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Polyphenols and Intestinal Permeability: Rationale and Future Perspectives

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ABSTRACT: Increasing evidence links intestinal permeability (IP), a feature of the intestinal barrier, to several pathological or dysfunctional conditions. Several host and environmental factors, including dietary factors, can affect the maintenance of normal IP. In this regard, food bioactives, such as polyphenols, have been proposed as potential IP modulators, even if the mechanisms involved are not yet fully elucidated. The aim of the present paper is to provide a short overview of the main evidence from *in vitro* and *in vivo* studies supporting the role of polyphenols in modulating IP and briefly discuss future perspectives in this research area.

KEYWORDS: *polyphenols, intestinal permeability, in vitro studies, animal studies, human studies*

INTRODUCTION

Over the last 10 years, there has been significant research effort to investigate the central role of gut function and properties in the promotion of human health and/or the development of several pathological conditions.

The intestine is the main organ involved in the absorption of nutrients and water, and it is the largest area of contact with environmental factors. It contains a large number of specialized immune cells that can coordinate with defensive responses that prevent or counteract exposure of the host and its immune system to luminal antigens of different origins (e.g., microbial and dietary origin).[1]

The definition and specific ontology related to the gut as a complex anatomical and functional system has been widely debated. Bischoff et al.[2] defined the intestinal barrier (IB) as a functional entity separating the gut lumen from the inner host and consisting of mechanical elements (mucus and epithelial layer), humoral elements [defensines and immunoglobulin A (IgA)], immunological elements (lymphocytes and innate immune cells), and muscular and neurological elements. Differently, intestinal permeability (IP), which contributes to the regulation of solute and fluid exchange between the lumen and tissues, should refer to a key feature of IB that is measurable as a whole or at a given site (e.g., evaluating specific molecule/factor flux rates). IP evaluation can be used to address a normal/stable or disturbed/compromised permeability related with IB function.[2] In this context, it is fundamental to underline that IB integrity and functionality can be affected also by the characteristics of the intestinal microbial ecosystem and mucosal immune system.

From an anatomical point of view, a well-organized monolayer of epithelial cells is required to form a selective permeability system mainly controlled by the transcellular and paracellular pathways.[3]

While the absorption and/or transport of nutrients (i.e., sugars, amino acids, vitamins, fatty acids, and minerals) occur through specific transporters or membrane channels (transcellular path),[3] a complex system of junctions crucial for the transport between adjacent cells [i.e., tight junction (TJ), gap junction (GJ), adherens junction (AJ), and desmosomes] constitutes the paracellular path.[4]

TJs have a composite molecular structure consisting of multiple protein complexes (with more than 50 proteins identified) that include a series of transmembrane tetraspan proteins, named occludin, claudins, and tricellulin, able to develop fibrils crossing the membranes and creating a connection with adjacent cell proteins. In addition, single-span transmembrane proteins are included and are mostly

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represented by junctional adhesion molecules (JAMs, belonging to the immunoglobulin superfamily). The claudin proteins are considered to be the structural pillar of TJs.[5] Specifically, TJ sealing, fundamental to avoid paracellular permeability, is provided by claudin-1, -3, -4, -5, and -8, while claudin-2 can form charge-selective pores. Less information is available for the specific activities of claudins-7, -12, and -15 and occludin.[6]

The transmembrane proteins strictly interact with the intracellular scaffold proteins, such as zonula occludens (ZO-1, ZO-2, and ZO-3) and cingulin tight fitting the actin cytoskeleton. In particular, increased paracellular permeability is activated by perijunctional actomyosin ring contraction induced by myosin light-chain kinase (MLCK). In addition, other signaling proteins, including protein kinase C (PKC) and mitogen-activated protein kinase (MAPK), together with phosphorylation are involved in the regulation pathways of assembly, disassembly, and maintenance of TJ-specific properties.[7] Finally, AJs, together with desmosomes and GJs located beneath the TJs, are involved in the cell-to-cell adhesion and intracellular signaling but do not seem to contribute to paracellular permeability.[8]

By considering the complex interplay of functions and activities of TJ proteins and signals regulating the fluxes/exchanges of molecules between the lumen and the environment, it is clear that TJ barrier integrity is essential for human health and metabolic homeostasis.

In fact, an impairment or defect in IB function can lead to modest (i.e., subclinical) but chronic immune system activation that might contribute to the pathogenesis of intestinal diseases, such as inflammatory bowel disease,[4] celiac disease,[9] intestinal bowel syndrome,[10] and colon cancer.[11] In addition, recent research showed a possible correlation of IB dysfunction with several clinical conditions, such as metabolic syndrome, obesity, non-alcoholic fatty liver disease (NAFLD),[12] diabetes,[13] and inflammatory joint diseases,[14] but also neurological conditions, such as major depression and degenerative disorders, such as Parkinson's disease[15] and multiple sclerosis (MS), involving the central nervous system (CNS).[16]

It is noteworthy that emerging experimental evidence suggests that an alteration of IB function and/or increased IP can actually occur also during aging, thus potentially representing a further mechanism underpinning the activation of the low-grade systemic inflammation process (also named inflammaging) identified in older subjects.[17] The alterations can take place at different levels of the IB, for example, induced by impairment of the epithelium (physical barrier) and/or the immune cell/function or by an alteration of the chemical barrier consisting of the thick mucus layer able to reduce the passage of bacteria through the epithelium (i.e., mucin secretion) or as a result of an inefficient/inadequate microbial barrier (represented by the commensal "protective" bacteria). In this regard, it has been demonstrated that age-associated microbial dysbiosis can increase gut microbiota lipopolysaccharide (LPS) production and promote IP with an increased risk of systemic endotoxemia and inflammation. In particular, bacteria LPS has been demonstrated to activate nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) by triggering the toll-like receptor 4 (TLR4) inflammatory cascade in immune cells (e.g., macrophages and monocytes).[18]

In addition, dysbiosis is not only an age-associated characteristic but can also be found in different clinical conditions associated with inflammation (e.g., obesity, diabetes, and NAFLD).

Thus, intestinal microbiota can be considered a critical regulator of the IP. Gut microorganisms may act directly on IP by affecting TJ properties and activities and indirectly by modulating inflammation, which is a well-recognized factor promoting IP impairment.[19] Consequently, the manipulation of the complex intestinal microbial ecosystem has been proposed as a novel strategy to restore IP.[2]

DIET AND IP

An adequate nutritional status is fundamental to maintain normal IB function (being able to affect all of the components of IB), and accordingly, malnutrition is associated with increased IP.[20] For example, Guerriero et al.[21] showed that a depletion of glutamine, tryptophan, and zinc could lead to increased IP.

Overall, it has been demonstrated that dietary patterns are a dominant factor in shaping the intestinal microbiota.[22] Hence, strategies to modify the relative abundance of specific bacterial groups by means of dietary interventions have been proposed with the aim also to modulate the concentrations of microbial metabolites in the gut, affecting inflammation.[23]

It has been demonstrated that the Western diet, characterized by high-energy and high-fat intake or high-fructose consumption, can alter IP by affecting the gut microbiota composition.[21] In addition, this dietary pattern often involves the consumption of food components, such as specific fatty acids, alcohol, additives, gliadin, and chitosan, and food-processing methods that are known to alter IB physical structure homeostasis and/or commensal microbial homeostasis. On the other hand, a healthy dietary pattern, such as the Mediterranean diet (MD), rich in fruit, vegetables, legumes, and unrefined cereals, has been suggested to positively affect IP and related conditions.[21] This may be related to an increased production of short-chain fatty acids (SCFAs), including acetate, propionate, butyrate, and valerate,[2] by gut commensal bacteria following fiber degradation provided by the MD dietary pattern. These metabolites have been suggested to play an important role as substrates for a functional colonic epithelium and the maintenance of the IB. For example, butyrate was shown to affect TJ integrity but also inhibit tumor necrosis factor α (TNF-α) release and inflammation.[23] In addition, butyrate has shown to increase expression of claudin-1 and ZO-1, to reverse the aberrant expression of ZO-1 and decrease LPS translocation, leading to inhibition of macrophage activation and pro-inflammatory cytokine production.[24] Moreover, plant-based dietary patterns, including MD, are also commonly abundant in bioactive compounds, such as polyphenols (PPs), that have been recently in the spotlight of research for their potential modulatory properties with respect to IP.[25]

RATIONALE FOR PP CONTRIBUTION TO A PROTECTIVE DIETARY PATTERN IN THE CONTEXT OF IP

PPs are secondary metabolites of plants, widely distributed in fruits, vegetables, and plant-derived foods. A diet rich in fruits, vegetables, and plant-based beverages has been estimated to provide about 1 g of PPs/day,[26] with significant variations depending also upon the extent of consumption of beverages rich in PPs (tea, wine, coffee, and fruit juices). The basic monomer in PPs is the phenolic ring. Phenols can be mainly classified into phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (flavons, flavanones, flavanols, flavonols, isoflavones, and anthocyanidins), stilbenes (i.e.,

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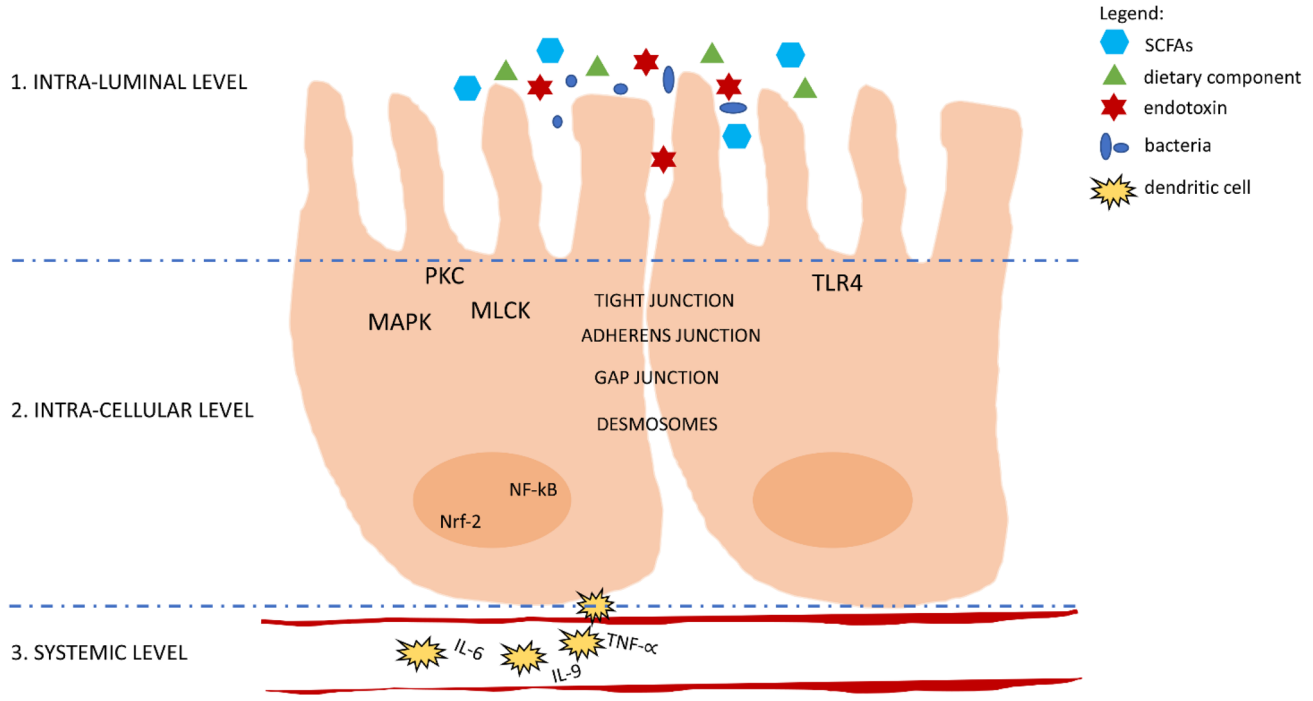

Figure 1. Putative effects of PPs on IP at different physiological levels: (1) intraluminal level, modulation of microbiota composition, endotoxin and/or SCFA production, redox status, and dietary component absorption and/or activity; (2) intracellular level, regulation of expression of TJ, AJ, GJ, and desmosome proteins, upregulation of kinases and Nrf-2, and dowregulation of NF-κB and TLR4; and (3) systemic level, maintenance of the functional immune system and regulation of inflammatory processes (toward a reduced pro-inflammatory status).

resveratrol), and lignans. PPs are recognized to be poorly bioavailable, rapidly absorbed, and extensively metabolized by gut microbiota.[27] Additional biotransformation can occur in the liver and kidneys through methylation, glucuronidation, and sulfation reactions of phenolic hydroxyl groups;[28] for these reasons, the concentration of the native compounds in the blood is low compared to their metabolic derivates (from nanomoles up to micromoles per liter).

PPs and their metabolites are widely studied for their numerous biological activities, including antimicrobial, antiproliferative, antioxidant, and anti-inflammatory functions.[29] These effects are exerted at both intestinal and systemic levels. In particular, PPs may exert their effects by downregulating inflammatory genes (i.e., NF-κB) and upregulating cytoprotective and antioxidant genes [i.e., nuclear factor erythroid 2-related factor 2 (Nrf-2)]. This modulation may bring a reduction of cytokine production [e.g., interleukin (IL)-8, IL-1β, and TNF-α] and boost the bodies' own antioxidant status [heme oxygenase 1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GPx)].[30] Furthermore, recent reviews[31,32] have shown that PPs may affect, in either a positive or negative way, pattern recognition receptors, such as toll-like receptors and nucleotide-binding oligomerization domain proteins, whose activation in epithelial cells may lead to intestinal inflammation. Moreover, PPs seem to be involved in the regulation of epigenetic factors through interaction with the enzymes responsible for DNA methylation and acetylation by reducing intestinal inflammation.[32]

Several studies documented the effects of PPs in the modulation of the intestinal microbial ecosystem. However, the mechanisms by which these compounds modulate the gut microbiota remain unclear. Some studies report that the interaction between PPs and microbiota may involve interference with enzymatic expression and activity and modulation of specific pathways related to antioxidant and anti-inflammatory activity.[33] In addition, PPs has been proposed to exert a prebiotic effect potentially inhibiting the pathogenic bacteria and stimulating the growth of beneficial microbes.[34−36] In fact, the microbiota can extensively metabolize PPs in numerous derivatives that could affect not only the composition of microbiota but also specific signaling pathways.[33] Another important aspect regards the possible involvement of PPs in the metabolism of colonic products, such as SCFAs, sterols (cholesterol and bile acids), and microbial products of non-absorbed proteins, which may directly or indirectly counteract or suppress pro-oxidant and/or pro-inflammatory responses with an overall improvement of gut health.[37]

To unravel the complex scenario related with PP−microbiota interaction *in vivo*, a combination of metabolomic, microbiome, and metagenomic approaches is strongly demanded.[33]

Finally, in the last few decades, specific research has been devoted to the evaluation of PPs as promising protective factors and regulators of epithelial homeostasis and the IB function. In particular, a direct/indirect effect of the regulation of TJ proteins has been investigated.

MECHANISMS OF PP REGULATION OF IP

At present, the exact mechanisms linking PPs with the intestinal epithelial barrier function have not yet been established (Figure 1). Some studies hypothesized a direct/indirect involvement of NF-κB signaling in the onset of IP. This pathway is recognized as one of the most important mediators of the inflammation; cytokines and interleukins have been shown to activate NF-κB and impair the epithelial barrier function by TJ disassembly. Conversely, PPs have documented to block NF-κB activation by inhibiting IκB kinase (IKK) phosphorylation and/or preventing proteasomal degradation of IκB.[38]

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Table 1. Summary of the Main *In Vitro* Studies Highlighting the Mechanisms of Action of PP Compounds in the Modulation of Barrier Integrity and Function

reference	cells	stimulation	PP source and dose	signaling pathway	response/marker	effect
Atkinson and Rao[40]	Caco-2	acetaldehyde	genistein (30−300 μM)	↓ tyrosine kinase	TEER,[a] occludin, and ZO-1[b]	↑ TEER ↑ occludin ↑ ZO-1
Watson et al.[59]	T84	IFN-γ[c]	epigallocatechin gallate (100 μM)	↓ STAT-1[d] ↓ MAPK[e]	TEER	↑ TEER
Amasheh et al.[51]	Caco-2		quercetin (0−200 μM)	↓ MLCK[f] and PKC[g]	TEER, occludin, claudin-1, claudin-3, claudin-4, and claudin-7	↑ TEER ↑ claudin-4 = claudin-1 = claudin-3 = claudin-7 = occludin
Suzuki and Hara[52]	Caco-2		quercetin (0−100 μM)	↓ PKCδ	ZO-2, occludin, claudin-1, and claudin-4	↑ ZO-2 ↑ occludin ↑ claudin-1 ↑ claudin-4
Amasheh et al.[60]	HT29/B6	TNF-α[h]	berberine (50 μM)	↓ NF-κB,[i] PI3K/Akt,[j] and tyrosine kinase	claudin-1 and claudin-2	↑ claudin-1 ↓ claudin-2
Chuenkitiyanon et al.[64]	ECV304	H_2O_2[k]	quercetin (10 μM)	↓ p38[l]	ZO-1 and occludin	↑ ZO-1 ↑ occludin
Rogoll et al.[61]	T84		(+)-catechin (10 μM) (−)-epicatechin (10 μM) quercetins (10 μM) phloretins (20 μM) D-(−)-quinic acids (10−50 μM) *p*-coumaric acids (10 μM) caffeic acids (20 μM)	↓ TJ permeability	TEER, ZO-1, occludin, and claudin-4	↑ TEER ↑ ZO-1 ↑ occludin ↑ claudin-4
Shin et al.[66]	HCT-116		anthocyanin mixture (45 μg/mL; delphinidin, cyanidin, petunidin, delphinidin, malvdin, peonidin-3,5-diglucoside, cyanidin, petunidin, peonidin, and malvidin-3-glucoside)	↑ p38	TEER, claudin-1, claudin-3, and claudin-4	↑ TEER ↓ claudin-1 ↓ claudin-3 ↓ claudin-4
Suzuki et al.[53]	Caco-2		kaempferol (100 μM)	↓ TJ permeability	TEER, ZO-1, ZO-2, occludin, claudin-1, claudin-3, and claudin-4	↑ TEER ↑ occludin ↑ claudin-1 ↑ claudin-3 ↑ claudin-4 ↑ ZO-1 ↑ ZO-2
Noda et al.[54]	Caco-2		chrysin, daidzein, genistein, hesperetin, luteolin, morin, and naringenin (100 μM)	↓ TJ permeability	TEER, ZO-1, ZO-2, JAM1, claudin-1, claudin-3, and claudin-4	↑ TEER (negative effect for chrysin) effect on TJ proteins was compound-dependent
Amasheh et al.[62]	HT-29/B6	IFN-γ and TNF-α	quercetin (200 μM)	↓ TJ permeability	TEER, claudin-1, claudin-2, claudin-3, claudin-4, claudin-7, and occludin	↑ TEER ↓ claudin-2 ↓ claudin-3 = claudin-1

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Table 1. continued

reference	cells	stimulation	PP source and dose	signaling pathway	response/marker	effect
						= claudin-4
						= claudin-7
						= occludin
Noda et al.[55]	Caco-2		naringenin (100 μM)	↑ Sp1[m]-dependent transcriptional regulation	TEER, ZO-1, ZO-2, occludin, JAM-A,[n] claudin-1, claudin-3, and claudin-4	↑ TEER
				↓ TJ permeability		↑ claudin-1
						↑ claudin-4
						↑ occludin
						= ZO-1
						= JAM-A
Cao et al.[56]	Caco-2	IFN-γ and TNF-α	berberine (100 μM)	↓ MLCK	occludin, claudin-1, ZO-1, and IP	↑ occludin
						↑ claudin-1
						↑ ZO-1
						↓ IP
Carrasco-Pozo et al.[57]	Caco-2	indomethacin	mix of quercetin (33 μM), resveratrol (438 μM), rutin (164 μM), and epigallocatechin gallate (218 μM)	↑ epithelial barrier function	TEER, FD4,[o] ZO-1, and occludin	↑ TEER (no effect with rutin)
						↓ FD4 (no effect with rutin)
						↑ ZO-1 after quercetin
						↑ occludin after quercetin
Piegholdt et al.[58]	Caco-2	TNF-α	biochanin A (50 μM) and prunetin (50 μM)	↓ NF-κB, ERK,[p] and tyrosine kinase	TEER, claudin-1, occludin, ZO-1, and E-cadherin	↑ TEER
						= claudin-1
						= ZO-1
						= E-cadherin
Park et al.[41]	Caco-2		theaflavin-3′-*O*-gallate (20 μM)	↓ MLCK	occludin, claudin-1, and ZO-1	↑ occludin
						↑ claudin-1
						↑ ZO-1
Contreras et al.[42]	Caco-2	TNF-α	(−)-epicatechin (0.5−5 μM)	↓ NF-κB, p-IKKα,[q] p-IkBα, and MLCK	occludin, ZO-1, and claudin-2	↑ ZO-1
						= occludin
						= claudin-2
Valenzano et al.[43]	Caco-2		berberine (50−200 μM) quercetin (100−400 μM)	↑ epithelial barrier function	TEER, claudin-1, claudin-2, claudin-3, claudin-4, claudin-5, claudin-7, occludin, tricellulin, and D-mannitol	↑ TEER (only berberine) quercetin (↑ claudin-2, claudin-4, and claudin-5 and ↓ tricellulin) berberin (↓ claudin-2 and D-mannitol)
Ling et al.[65]	IPEC-J2	deoxynivalenol	resveratrol (0−200 μM)	↓ p38, ERK, and p-JNK[r]	TEER, FD4, claudin-1, claudin-3, claudin-4, claudin-7, occludin, and ZO-1	↑ TEER
						↑ occludin
						↑ claudin-3
						↑ claudin-4
						↓ FD4
						= claudin-1
						= claudin-7
Wang et al.[44]	Caco-2		PP-rich propolis extract (25 and 50 μg/mL)	↑ AMPK-α,[s] ERK1/2, Akt, and p38	ZO-1 and occludin	↑ TEER
						↑ occludin
						↑ ZO-1

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Table 1. continued

reference	cells	stimulation	PP source and dose	signaling pathway	response/marker	effect
Azzini et al.[45]	Caco-2		three different PP-rich extracts from chicory (0.2, 1.3, 10, 17, 34, and 70 μM)	↑ epithelial barrier function	TEER	↑ TEER
Luescher et al.[38]	Caco-2	TNF-α	xanthohumol (chalcone, 10 μM) and isoxanthohumol (prenylflavone, 10 μM)	↓ TJ permeability	TEER	↑ TEER
Cremonini et al.[46]	Caco-2	TNF-α	cyanidin, delphinidin, malvidin, petunidin, or peonidin-3-*O*-glucoside (0.25−1 μM)	↓ IKK and p65 phosphorylation	TEER	↑ TEER (only cyanidin, delphinidin, and ACN-rich plant extracts)
		IFN-γ	crowberry extract (1−10 μg/mL)			
			anthocyanin-rich plant extracts [black chokeberry, black kernel rice, wild blueberry, bilberry, crowberry, domesticated blueberry, and red grape (5 μg/mL)]			
Rybakovsky et al.[47]	Caco-2	^{14}C-D-mannitol	theaflavins (5−20 μg/mL)	↑ membrane permeability	claudin-1, claudin-2, claudin-4, and claudin-5	↑ TEER (quercetin)
			quercetin (100−400 μM)			↓ transepithelial mannitol permeability (quercetin)
			berberine (50−200 μM)			↑ claudin-2
						= claudin-1
						= claudin-4
						= claudin-5
Van Buiten et al.[48]	Caco-2		decaffeinated green tea PPs (0−100 μg/mL)	↓ paracellular permeability	TEER, IL-6,[t] and IL-8	↑ TEER
						↓ IL-6
						↓ IL-8
Li et al.[67]	MODE-K	LPS[u]	naringin (50−200 μM)	↓ NF-κB and MLCK/MLC	TNF-α, IL-10, IL-6, MLCK, p-MLC/MLC, p-p65/p65, and p-IkBα/IkBα	↓ TNF-α
						↓ IL-10
						↓ IL-6
						↓ MLCK
						↓ p-MLC/MLC
						↓ p-p65/p65
						↓ p-IkBα/IkBα
Cremonini et al.[49]	Caco-2	TNF-α	(−)-epicatechin	↑ ERK1/2, and AMPK and ↓ NF-κB	NOX1/NOX4,[v] FITC[w]−dextran transport, and TEER	↑ TEER
						↓ FITC
						↓ NOX1/NOX4
Vazquez-Olivo et al.[50]	Caco-2		four PP-rich mango extracts (100 μg/mL)	↑ membrane permeability	Papp	↑ improvement of apparent membrane permeability
			gallic acid (100 μg/mL)			
Nunes et al.[63]	HT-29	TNF-α, IL-1, and IFN-γ	non-alcoholic polyphenolic red wine extract (catechin, oligomeric procyanidins, anthocyanin, phenolic acids, ethyl cinnamate, and condensed tannin), 200, 400, and 600 μg/mL	↓ paracellular permeability	occludin, claudin-5, and ZO-1	↑ occludin
						↑ claudin-5
						↑ ZO-1

[a]TEER = transepithelial eletrical resistance. [b]ZO-1 = zonula occludens 1. [c]IFN-γ = interferon γ. [d]STAT-1 = signal transducer and activator of transcription 1. [e]MAPK = mitogen-activated protein kinase. [f]MLCK/MLC = myosin light-chain kinase. [g]PKC = protein kinase C. [h]TNF-α = tumor necrosis factor α. [i]NF-κB = nuclear factor κB. [j]PI3K/Akt = phosphoinositide 3-kinase. [k]H_2O_2 = hydrogen peroxide. [l]p38 = p38 pathway. [m]SP-1 = specific protein transcription factor 1. [n]JAM-A = junctional adhesion molecule A. [o]FD4 = fluorescein isothiocyanate-labeled dextran. [p]ERK1/2 = extracellular signal-regulated kinases 1 and 2. [q]p-IKKα = phosphorylated IκB kinase α. [r]JNK = c-Jun N-terminal kinase. [s]IL-6, IL-8, and IL-10 = interleukins 6, 8, and 10. [t]AMPK = AMP-activated protein kinase. [u]LPS = lipopolysaccharide. [v]NOX = nicotinamide adenine dinucleotide oxidase. [w]FITC = fluorescein isothiocyanate.

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Other important factors potentially involved in increasing IP are the multiple protein kinases, such as MAPK, phosphoinositide-3-kinase (PI3K)/Akt, PKC, tyrosine kinase, MLCK, and adenosine monophosphate (AMP)-activated protein kinase (AMPK). Most of them are regulators of fundamental biological processes in epithelial cells, including barrier function, primarily through regulating TJ expression. Some PPs (e.g., quercetin, curcumin, epigallocathechin 3-gallate, and myricetine) have shown to improve the epithelial barrier function through the inhibition of different kinases (PKC and MLCK) involved in phosphorylation of target proteins controlling IP.[3,30,39]

To ascertain the availability of data supporting the role of PPs on IP, a literature search has been performed using the following terms "intestinal permeability" OR "intestinal barrier" AND "polyphenols" OR "bioactives" OR "phenolics" as keywords in PubMed. The use of the word "polyphenols" as a specific keyword consistently reduced the number of results. On the contrary, a more appropriate search with single PP subclasses AND "intestinal permeability" provided a larger number of *in vitro* and animal studies mainly summarized in Table 1 and 2 and an apparent lack of human intervention studies.

***In Vitro* Studies.** The main lines of evidence on the *in vitro* effects of PPs in the modulation of the potential mediators and regulatory pathways involved in the IP are reported in Table 1. Most of the studies are performed on the Caco-2 cell line,[38,40−58] as a model of the IB, followed by T84 and HT29/B6 cells (colonic adenocarcinoma cell line),[59−63] IPEC-J2 cells (intestinal porcine enterocytes), and ECV304 cells (human endothelial cell line).[64,65] The main evidence of protection is available for berberine, quercetin, and catechin tested in a range of concentrations between 10 and 200 μM (from physiological to pharmacological concentrations). Other PPs tested included genistein, anthocyanins, resveratrol, theaflavin, and a mix of PPs. Most the studies have shown an increase in transepithelial electrical resistance (TEER) across a cellular monolayer, confirming the integrity and functional permeability of the membranes.[38,43−49,53−55,57,58,62,65,66] In addition, most the PPs tested have shown to increase the expression and/or production of numerous TJ proteins, including ZO-1, occludin, and the family of claudins, whose alteration may result in increased paracellular permeability.[41,42,44,53,55−57,63,65] Finally, some studies have reported the capacity of PP to counteract the inflammatory process induced by TNF-α and interferon γ (IFN-γ) downregulating the expression of several interleukins, such as IL-8 and IL-6.[48,67]

Animal Studies. Table 2 reported the effects of PPs and PP-rich extracts in the modulation of IP in animal models.[44,49,67−74] Most of the studies were performed in healthy rat models (i.e., Wistar rats and Sprague Dawley rats), and IP was induced by stimuli, such high-fat diets, mannitol, inflammatory cytokines, or chemicals.[44,72,74] Two studies used mice with IL-10 deficiency to test the effect on IP.[69,70]

The main PPs used were obtained from grape seed extracts (1% GSE; grams of GSE per gram of dry food weight)[69,70] and grape seed proanthocyanidin extracts (5−50 mg/kg).[74] Other studies included berberine (200 mg/kg),[68] (−)-epicatechin (2−20 mg/kg),[49] and epigallocatechin-3-gallate (about 3 mg/mL).[73] Some studies were performed by testing anthocyanin-rich raspberry extract, PP-rich propolis extract, and oregano essential oil.[44,72] The doses administered ranged from nearly physiological (epicatechin) up to supraphysiological (i.e., berberine). The duration of the intervention varied from a few days (3−10 days) up to several weeks (15−16 weeks).

On the whole, the results obtained support an improvement of IP following the intervention with PPs and PP-rich extracts. In particular, the studies showed the capacity of PPs to upregulate some important genes, such as AMPK and ERK, and downregulate NF-κB as pathways involved in the inflammation process. In line with the observations reported in the *in vitro* studies, the compounds tested have shown to increase the expression of ZO-1, occludin, and several claudins involved in the functioning of TJs.

Human Studies. The number of human intervention studies with IP as primary or secondary outcome increased in the last few years, as also documented by the number of trials made available and reported in public registers (i.e., ISRCTN and ClinicalTrial.gov).

Most of these studies were performed or are ongoing using probiotics, prebiotic fibers, dietary supplements, and sugars. Only four studies seem to have explored the potential beneficial effects of PPs/PP-rich foods on IP in humans (Table 3).[75−78] The studies differ in terms of population (overweight/obese, cyclists, and older subjects), foods administered (green tea, flavonoid-rich beverage, and mix of PP-rich foods), dose of bioactives (650 mg of flavonoids and 750 mg of PPs), duration of intervention (from 2 weeks up to 8 weeks), and marker of IP selected (endotoxin, lactulose/mannitol ratio, and zonulin levels). The trials are still ongoing, and the results will be useful to increase the understanding on the actual role of PPs and PP-rich foods in humans, where a large number of factors can interact, affecting IP. For example, it is well-recognized that PPs are poorly bioavailable and are biotransformed by gut microbiota into metabolites that can be absorbed in the colon. At the same time, PPs may modulate the composition of the gut microbial community, shaping toward protective symbionts and reducing pathobionts. The complex and not fully elucidated two-way interaction between PPs and gut microbiota is postulated to play a potential direct/indirect role on IP regulation.

In this context, the MaPLE project (Microbiome mAnipulation through Polyphenols for managing gut Leakiness in the Elderly) has been developed with the aim to test the hypothesis that changing the diet of older subjects with established enhanced IP by increasing their PP consumption can alter the intestinal microbial ecosystem (IME) in a way that is beneficial for IB function, resulting in reduced IP and decreased translocation of inflammogenic bacterial factors from the digestive tract into the bloodstream.[78] To test this hypothesis, a multidisciplinary approach has been used (i) to evaluate the impact of a PP-rich dietary pattern on IB, IP, and IME in a target group of older subjects and (ii) to investigate the possible mechanisms involved in the PP−microbiota−IP interactions through *in vitro* and animal models.

Findings obtained from our and other studies will be "pivotal" for the development of new and advanced hypothesis and experimental approaches in this complex area of research.

SOME CONSIDERATIONS ON IP ASSESSMENTS IN DIFFERENT CONTEXTS

IP can be evaluated through numerous methodologies, and consequently, data obtained can differ among studies. The techniques vary depending upon the setting (*in vitro*, *ex vivo*, or *in vivo* models), the models (cells, animals, and humans), and the markers (i.e., ions, macromolecules, bacteria, and bacterial products) but also the compartments (i.e., tissues, blood, and urine). The measurement of IP can be performed through *ex*

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Table 2. Summary of the Main Evidence from Animal Models Reporting the Effects of PPs and PP-Rich Extracts in the Modulation of Barrier Integrity and Function

reference	animal model	diet	PP source and dose	signaling pathway	response/marker	main findings
Gu et al.[68]	male C57BL/6 mice	BBR versus C	BBR, berberine (200 mg/kg)	↓ MLCK[a]	IP	↑ ZO-1
		LPS stimulation	C, control diet		claudin-1	↑ occludin
			7 days		claudin-4	↑ claudin-1
					occludin	↑ claudin-4
					ZO-1[b]	↓ IP
Yang et al.[69]	C57BL/6 (WT) and IL-10[c]-deficient ($IL\text{-}10^{-/-}$, IL10KO) female mice	GSE versus C	GSE, grape seed extract (0 or 1% GSE)[d]	↓ NF-κB[e]	claudin-1	↑ claudin-1
		dextran sulfate sodium stimulation	C, standard rodent diet		claudin-2	↓ claudin-2
			16 weeks			
Wang et al.[70]	IL10-deficient mice (IL10KO)	GSE versus C	GSE, grape seed extract (0 or 1% GSE)[d]	↓ AMPK[f]	claudin-1	↑ claudin-1
		dextran sulfate sodium stimulation	C, standard rodent diet		claudin-2	↓ claudin-2
			16 weeks			
Li et al.[71]	BALB/c mice	ARF versus C	ARF, anthocyanin-rich raspberry extract (20 mg/kg)	↓ NF-κB	colonic histological architecture	↑ colonic histological architecture
		dextran sulfate sodium stimulation	C, saline solution as control treatment	↓ MAPKs[g]		
			10 days			
Wei et al.[72]	male Wistar rats	OEO versus C	OEO, oregano essential oil (5 or 20 mg/kg of BW)	↓ SOD[h]	ZO-1	↑ ZO-1
		diquat stimulation	C, saline solution as control treatment	↓ GSH-Px[i]	occludin	↑ occludin
			14 days			
Wang et al.[44]	male Sprague Dawley rats	PPE versus C	PPE, PP-rich propolis extract (0.3%, w/w)[d]	↑ AMPK	ZO-1	↑ ZO-1
		2,4,6-trinitrobenzenesulfonic acid stimulation	C, control diet	↑ ERK[j]	occludin	↑ occludin
			14 days			
Bitzer et al.[73]	male CF-1 mice	DSS[k] treatment + D (0.5% citric acid)	EGCG, epigallocatechin-3-gallate (3.2 mg/mL)		GLP-2[l]	↓ GLP-2
		DE (DDS + EGCG) + D (0.5% citric acid)	C, control diet		LAC/RHA[m]	↓ LAC/RHA
		C diet	3 days		SUC/ERY[n]	↓ SUC/ERY
Gil-Cardoso et al.[74]	female Wistar rats	CAF	CAF, cafeteria diet[d]		ZO-1	↑ ZO-1
		CAF + GSPE	CAF + GSPE, (cafeteria diet + grape seed proanthocyanidin extract, 5−50 mg/kg)		occludin	
		C group	C, control diet		claudin-1	
			15 weeks CAF		JAM-A[o]	
			3 weeks CAF + GSPE			
Cremonini et al.[49]	C57BL/6J mice	HF versus C	CE, (−)-epicatechin (2−20 mg/kg)	↑ ERK1/2	p65[p]	↑ p65 (HF)
		HFE20 versus CE	C, control diet	↑ NF-κB (p65)	GLP-2	↑ GLP-2 (CE and HFE20)
			HF, high-fat diet (60% total calories from fat); HFE20, high-fat diet + 20 mg/kg of epicatechin	↑ AMPK	NOX1/NOX4[q]	↑ NOX1/NOX4 (HF)
			15 weeks			
Li et al.[67]	male Kunming mice	CLP[r] + vehicle	NG, naringin (30 and 60 mg/kg)		TEM[s]	↑ survival CLP + NG (30−60)
		CLP + NG (30)	C, no control diet		FITC[t]−dextrane	↑ IM impairment CLP + vehicle
		CLP + NG (60)	24−72 h		D-lactate	CLP ↑ FITC−dextrane and D-lactate
						CLP + NG ↓ FITC−dextrane (dose-dependent)

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Table 2. continued

[a]MLCK/MLC = myosin light-chain kinase. [b]ZO-1 = zonula occludens 1. [c]IL = interleukin. [d]Data on PP characterization were not provided. [e]NF-κB = nuclear factor κB. [f]AMPK = AMP-activated protein kinase. [g]MAPK = mitogen-activated protein kinase. [h]SOD = superoxide dismutase. [i]GSH-Px = glutathione peroxidase. [j]ERK1/2 = extracellular signal-regulated kinases 1 and 2. [k]DSS = dextran sulfate sodium. [l]GLP-2 = glucagon-like peptide 2. [m]LAC/RHA = lactulose/rhamnose ratio. [n]SUC/ERY = sucralose/erythritol ratio. [o]JAM = junctional adhesion molecule. [p]p65 = transcription factor p65. [q]NOX1/NOX 4 = NADPH oxidases. [r]CLP = cecal ligation and puncture. [s]TEM = transmission electron microscopy. [t]FITC = fluorescein isothiocyanate.

vivo and *in vivo* approaches.[79] An example of an *ex vivo* approach includes the use of an Ussing chamber able to measure the transport of ions and molecules (i.e., nutrients and drugs) across various epithelial tissues using fresh intestinal tissue. *In vivo*, the assessment of IP can be performed through permeability assays (i.e., evaluation of the ratio of lactulose/mannitol, sucralose, sucrose, polyethylene glycols, or ^{52}Cr-EDTA in urine), analysis of bacterial-related markers (i.e., endotoxin test, EndoCAb, D-lactate, and butyrate production), markers of epithelial damage (i.e., citrullin, fatty-acid-binding protein, and claudin-3), and/or other related markers (i.e., fecal calprotectin). Finally, histological approaches measuring, for example, globet cell analysis, shedding of the epithelium, or Paneth cell loss, can be performed.[2]

On the whole, on the basis of revised literature, it can be assumed that current *in vitro* permeability models are still far from reflecting an *in vivo* situation. This limits the relevance of data obtained within the cell culture and the possibility to transfer the results to humans. In fact, the comparison between *in vitro* and *in vivo* permeability data is difficult and dependent upon numerous factors, including the type of cells used, the molecule under study, the transport route evaluated, and the method used for the assessment of IB function and permeability [i.e., mainly transepithelial electrical resistance (TEER) and biomarkers of epithelial integrity], which can significantly affect the results obtained, making it difficult to identify the best approach.

A novel biomarker of IP *in vivo* is zonulin, a protein secreted by enterocytes but also from other type of cells (i.e., epithelial cells), known to be a physiological modulator and, thus, to control IP reversibly via intercellular TJs.[80] Increased zonulin serum levels have been observed in many gut-related diseases, and emerging evidence suggests an increased zonulin level in specific subjects (e.g., older persons)[81] and in different diseases or conditions (e.g., diabetes and obesity).[82,83] The reliability and accuracy of the different markers to assess IP is clearly a fundamental part of the recent discussion and a hot topic considering the increasing demand for non-invasive diagnosis tools.[84] In this regard, the concurrent evaluation of different markers of IP seems highly recommendable to improve reliability of findings on IB function.

CONCLUSIONS AND FUTURE PERSPECTIVES

There is increasing demand for non-invasive strategies able to modulate critical regulatory functions for human health, such as IP, which can play a role in the pathogenesis of intestinal and systemic diseases. The improvement or manipulation of the diet, for example, increasing or reducing specific nutrients and/or including food bioactives, such as PPs, is recognized as a potential powerful tool to be explored also in the context of IP. From data available, PP activity seems to be plausibly a consequence of multiple mechanisms, which may also depend upon the type and amount of compounds considered. The results from *in vitro* studies have shown the capacity of PPs to increase the expression and/or production of numerous TJ proteins and to reduce the release of several interleukins/cytokines. These results are partially in line with the findings obtained in the animal models, showing the capacity of PPs to up-/downregulate some important genes involved in the inflammatory process. With regard to human studies, recent literature suggests that PPs may modulate IP through a number of direct and indirect effects, including the impact on the intestinal ecosystem and immune system. This type of research

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Table 3. Summary of the Ongoing Human Studies Evaluating the Effect of PPs and PP-Rich Foods on IP

title	source	subject number/characteristics and inclusion criteria	study design	intervention	duration of intervention	markers under study
dietary green tea confection for resolving gut permeability-induced metabolic endotoxemia in obese adults	ClinicalTrials.gov NCT03413735	40 overweight/obese (BMI = 28−40 kg/m^2)	randomized parallel design	test group, green tea extract (GTE)-rich confection	4 weeks	primary outcome: Endotoxin
		fasting glucose of <126 mg/dL		placebo group, no GTE-rich confection		secondary outcome, gut permeability (lactulose/mannitol ratio and sucralose/erythritol ratio)
	75	normotensive (blood pressure of <140/90 mmHg)		dose, daily (no information about the amount provided in terms of PPs)		microbiota Firmicutes/Bacteroidetes ratio)
		non-dietary supplement user				calprotectin
		non-smoker				green tea PP bioavailability
effect of flavonoids on gut permeability in cyclists	ClinicalTrials.gov	22 male or female of any race or ethnicity between 18 and 49 years of age	randomized crossover design	test group, a high-flavonoid, sports nutrition recovery beverage will be prepared from milk (78%), sugar (8.6%), maltodextrin (8.6%), blueberry powder (2.4%), cocoa powder (1.6%), green tea extract (0.1%), and whey protein isolate (0.6%) containing approximately 620 mg of flavonoids per serving	2 weeks	primary outcome, urinary lactulose/mannitol ratio
	NCT03427879	competed in a road race or triathlon in past 12 months		placebo group, a low-flavonoid, sports nutrition recovery beverage will be prepared from milk (78%), sugar (8.6%), maltodextrin (8.6%), placebo blueberry powder (2.4%), alkalized cocoa powder (1.6%), and whey protein isolate (0.6%), containing approximately 5 mg of flavonoids per serving		plasma intestinal fatty-acid-binding protein
	76	free of chronic disease and gut inflammation conditions		dose, 330 mL/day		secondary outcome, fecal calprotectin
		train at least 3 times per week, 1 h at a time on average				urinary sucralose/mannitol ratio
		willing to prepare and consume provided pre-workout beverage daily				inflammatory markers (TNF-α[b] and IL-10)[c]
		maintain weight (no more/less than 5 kg change)				endotoxin
		willing to avoid consumption of high flavonoid foods/supplements, large dose vitamin and mineral supplements, and NSAIDs[a] or other medications known to affect inflammation during the study period				other variables related to exercise performance
effect of dietary flavonoids on intestinal microbiota, intestinal inflammation and metabolic syndrome	ClinicalTrials.gov NCT02728570 77	30 overweight/obese (BMI[d] = 25−35 kg/m^2)	randomized crossover design	test group, prepared diet with high levels of dietary flavonoids (340 mg of flavonoids/1000 kcal) with a macronutrient composition of 17% energy from protein, 30% energy from fat, and 53% energy from carbohydrate	6 weeks	primary outcome, fecal calprotectin
				control group, prepared diet with high levels of dietary flavonoids (10 mg of flavonoids/1000 kcal) with a macronutrient composition of 17% energy from protein, 30% energy from fat, and 53% energy from carbohydrate		serum PCR[e]
						serum TNF-α
						serum insulin
						secondary outcome, fecal microbiome composition, SCFAs, eosinophil protein X, and myeloperoxidase
						IP by four sugar differential absorption tests
						serum endotoxin, IL-6, soluble TNFr-2,[f] and fasting glucose

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Table 3. continued

title	source	subject number/characteristics and inclusion criteria	study design	intervention	duration of intervention	markers under study
						calculated homeostatic model assessment for insulin resistance
						serum C peptide
						plasma lipid profile
						blood pressure
						other outcome measures, serum resistin, visfatin, adiponectin, and leptin
						body weight
effect of a polyphenol-rich diet on leaky gut in the elderly	ISRCTN registry	60 healthy older subjects	randomized crossover design	test group, habitual diet + PP-rich products (berries and derived products, blood oranges and derived products, pomegranate juice, Renetta apple and purée, green tea, and dark chocolate products)	8 weeks	primary outcome, zonulin serum levels
	ISRCTN10214981	age of >60 years old		control group, comparable diet without the PP-rich products		secondary outcome, total blood bacterial load
	78	IP evaluated by zonulin serum level		dose, three portions of PP-rich food products daily (about 750 mg of PPs)		fecal microbiota composition and metabolism
		adequate nutritional status evaluated with a mini nutritional assessment (MNA) score of ≥24				SCFAs and PP-derived metabolites
		good cognitive status tested with mini mental state evaluation (MMSE) score of ≥24				inflammatory, oxidative stress, and related markers
		self-sufficiency assessed with validated tests (e.g., Barthel index for activities of daily living and Tinetti balance assessment)				endotoxin
						LPS-BP[g]
						metabolomic markers
						metabolic and anthropometric markers

[a]NSAID = non-steroidal anti-inflammatory drug. [b]TNF-α = tumor necrosis factor α. [c]IL-10 = interleukin 10. [d]BMI = body mass index. [e]PCR = C-reactive protein. [f]TNFr-2 = tumor necrosis factor receptor 2. [g]LPS-BP = lipopolysaccharide-binding protein.

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is still in its infancy by considering the few human studies available. Future research should be targeted to identify the PPs and/or their metabolites eventually involved in the modulation of IP while also demonstrating their specific dose-dependent mechanisms of action. Meanwhile, *in vivo* studies should be performed to increase understanding of the diet−microbiota−IP axis possibly through the development of well-controlled dietary intervention studies. Finally, by considering the wide discussion in the literature on IP evaluation, further effort is needed to better define the reliability of the already available IP biomarkers and the potential exploitation of new and/or improved candidate biomarkers.

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ABBREVIATIONS USED

IP, intestinal permeability; IB, intestinal barrier; IME, intestinal microbial ecosystem; TJ, tight junction; GJ, gap junction; AJ, adherens junction; JAM, junctional adhesion molecule; ZO, zonula occludens; MLCK, myosin light-chain kinase; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; TLR4, toll-like receptor 4; NAFLD, non-alcoholic fatty liver disease; MS, multiple sclerosis; CNS, central nervous system; TNF-α, tumor necrosis factor α; MD, Mediterranean diet; SCFA, short-chain fatty acid; PP, polyphenol; NF-κB, nuclear factor κB; Nrf-2, nuclear factor erythroid 2-related factor 2; IL, interleukin; HO-1, heme oxygenase 1; SOD, superoxide dismutase; GPx, glutathione peroxidase; DNA, deoxyribonucleic acid; IKK, IκB kinase; PI3K, phosphoinositide-3-kinase; AMPK, AMP-activated protein kinase; TEER, transepithelial electrical resistance; INF-γ, interferon γ; ERK, extracellular regulated kinase; MAPLE, microbiome manipulation through polyphenols for managing gut leakiness in the elderly

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