tails, one PDZ binding motif	Cell–cell adhesion; cation and anion selective pores formation
Occludin	Four transmembrane domains, two extracellular loops, short cytoplasmic N-terminus and long cytoplasmic C-terminus, one PDZ binding motif	Cell–cell adhesion; regulation of flux of macromolecules
Tricellulin	Homologous to occludin	Cell–cell adhesion; critical for assembly of three cells
Junctional adhesion molecules (JAMs)	Immunoglobulin superfamily, one N-terminal extracellular region containing two Ig domains, one transmembrane region, one C-terminal cytoplasmic tail with PDZ binding motif	Cell–cell adhesion; anchors TJ to actin cytoskeleton through binding to ZO-1
Coxsackie and adenoviral serotype receptor (CAR)	Immunoglobulin superfamily, single membrane spanning domain, PDZ binding motif	Mediates cell–cell binding, interacts with ZO-1 (directly or indirectly), MAGI-1 and MUPP1
<i>Cytoplasmic plaque proteins</i>		
Zonula occludens (ZO-1-3)	Three PDZ domains, one SH3 domain, one GUK domain, one actin-binding region	ZO-1 is the central structural protein of the tight junction, serves as intracellular scaffold, ZO-1-3 associate transmembrane proteins to cytoskeleton and signalling molecules; ZO-1 depletion increases flux of macromolecules
Cingulin	Homodimer, each subunit contain globular N-terminal head, long coiled-coil rod and C-terminal tail	Interacts with ZO-1, actin and myosin
Membrane associated guanylate kinase inverted (MAGI)	6 PDZ-domains, one GUK domain	Scaffolding molecule
Multi-PDZ domain protein 1 (MUPP1)	13 PDZ-domains	Binds to claudin-1 and JAM
Partitioning proteins (PAR-3/-6)	10 PDZ-domains	Binds to JAM

PDZ, Post synaptic density 95, Disc large and ZO-1 domain; GUK, guanylate kinase domain.

TJ to the actin cytoskeleton, by binding to ZO-1 and cingulin. Extracellular homophilic and heterophilic interactions of JAMs are responsible for paracellular permeability.

3.2. Cytoplasmic plaque proteins

In addition to the integral membrane proteins, TJs are formed by several peripheral proteins localised in the cytoplasm and comprising adaptor/scaffolding proteins, signalling molecules and transcriptional regulators. Adaptor proteins form the link between the integral membrane proteins and cytoskeleton. Many members of this group of proteins contain PDZ domains, which enable multiple protein–protein interactions. ZO-1, ZO-2 and ZO-3 are members of the membrane associated guanylate kinase (MAGUK) family proteins. They share characteristic features of all MAGUK proteins: PDZ domains and an SH3 domain, as well as a carboxyl terminal containing an acidic domain and proline-rich regions. Due to their modular organisation, they function as scaffolds; they bind to transmembrane TJ proteins, the cytoskeleton and signal transduction molecules. The central role of ZO-1 in protein–protein interaction is illustrated in Fig. 3. ZO-1 interacts with claudins through its first PDZ domain, with ZO-2 or ZO-3 through its second PDZ domain, with occludin and tricellulin through the guanylate kinase (GUK) homology domain and with F-actin and α -catenin through its large C-terminal domain. In addition, via its SH3, ZO-1 domain binds to several signalling proteins such as a serin/threonine protein kinase, heat shock protein Apg2, transcription factor ZONAB (ZO-1 associated nucleic acid binding protein), and therefore regulates gene transcription and cell proliferation (Van Itallie et al., 2009). ZOs shuttle between the TJ and the nucleus, where they may regulate gene expression. The role of ZO-1 in paracellular permeability was revealed by Van Itallie et al., who found that ZO-1 depletion increases flux in the leak pathway for solutes larger than claudin pore. At the same time it does not decrease TEER values, which means that the flux through pore pathway remains unchanged (Van Itallie et al., 2009).

ZO-1 incorporates cingulin to TJ. Cingulin has a globular head domain and a coiled coil domain. It also interacts with ZO-2, ZO-3, JAM, actin and myosin (Assimakopoulos, Papageorgiou, & Charonis, 2011). In addition to ZO 1-3, several PDZ domain containing proteins such as membrane-associated guanylate kinase inverted (MAGI-1, MAGI-2, MAGI-3),

multi-PDZ domain protein 1 (MUPP1), partitioning-defective (PAR-3 and PAR-6) and PALS-1-associated tight junction (PATJ) proteins are involved in TJ formation (Hamazaki, Itoh, Sasaki, Furuse, & Tsukita, 2002). The roles of the above mentioned proteins are still under investigation; however, their association with TJs has been confirmed.

Cytoplasmic plaque proteins also recruit regulatory and signalling proteins to TJ. Signalling molecules involved in TJ formation play a role in maintenance of epithelial barrier integrity. They include the Rho family of small GTPases, protein kinases and protein and lipid phosphatases, amongst others (Terry et al., 2010). In addition, transcription

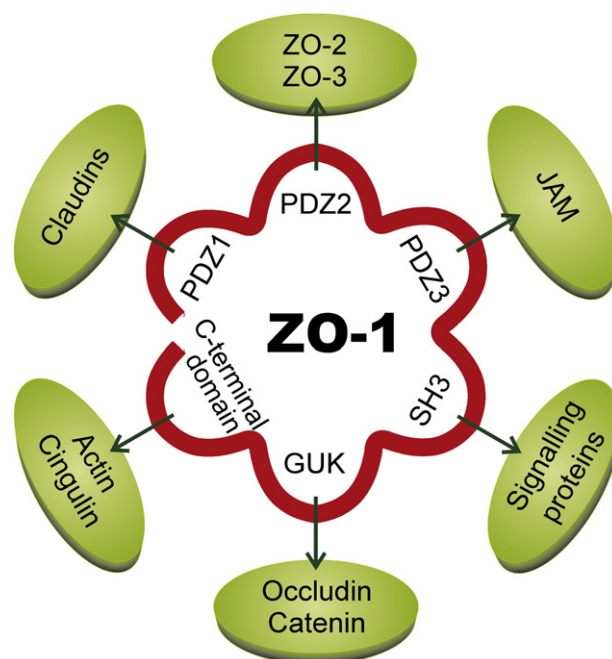


Fig. 3. Schematic diagram of interactions of ZO-1 (zonula occludens-1) with transmembrane, cytosolic and cytoskeletal proteins. JAM, junctional adhesion molecule; PDZ, Post synaptic density 95, Disc large and ZO-1 domain; SH3, Src homology domain; GUK, guanylate kinase domain.

factors are also associated to TJs by scaffolding proteins *i.e.* ZONAB, a transcription factor involved in cell growth.

3.3. Cytoskeletal proteins

ZO-1, ZO-2, ZO-3 and cingulin bind to F-actin. The actin cytoskeleton, in association with myosin II, forms a belt encircling the cell, known as PAMR (Rodgers & Fanning, 2011).

3.4. Adherens junctions

AJs are formed by interactions between transmembrane proteins, intracellular adaptor proteins and the cytoskeleton. The predominant interactions are the cadherin–catenin interactions. The calcium dependent adhesion molecules, E-cadherins, are single transmembrane spanning glycoproteins with intracellular C-terminus and extracellular N-terminus. The extracellular domain forms homotypical interactions with the cadherin of neighbouring cells. The intracellular domain of cadherin binds to catenin, which links the AJ to the cytoskeletal network. It is possible that this is achieved by a direct binding to the C-domain of F-actin or indirectly through interactions with another adaptor protein, specifically, afadin. Cadherin–catenin complexes are important for linking adjacent cells but also for maintaining cell polarity, regulating epithelial migration and proliferation (Hartsock & Nelson, 2008).

4. Modulation of TJ

TJ has a dynamic and complex multiprotein structure. The modulation of paracellular permeability must always be considered from two distinct points of view. Firstly, TJs play an important role in the pathogenesis of Crohn's disease, inflammatory bowel disease, ulcerative colitis and food allergies, and TJ can tighten for the therapeutic reason of diminishing the risk of disease onset (Assimakopoulos *et al.*, 2011; Förster, 2008; Groschwitz & Hogan, 2009). Secondly, intentional opening of paracellular space can be applied to increase the absorption of poorly permeable drugs and bioactive compounds (Deli, 2009; Kang, Cho, Shim, Kim, & Lee, 2009; Salama, Eddington, & Fasano, 2006; Ward *et al.*, 2000). TJ is selectively permeable to certain hydrophilic molecules. The paracellular route of absorption is much less efficient than any other pathway. However, increased paracellular permeability could be of vital importance for enhancing absorption of a wide range of poorly absorbed drugs and food substances.

The permeability of TJ is physiologically regulated (Ballard *et al.*, 1995). It can be affected by both intracellular and extracellular events. Intracellular stimuli are related to energy depletion and cAMP level changes. ATP depletion downregulates TJs, whereas cAMP reduces paracellular permeability. Extracellular events include interactions with other cellular proteins, interaction to external antigens, cytokines, oxidative stress, calcium level imbalance, amongst others (Steed *et al.*, 2010). The intestinal TJs are more permeable after a meal, presumably in order to allow hydrophilic nutrients like glucose and amino acids to pass through paracellular spaces. The change in permeability is probably induced by PAMR contractions (Rodgers & Fanning, 2011). Furthermore, cytokines, such as tumour necrosis factor α (TNF α), interferon gamma (IFN γ), hepatocyte growth factor (HGF), interleukin 4 (IL-4) and 13 (IL-13) have been reported to decrease barrier function (Al Sadi & Ma, 2007; Juuti-Uusitalo *et al.*, 2011). Even the presence of nutrients such as glucose and alanine increases permeability of high molecular weight markers (Sadowski & Meddings, 1993). This observation has led to the presumption that due to its continuous exposure to food components, functions of the intestinal epithelium might be affected or even regulated by food substances.

Numerous studies have demonstrated that the tightness of cultured epithelial monolayers can be increased or decreased by changing the expression profiles of specific protein components of the TJ

(Al-Sadi *et al.*, 2011; González-Mariscal *et al.*, 2008; Noda *et al.*, 2012; Raleigh *et al.*, 2011). Once the structure and role of individual TJ proteins was revealed, it became possible to intentionally alter their functioning. This can be achieved by down or up regulation of specific genes responsible for the expression of individual TJ proteins, or affecting signalling pathways. It has been reported that a pore pathway is dependent on claudin expression, whereas flux through a leak pathway is sensitive to cytoskeletal disruption and can be enhanced by proinflammatory cytokines without altering the pore pathway (Anderson & Van Itallie, 2009; Shen *et al.*, 2011). Signalling pathways involved in TJ regulation, and interactions between transmembrane proteins and the PAMR are controlled by several signalling proteins including PKC, MAPK, MLCK and the Rho family of small GTPases. Phosphorylation of TJ proteins and displacement (contraction or relaxation) of the PAMR have been shown to affect epithelial barrier function (Ward *et al.*, 2000).

4.1. Food components as absorption enhancers

4.1.1. Plant extracts

Screening studies of food extracts to determine their effect on TJ integrity have been carried out. Table 2 compiles data of food related modulators of TJ, both enhancing and decreasing epithelial permeability. As early as in 1994 Hashimoto *et al.* examined aqueous extracts of 32 different kinds of vegetable (Hashimoto *et al.*, 1994). From this vast selection, only sweet pepper and ginger extract significantly decreased the TEER values. However, in this study the effect on LY flux was not evaluated. It should be mentioned that ginger reduced viability of the cells whereas sweet pepper did not. Thus, only sweet pepper extract was suggested to contain substances that increase the TJ permeability without exerting cytotoxic effect on cells. More than 300 food materials such as vegetables, fruits, seaweeds, teas, spices, and other edible plants were examined by Konishi in the context of their effect on TEER value and LY flux (Konishi, 2003). Aqueous extracts of galangal, marigold, Nikko maple and hops were able to decrease TEER and simultaneously increase LY flux, without exerting adverse effects on cell viability. However, linden, star anise, black tea and dwarf sugar palm extracts strengthened the barrier integrity. Unfortunately, quite often the effect of food components on intestine permeability was not the main objective of the studies carried out, and the results available resulted from side observations, which were not further approached. In the course of absorption studies some authors noticed considerably lowered TEER values as a result of the application of food components or extracts on Caco-2 cell monolayers. However, the reasons and mechanisms of this phenomenon were not further studied. Digested tomato and mango juice as well as dried tomato, mango and papaya caused significant decreases in the TEER values (Epriliati, D'Arcy, & Gidley, 2009). Also Laitinen *et al.* (2004) reported that herb extracts affected paracellular diffusion of compounds through Caco-2 monolayers. The authors suggested that the herb extracts caused partial opening of paracellular spaces between Caco-2 cells. At the same time no effect of bilberries, cowberries, and raspberry phenolic compound extracts on Caco-2 TEER was observed. Similarly, the addition of grapefruit juice did not affect Caco-2 monolayer integrity measured by TEER, whereas pummelo juice addition increased TEER value and lime and lemon juices addition resulted in a drop of TEER associated with decreased cell viability (Xu, Go, & Lim, 2003). Ginger, carrot and shimeji (edible mushroom) aqueous extracts decreased TEER value by 40–50% without toxic effects (Eguchi, Murakami, & Ohigashi, 2005).

4.1.2. Isolated food components

Lectins isolated from Japanese jack bean and wheat germ resulted in significantly decreased TEER values and increased calcium ions, isoflavones and quercetin glycosides transport across epithelium (Ohno, Naganuma, Ogawa, & Muramoto, 2006). Capsaicin, a major

Table 2

Food related modulators of tight junction integrity.

Modulating substance	Concentration	Effect on TEER value	Effect on macromolecule flux	Reference
<i>Plant extracts</i>				
Sweet pepper	5 mg/mL	↓	n.e.	Hashimoto et al. (1994)
Galangal, marigold, maple Nikko, hops	5 mg/mL	↓	↑ LY	Konishi (2003)
Linden, star anise, black tea, dwarf sugar palm	5 mg/mL	↑	↓	Konishi (2003)
Mango juice, dried tomato, mango, papaya digests	–	↓	↑ mannitol	Epriliati et al. (2009)
Sage, rosemary, oregano	1 mg/mL	↓	↑ mannitol	Laitinen et al. (2004)
Ginger, carrot and <i>shimeji</i>	0–25%	↓	n.e.	Eguchi et al. (2005)
Aloe leaves	0.5–2.0%	↓	↑ atenolol, ↑FITC dextran	Chen et al. (2009); Lebitsa et al. (2012)
Apple extract and digest	–	↑	n.e.	Vreeburg et al. (2012)
<i>Isolated food components</i>				
Lectins	0–180 µg/mL	↓	n.e.	Ohno et al. (2006)
Capsaicin	100–500 µM	↓	n.e.	Isoda et al. (2001); Tsukura et al. (2007)
Taurine	0.5–2.0%	↓	n.e.	Cho et al. (2002)
Sinomenin	0.5–2.0%	↓	n.e.	Lu et al. (2010)
Nondigestible saccharides	50–100 mM	↓	↑ LY	Suzuki and Hara (2004); Suzuki et al. (2010)
Glutamine	–	↑	n.e.	Li et al. (2004)
Curcumin	5 µM	↑	n.e.	Al Sadi and Ma (2007); Rapin and Wiernsperger (2010)
Casein peptide	0.001–0.1 mM	↑	n.e.	Yasumatsu and Tanabe (2010)
Sodium caprate	10 mM	↑	n.e.	Soderholm et al. (1998)
<i>Phenolic compounds</i>				
Chrysin	100 µM	↓	↑ FITC dextran	Noda et al. (2012)
Luteolin, daidzein, genistein, hesperetin, naringenin, morin	100 µM	↑	~ FITC dextran	Noda et al. (2012)
Ferulic, isoferulic and <i>p</i> -coumaric acids	20–100 µM	↑	n.e.	Bergmann et al. (2009)
ECG	50–200 µM	↓	↑ mannitol	Vaidyanathan and Walle (2003)
Green tea extract	276 µg/mL	↓ ^a	n.e.	Zhang et al. (2006)
EC and EGC	>300 µM	↓	n.e.	Chan et al. (2007)
A-type procyanidins from cranberries	0.3–1 mM	↓	n.e.	Ou et al. (2012)
Hexameric procyanidins	20 µM	↑	n.e.	Erlejan et al. (2006)
Procyanidins	1 mM	↑	n.e.	Déprez et al. (2001)
Quercetin, quercitrin	100–200 µM	↑	~ mannitol	Amasheh et al. (2008); Suzuki and Hara (2009)
Quercetin, myrecitin	10–100 µM	↑	↓ LY	Suzuki and Hara (2009)
Kaempferol	10–100 µM	↑	~LY, FITC dextran	Suzuki et al. (2011)

EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; FITC dextran, fluorescein isothiocyanate dextran; LY, lucifer yellow; TEER, transepithelial electrical resistance.

↓ decrease.

↑ increase.

~ not influenced.

n.e. not evaluated.

^a In the case of secretion transport.

component of hot pepper caused reversible opening of the paracellular route (Isoda et al., 2001; Tsukura et al., 2007). Taurine, naturally occurring in meat and seafood, decreased TEER value and enhanced transport of heparin disaccharide. It has been suggested that it affected the level of intercellular Ca^{2+} , which plays a role in regulation of TJ by activation of protein kinase C (Cho et al., 2002). Sinomenine, an alkaloid extracted from the stem of *Sinomenium acutum*, significantly decreased TEER values. Therefore, transepithelial transport of vitamin C, luteolin, rutin and insulin was significantly increased. This effect was rapidly reversed after removal of sinomenine (Lu, Chen, Viljoen, & Hamman, 2010).

Leaves of two aloe species increased TEER values as well as transport of atenolol (Chen, Lu, Viljoen, & Hamman, 2009; Lebitsa, Viljoen, Lu, & Hamman, 2012). The ability to open TJ was assigned to polysaccharides present in aloe. It is worth mentioning that the opening was fully reversible. Nondigestible saccharides have also been reported to increase paracellular absorption of Ca^{2+} both in Caco-2 model (Suzuki & Hara, 2004) and in the small intestine of rat (Suzuki et al., 2010). The mechanism of action was studied in the second case, and it was reported that epilactose activates paracellular Ca^{2+} absorption through the induction of MLC phosphorylation and thus, contractions of the PAMR in the intestinal cells.

4.1.3. Miscellaneous

Yeast cells have been reported to increase the permeability of Caco-2 cell monolayers in a non-toxic and reversible manner (Fuller, Duckham,

& Wood, 2007). The suggested mode of action was linked with translocation of ZO-1 and occludin associated proteins from the membrane to cytoskeletal cell areas, which could involve protein kinase C activation.

4.2. Food components as enhancers of barrier function

Glutamine and curcumin have been reported to have a therapeutic effect on 'leaky gut' syndrome, by decreasing paracellular permeability (Rapin & Wiernsperger, 2010). Deprivation of glutamine decreased claudin-1, occludin, and ZO-1 protein expression (Li, Lewis, Samuelson, Liboni, & Neu, 2004). However, at the same time, a disappearance of perijunctional claudin-1 and a reduction of occludin was observed, but surprisingly no effect on ZO-1 was reported. In turn, curcumin prevented IL-1 β induced changes in intestinal epithelial permeability (Al Sadi & Ma, 2007). The effect of apple digest and crude extract on Caco-2 barrier function was investigated by Vreeburg et al. (2012). They observed an increase in paracellular resistance of the Caco-2 monolayer, which was not evoked by phosphokinase A, phosphokinase C, phosphokinase G, myosin light chain kinase or intracellular calcium signalling. The authors concluded that increase in resistance was due to increased expression of claudin-4. Another study demonstrated that casein peptide Asn-Pro-Trp-Asp-Gln enhanced epithelial barrier function (TEER) due to the ability to up-regulate occludin expression (Yasumatsu & Tanabe, 2010).

4.3. Phenolic compounds as modulators of intestinal permeability

Phenolic compounds are mainly found in fruits, vegetables and cereals, as well as in tea and wine (Aaby, Ekeberg, & Skrede, 2007; Kosińska et al., 2012; Wu, Xu, Hérítier, & Andlauer, 2012). Their health benefits have been widely studied, and attributed mainly to their antioxidant activities (Blomhoff, Carlsen, Andersen, & Jacobs, 2006; Boyer & Liu, 2004; Seeram, 2008; Seeram et al., 2006). However, in recent years, it has been pointed out that they usually occur in systemic circulation at a concentration much lower than required for effective antioxidant activity. Thus, it has been acknowledged that their biological functions are mainly related to their ability to affect enzyme activities as well as signalling molecules, nuclear receptors and gene expression (Gonzalez et al., 2011; Sharma et al., 2010; Son, Camandola, & Mattson, 2008; Vreeburg, Bastiaan-Net, & Mes, 2011; Weinreb, Amit, Mandel, & Youdim, 2009). Accordingly, the ability of phenolic compounds to affect TJ functioning can be assumed. In general, phenolic compounds cannot be easily classified as permeability or barrier function enhancers, since they may exert both effects on barrier integrity.

Phenolic acids, such as ferulic, isoferulic and *p*-coumaric acids have been found to increase initial TEER values of colon epithelial cell monolayers (Bergmann, Rogoll, Scheppach, Melcher, & Richling, 2009). Flux of macromolecular marker was not measured. Moreover, the above mentioned phenolic acids, their respective esters (4-*p*-coumaroylquinic acid, 1-caffeoylquinic acid, and 5-caffeoylquinic acid) and investigated flavonoids such as quercetin, quercetin 3-*O*-rhamnoside, phloretin, phloretin 2'-*O*-glucoside, and phloretin 2'-*O*-xyloglucoside besides the flavan-3-ols such as (–)-epicatechin and (+)-catechin were able to reverse the TJ opening effect of capric acid. The authors reported direct induction of the expression of genes encoding the TJ components: ZO-1, occludin and claudin-4 by ferulic and isoferulic acids.

The effect of seven flavonoids: chrysin, luteolin, daidzein, genistein, hesperetin, naringenin, and morin on TJs was investigated by Noda et al. (Noda et al., 2012). Chrysin enhanced transepithelial permeability which was reflected by decreased TEER and at the same time increased flux of conjugated dextran of average molecular weight of 4000 Da. It was consistent with the effect of chrysin on TJ protein expression *i.e.* occludin, JAM-1 and claudin-1, -3 and -4 in the cells incubated with chrysin was lower than in control cells. On the contrary, daidzein, hesperetin, morin and naringenin significantly increased TEER, but showed no significant change of dextran flux. Genistein and luteolin normalised TEER after its transient decrease. The effect of quercetin and myricetin on epithelial barrier properties was demonstrated by Suzuki and Hara (2009). Both compounds increased TEER of Caco-2 monolayers and lowered LY flux. Quercetin enhanced barrier function through the assembly of ZO-2, claudin-1 and occludin resulting from direct inhibition of PKC δ . The activity of PKC isoforms plays a role in phosphorylation of TJ proteins, which is one of the most potent ways of influencing TJ functioning (Deli, 2009; Ward et al., 2000). Also Amasheh et al. reported enhanced barrier properties of Caco-2 cell monolayers as a result of quercetin and quercetrin treatment (Amasheh et al., 2008). However, the observed effect was brought about by induced claudin-4 expression. At the same time, claudin-1, -3, -7 and occludin expression remained unchanged. Similarly, kaempferol-mediated increment of TEER has been related to the promotion of the cytoskeletal association and expression of ZO-1, ZO-2, occludin, claudin-1, claudin-3, and claudin-4 (Suzuki et al., 2011).

Vaidyanathan and Walle noticed increased permeability of mannitol and decreased TEER value at higher concentration of ECG. As an explanation, the authors suggested the opening of TJ (Vaidyanathan & Walle, 2003). In another example, loading of green tea extract to the basal side of transwell system led to a significant drop of TEER value, indicating the effect on the monolayer integrity (Zhang, Chow, & Zuo, 2006). No effect was observed when the same

concentration was loaded at the apical side and no cytotoxicity was noted. Damage of the integrity of monolayers may have occurred with greater loading concentration of EC and EGC, concurrently showing a drop in TEER values (Chan, Zhang, & Zuo, 2007). Similarly, A-type procyanidins of cranberries has been found to cause a decrease in TEER values. The authors did not pursue the issue and excluded those monolayers from the experiment (Ou, Percival, Zou, Khoo, & Gu, 2012). Shojí et al. (2006) reported that high molecular weight polymers from apples positively influenced the absorption of PC oligomers in rats, although they were not absorbed themselves. Similar effects of tetramers on absorption of procyanidin B2 by rats was observed by Appeldoorn, Vincken, Gruppen, & Hollman (2009). Also Kosińska and Andlauer (2012) reported that incubation with cocoa procyanidins enhanced Caco-2 monolayer permeability, reflected in increased LY flux. Conversely, Erlejan, Fraga, and Oteiza (2006) observed a significant increase in TEER value as a result of incubation with hexameric fraction of procyanidins, which at the same time did not affect transcellular and paracellular transport in Caco-2 monolayers. Moreover, procyanidins protected Caco-2 monolayers from bile acids induced cytotoxicity and alterations in TJ protein (ZO-1) distribution and barrier integrity. Similarly, Déprez et al. showed increased TEER as an effect of addition of procyanidins (Déprez et al., 2001). Striking changes in the actin cytoskeleton of the polarised intestinal epithelial cell line Caco-2 upon cranberry treatment has been revealed but the mechanism of this phenomenon was not characterised (Harmidy, Tufenkji, & Gruenheid, 2011).

5. Concluding remarks

The paracellular flux of molecules is very limited, and involves mainly hydrophilic molecules. A wide range of bioactive compounds, including a large percentage of phenolic compounds, might be classified as hydrophilic, and their low intestinal absorption has been demonstrated (Déprez et al., 2001; Scalbert et al., 2000; Shoji et al., 2006). Small changes in the tightness of paracellular spaces can significantly affect the permeability of paracellularly permeating molecules (Laitinen et al., 2004). The idea of influencing TJ integrity to increase the absorption of beneficial compounds is intriguing. Opening of TJs must be safe; for this reason, food extracts and food components are of high interest. Simultaneously, TJ opening has to be reversible and occur at a suitable time to diminish the risk that the opening of TJs for a molecule of interest results in the enhanced transport of undesirable molecules, toxins or pathogens across protective barriers of the gastrointestinal tract. The need of adequate cell models to study TJ modulations is acute and undeniable.

It appears that efforts to transiently weaken the paracellular barrier in order to enable the passage of macromolecules could aim to affect the leak pathway rather than the pore pathway. As discussed in the preceding sections, the leak pathway is associated with ZO-1 and actomyosin changes. Two main possible mechanisms of action could be suggested: direct targeting of TJ proteins or signalling pathways regulating TJ function. The final effect of modulation of these signalling pathways is the phosphorylation of TJ proteins or the contraction/relaxation of the PAMR. Nevertheless, the mechanism of TJ opening due to the action of food components is still to be explored in order to enable its target application of increasing absorption of beneficial compounds. This aspect is worth considering in the design of functional food, since the idea of TJ modulation as an approach to modify intestinal absorption of bioactive compounds is extremely promising.

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