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 (IB), 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 fully elucidated yet. 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.

Introduction

Over the last ten 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, epithelial layer), humoral elements (defensines, IgA), immunological elements (lymphocytes and innate immune cells), 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 molecules/factors 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 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 the paracellular pathways [3].

While the absorption and/or transport of nutrients (i.e. sugars, amino acids, vitamins, fatty acids, 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 junctions (GJ), adherent junctions (AJ), and desmosomes) constitute the paracellular path [4].

TJs have composite molecular structure consisting of multiple protein complexes (with more than 50 proteins identified) that include a series of transmembrane tetra-span proteins, named occludin, claudins and tricellulin, able to develop fibrils crossing the membranes and creating a connection with adjacent cells proteins. In addition, single span transmembrane proteins are included and are mostly represented by junctional adhesion molecules (JAM, belonging to the immunoglobulin superfamily). The claudin proteins are considered to be the structural pillar of TJ [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, -15 and occludin [6].

The transmembrane proteins strictly interact with the intracellular scaffold proteins such as zonula occludens (ZO-1, ZO-2, 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 signalling proteins, including protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) together with phosphorylation are involved in the regulation pathways of assembly, disassembly, and maintenance of TJ specific properties [7]. Finally, adherent junctions, together with desmosomes and gap junctions located beneath the TJ are involved in the cell-to-cell adhesion and intracellular signalling but seem not 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. sub-clinical) 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] up to 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] , 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 intestinal barrier: for example, induced by impairment of the epithelium (physical barrier) and/or of the immune cells/function, or by an alteration of the chemical barrier consisting in the thick mucus layer able to reduce the passage of bacteria through the epithelium (i.e. mucin secretion) or due to an inefficient/inadequate microbial barrier (represented by the commensal "protective" bacteria). In this regard, it has been demonstrated that age-associated microbial dysbiosis can promote IP and consequently inflammation. In addition, dysbiosis is not only an age-associated characteristic but it can be found in different clinical conditions associated with inflammation (e.g. obesity, diabetes, NAFLD).

Thus, intestinal microbiota can be considered a critical regulator of the IP. Gut microorganisms may act directly on IP by affecting tight junction properties and activities and indirectly by modulating inflammation, which is a well-recognized factor promoting IP impairment [18]. 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 the components of IB) and accordingly, malnutrition is associated with increased IP [19]. For example, Guerriero et al [20] 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 [21]. Hence, strategies to modify the relative abundance of specific bacterial groups by means of dietary interventions has been proposed with the aim also to modulate the concentrations of microbial metabolites in the gut e.g. butyrate affecting tight junction integrity but also inhibiting TNF-α release and inflammation [22].

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 [20]. In addition, this dietary pattern often involves the consumption of food components like specific fatty acids, alcohol, additives, gliadin, 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 [20]. This may be related to an increased production of short chain fatty acids (SCFAs) by gut commensal bacteria following fiber degradation provided by MD dietary pattern. Moreover, plant based dietary patterns including MD are also commonly abundant of bioactive compounds such as polyphenols that have been recently on the spotlight of research for their potential modulatory properties with respect to IP [23].

Rationale for polyphenols contribution to a protective dietary pattern in the context of IP

Polyphenols (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 polyphenols/day [24], with significant variations depending also on the extent of consumption of beverages rich in polyphenols (tea, wine, coffee, fruit juices). The basic monomer in polyphenols 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. resveratrol) and lignans. PPs are recognized to be poorly bioavailable, rapidly absorbed and extensively metabolized by gut microbiota [25]. Additional biotransformation can occur in liver and kidney through methylation, glucuronidation and sulfation reactions of phenolic hydroxyl groups [26] or 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 function [27]. These effects are exerted both at intestinal and systemic levels. In particular, PPs may exert their effects by down regulating inflammatory genes (i.e. nuclear factor-kB, NF-kB) and up-regulating cytoprotective and antioxidant genes (i.e. nuclear factor erythroid 2–related factor 2, Nrf-2). This modulation may bring to a reduction of cytokines production (e.g., IL-8, IL-1β, and TNF-α) and boost the bodies' own antioxidant status (HO-1, SOD, and GPx) [28]. Furthermore, recent studies have shown the capacity of PPs to modulate 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 [29].

Several studies documented the effects of PPs in the modulation of 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 anti-oxidant and anti-inflammatory activity [30]. In addition, PPs has been proposed to exert a prebiotic effect potentially inhibiting the pathogenic bacteria and stimulating the growth of beneficial microbes [31–33]. In fact, the microbiota can extensively metabolize PPs in numerous derivatives that could affect not only the composition of microbiota but also specific signalling pathways [30]. Another important aspect regards the possible involvement of PPs in the metabolism of colonic products, such as short chain fatty acids (SCFA), 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 [34].

To unravel the complex scenario related with PP-microbiota interaction in vivo, a combination of metabolomic, microbiome and metagenomic approaches are strongly demanded [30].

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

Mechanisms of polyphenols regulation of IP

At present, the exact mechanisms linking PPs with intestinal epithelial barrier function have not been established yet. Some studies hypothesized a direct/indirect involvement of nuclear factor-κB (NF-κB) signalling in the onset of intestinal permeability. This pathway is recognized as one of the most important mediators of the inflammation; cytokines and interleukins have shown to activate NF-κB and impair the epithelial barrier function by tight junction disassembly. Conversely, PPs have documented to block NF-κB activation by inhibiting IKK (kinase) phosphorylation and/or preventing proteasomal degradation of IκB [35].

Other important factors potentially involved in increasing intestinal permeability are the multiple protein kinases such as mitogen-activated protein kinases (MAPK), phosphoinositide-3-kinases (PI3K)/Akt, protein kinase C (PKC), tyrosine kinases, myosin light chain kinase (MLCK) and 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 have shown to improve the epithelial barrier function through inhibition of the activation of numerous kinases [28].

In order 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 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 **Tables (1-2)** 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 Caco-2 cell line [35–54], as a model of the intestinal barrier, followed by T84, HT29/B6 cells (colonic adenocarcinoma cell line) [55–59], IPEC-J2 cells (intestinal porcine enterocytes) and ECV304 cells (human endothelial cell line) [60,61]. The main evidence of protection are available for berberine, quercetin and catechin tested in a range of concentration between 10 and 200 μM. Other PPs tested included genistein, anthocyanins, resveratrol, theaflavin and 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 [35,39–45,49–51,53,54,58,61,62]. In addition, most the

PPs tested have shown to increase the expression and/or production of numerous TJ proteins including zonula occludens (ZO)-1, occludin, and the family of claudins whose alteration may result in increased paracellular permeability [37,38,40,49,51–53,59,61]. Finally, some studies have reported the capacity of PP to counteract inflammatory process induced by TNF-α and INF-γ down-regulating the expression of several interleukins such as IL-8 and IL-6 [44,63].

Animal studies

In **Table 2** are reported the effects of polyphenols and polyphenol-rich extracts in the modulation of IP in animal models [40,45,63–70]. Most of the studies were performed in healthy rat models (i.e. Wistar rats, Sprague-Dawley rats) and intestinal permeability was induced by stimuli such high fat diets, mannitol, inflammatory cytokines, or chemicals [45,68,70]. Two studies used mice with IL-10 deficiency in order to test the effect on intestinal permeability [65,66].

The main PPs used were obtained from grape seed extracts (1% GSE; g GSE per g dry food weight) [65,66] and grape seed proanthocyanidin extracts (5-50 mg/kg) [70]. Other studies included berberine (200 mg/kg) [64], (-)-epicatechin (2-20 mg/kg) [45] and epigallocatechin-3-gallate (about 3 mg/ml) [69]. Some studies were performed by testing anthocyanins-rich raspberry extract, polyphenol-rich propolis extract, and oregano essential oil [40,68]. The doses administered ranged from nearly physiological (epicatechin) up to supra- physiological (i.e. berberine). The duration of the intervention varied from few days (3-10 days) up to several weeks (15-16 weeks).

On the whole, the results obtained support an improvement of intestinal permeability following the intervention with PPs and PP-rich extracts. In particular, the studies showed the capacity of PPs to up-regulate some important genes such as AMPK and ERK and down-regulate NF-kB 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 zonula occludens (ZO)-1, occludin, and several claudins involved in the functioning of tight junctions.

244

245 ***Human studies***

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

249 Most of these studies were performed, or are ongoing, by using probiotics, prebiotic fibers, dietary
250 supplements, and sugars. Only 4 studies seem to have explored the potential beneficial effects of
251 polyphenols/polyphenol-rich foods on intestinal permeability in humans **(Table 3)** [71–74]. The studies
252 differ in terms of population (overweight/obese, cyclists, older subjects), foods administered (green
253 tea, flavonoid-rich beverage, mix of polyphenol-rich foods), dose of bioactives (650 mg of
254 flavonoids, 750 mg of polyphenols), duration of intervention (from 2 weeks up to 8 weeks), marker
255 of intestinal permeability selected (endotoxin, lactulose:mannitol ratio, zonulin levels). The trials
256 are still ongoing, and the results will be useful to increase understanding on the actual role of
257 polyphenols and polyphenol-rich foods in humans where a large number of factors can interact
258 affecting intestinal permeability.

259 In this context, the MaPLE project (Microbiome mAnipulation through Polyphenols for managing
260 gut Leakiness in the Elderly) has been developed with the aim to test the hypothesis that changing
261 the diet of older subjects with established enhanced IP by increasing their polyphenols consumption
262 can alter IME in a way that is beneficial for IB function, resulting in reduced IP and decreased
263 translocation of inflammogenic bacterial factors from the digestive tract into the bloodstream [74]. To
264 test this hypothesis, a multidisciplinary approach has been used (i) to evaluate the impact of a
265 polyphenol-rich dietary pattern on IB, IP and IME in a target group of older subjects; and (ii) to
266 investigate the possible mechanisms involved in the polyphenol-microbiota-IP interactions through
267 *in vitro and in 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 on the setting (in vitro, ex-vivo or in vivo models), the models (cells, animals, humans), the markers (i.e. ions, macromolecules, bacteria and bacterial products) but also the compartments (i.e. tissues, blood, urines). The measurement of IP can be performed through *ex vivo* and *in vivo* approaches [75]. An example of ex vivo approach includes the use of an Ussing chamber able to measure the transport of ions and molecules (i.e. nutrients, drugs) across various epithelial tissues by using fresh intestinal tissue. In vivo, the assessment of IP can be performed through permeability assays (i.e. evaluation of ratio lactulose/mannitol, sucralose, sucrose, polyethylene glycols or 52Cr-EDTA in urines), analysis of bacterial related markers (i.e. endotoxin test, EndoCAb, D-lactate, butyrate production), markers of epithelial damage (i.e. citrullin, fatty acid binding protein, cludin-3), and/or other related markers (i.e. faecal calprotectin). Finally, histological approaches measuring for example Globet cell analysis, shedding of epithelium or Paneth cell loss, can be performed [2].

A novel biomarker of IP 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 [76]. 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) [77] and in different diseases (e.g. diabetes, obesity) [78,79]. 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 [80].

Conclusion 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 recognised as a potential powerful tool to be explored also in the context of IP. From data available PPs activity seems to be plausibly a consequence of multiple mechanisms which may also depend on the type and amount of compounds considered. Recent literature suggests that PPs may modulate IP through a number of direct and indirect effects including the impact on intestinal ecosystem and immune system. Thus, future research should be directed to increase understanding of the diet-microbiota-intestinal permeability axis possibly through the development of well controlled dietary intervention studies. This type of research is still in its infancy by considering the few human studies available. Finally, by considering the wide discussion in literature on IP evaluation, a 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.

Abbreviations used

IP, intestinal permeability; IB, intestinal barrier; IME, intestinal microbial ecosystem; TJ, tight junction; GJ, gap junction; AJ, adherent junction; JAM, junctional adhesion molecules; ZO, zonula occludens; MLCK, myosin light chain kinase; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; NAFLD, non-alcoholic fatty liver disease; MS, multiple sclerosis; CNS, central nervous system; TNF, tumor necrosis factor; MD, mediterranean diet; SCFAs, short chain fatty acids; PPs, polyphenols; NF- kB, nuclear factor-kB; Nrf-2, nuclear factor erythroid 2–related factor 2; IL, interleukine; HO, oxigenase enzyme; SOD, superoxide dismutase; GPx, glutathione peroxidase; DNA, deoxyribonucleic acid; IKK, ikB-kinase; PI3K, phosphoinositide-3-kinases; 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.

Funding: This work was developed as part of the MAPLE project (Gut and blood microbiomics for studying the effect of a polyphenol-rich dietary pattern on intestinal permeability in the elderly) supported within the European Joint Programming Initiative "A Healthy Diet for a Healthy Life" (JPI- HDHL - http://www.healthydietforhealthylife.eu/) granted by Mipaaft (Italy; D.M. 8245/7303/2016), MINECO (Spain, PCIN-2015-238), BBSRC (UK, BB/R012512/1). CAL thanks 2017SGR1546 from the Generalitat de Catalunya's Agency AGAUR, funds from CIBERFES (co-funded by the FEDER Program from EU) and ICREA Academia award 2018.

Acknowledgments:

European Cooperation for Science and Technology (COST Action) CA16112 "NutRedOx: Personalized Nutrition in Aging Society: Redox Control of Major Age-related Diseases" is acknowledged.

Table 1- Summary of the main in vitro studies highlighting the mechanisms of action of polyphenol compounds in the modulation of barrier integrity and function

Reference	**Cells**	**Stimulation**	**Polyphenol source and dose**	**Signaling Pathway**	**Response/Marker**	**Effect**
Atkinson and Rao 2001 [36]	Caco-2	Acetaldehyde	Genistein (30–300 μM)	↓ tyrosine kinase	TEER, occludin, ZO-1	↑ TEER ↑ occludin ↑ ZO-1
Watson et al., 2004 [55]	T84	IFN-γ	Epigallocatechin gallate (100 μM)	↓STAT-1 ↓MAPK	TEER	↑ TEER
Amasheh et al., 2008 [47]	Caco-2	-	Quercetin (0-200 μM)	↓MLCK, PKC	TEER, occludin, claudin-1, claudin-3, claudin-4, claudin-7	↑ TEER ↑ claudin-4 = claudin-1 = claudin-3 = claudin-7 = occludin

Suzuki and Hara 2009 [48]	Caco-2	-	Quercetin (0-100 µM)	↓ PKCδ	ZO-2, occludin, claudin-1, claudin-4	↑ ZO-2 ↑ occludin ↑ claudin-1 ↑ claudin-4
Amasheh et al., 2010 [56]	HT29/B6	TNF-α	Berberine (50 µM)	↓ NF-Kb, PI3K/Akt, tyrosine kinase	Claudin-1, claudin-2	↑ claudin 1 ↓ claudin 2
Chuenkitiyanon et al., 2010 [60]	ECV304	H_2O_2	Quercetin (10 µM)	↓p38	ZO-1, occludin	↑ ZO-1 ↑ occludin
Rogoll et al., 2010 [57]	T84	-	(+)-Catechin (10 µM) (-)-epicatechin (10 µM) Quercetins (10 µM) Phloretins (20 µM)	↓Tight junction permeability	TEER, ZO-1, occludin, claudin-4	↑TEER ↑ ZO-1 ↑ occludin ↑ claudin-4

			D-(-)-quinic acids (10-50 µM) p-coumaric acids (10 µM) caffeic acids (20 µM)			
Shin et al., 2011 [62]	HCT-116	-	Anthocyanin mixture (45 µg/mL; delphinidin, cyanidin, petunidin, delphinidin, malvdin, peonidin-3,5-diglucoside, cyanidin, petunidin, peonidin, malvidin-3-glucoside)	↑p38	TEER, claudin-1, claudin-3, claudin-4	↑ TEER ↓ claudin 1 ↓ claudin 3 ↓ claudin 4
Suzuki et al., 2011 [49]	Caco-2	-	Kaempferol (100 µM)			↑ TEER

				↓Tight junction permeability	TEER, ZO-1, ZO-2, occludin, claudin-1, claudin-3, and claudin-4	↑ occludin ↑ claudin 1 ↑ claudin 3 ↑ claudin 4 ↑ ZO-1 ↑ ZO-2
Noda et al., 2012 [50]	Caco-2	-	Chrysin, daidzein, genistein, hesperetin, luteolin, morin, and naringenin (100 μM)	↓Tight junction permeability	TEER, ZO-1, ZO-2, JAM1, claudin-1, claudin-3, claudin-4	↑ TEER (negative effect for chrysin) Effect on tight junction proteins was compound dependent
Amasheh et al., 2012 [58]	HT-29/B6	IFN-γ, TNF-α	Quercetin (200 μM)	↓Tight junction permeability	TEER, claudin-1, claudin-2, claudin-3, claudin-4,	↑ TEER ↓claudin-2 ↓claudin-3

					claudin-7, occludin	= claudin-1 = claudin-4 =claudin-7 =occludin
Noda et al., 2013 [51]	Caco-2	-	Naringenin (100 µM)	↑Sp1-dependent transcriptional regulation ↓Tight junction permeability	TEER, ZO-1, ZO-2, occludin, JAM-A, claudin-1, claudin-3, claudin-4	↑ TEER ↑claudin-1 ↑claudin-4 ↑occludin = ZO-1 = JAM-A
Cao et al., 2013 [52]	Caco-2	IFN-γ, TNF-α	Berberine (100 µM)	↓MLCK	Occludin, claudin-1, ZO-1, intestinal permeability	↑ Occludin ↑ claudin-1 ↑ ZO-1 ↓ intestinal permeability

Carrasco-Pozo et al., 2013 [53]	Caco-2	Indomethacin	Mix of quercetin (33µM), resveratrol (438µM), rutin (164µM), epigallocatechin gallate (218µM)	↑epithelial barrier function	TEER, FD4, ZO-1, occludin	↑TEER (no effect with rutin) ↓FD4 (no effect with rutin) ↑ ZO-1 after quercetin ↑ occludin after quercetin
Piegholdt et al., 2014 [54]	Caco-2	TNF-α	Biochanin A (50 µM), prunetin (50 µM)	↓ NF-Kb, ERK, tyrosine kinase	TEER, claudin 1, occludin, ZO-1, E-cadherin	↑ TEER = claudin 1 = ZO-1 = E-cadherin
Park et al., 2015 [37]	Caco-2	-	Theaflavins-3'-0-gallate (20 µM)	↓MLCK	Occludin, claudin-1, ZO-1	↑occludin ↑claudin-1

						↑ZO-1
Contreras et al., 2015 [38]	Caco-2	TNF-α	(-)-Epicatechin (0.5–5 μM)	↓ NF-Kb, p-IKKα, p-IkBα, MLCK	Occludin, ZO-1, claudin-2	↑ZO-1 = occludin =claudin-2
Valenzano et al., 2015 [39]	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, D-mannitol	↑ TEER (only berberine) Quercetin (↑claudin 2, claudin-4, claudin-5, ↓tricellulin) Berberin (↓claudin-2, D-mannitol)
Ling et al., 2016 [61]	IPEC-J2	Deoxynivalenol	Resveratrol (0-200 μM)			↑ TEER

				↓p38, ERK, p-JNK	TEER, FD4, Claudin-1, Claudin-3, Claudin-4, Claudin-7, occludin, ZO-1	↑ occludin ↑ claudin-3 ↑ claudin-4 ↓FD4 = claudin-1 = claudin-7
Wang et al., 2016 [40]	Caco-2	-	Polyphenol-rich propolis extract (25 and 50 µg/mL)	↑AMPK-α, ERK1/2, Akt, p38	ZO-1, occludin	↑ TEER ↑ occludin ↑ ZO-1
Azzini et al., 2016 [41]	Caco-2	-	3 different polyphenol-rich extracts from Chicory (0.2, 1.3, 10, 17, 34, 70 µM)	↑epithelial barrier function	TEER	↑TEER

Luescher et al., 2017 [35]	Caco-2	TNF-α	Xanthohumol (chalcone; 10 μM), isoxanthohumol (prenylflavone; 10 μM)	↓Tight junction permeability	TEER ↑TEER
Cremonini et al., 2017 [42]	Caco-2	TNF-α IFN-γ	cyanidin, delphinidin, malvidin, petunidin, or peonidin- 3-O-glucoside (0.25–1 μM) crowberry extract (1–10 μg/mL) ACN-rich plant extracts (black chokeberry, black kernel rice, wild blueberry, bilberry, crowberry, domesticated	↓IKK and p65 phosphorylation	TEER ↑TEER (only cyanidin and delphinidin, and ACN-rich plant extracts)

			blueberry, red grape (5 μg/mL))			
Rybakovsky et al., 2017 [43]	Caco-2	^{14}C–D-mannitol	Theaflavins (5-20 μg/mL) Quercetin (100-400 μM) Berberine (50-200 μM)	↑ membrane permeability	Claudin-1,claudin-2, Claudin-4, claudin-5	↑TEER (quercetin) ↓Transepithelial Mannitol Permeability (quercetin) ↑ claudin-2 = claudin-1 = claudin-4 = claudin-5
Van Buiten et al., 2018 [44]	Caco-2	-	Decaffeinated green tea polyphenols (0-100 μg/mL)	↓paracellular permeability	TEER, IL-6, IL-8	↑TEER ↓IL-6 ↓IL-8

Li et al., 2018 [63]	MODE-K	LPS	Naringin (50-200 μM)	↓NF-kB, MLCK/MLC	TNF-α, IL-10, IL-6, MLCK, p-MLC/MLC, p-p65/p65, p-IkBα/IkBα	↓TNF-α ↓ IL-10 ↓ IL-6 ↓ MLCK ↓ p-MLC/MLC ↓ p-p65/p65 ↓ p-IkBα/IkBα
Cremonini et al., 2018 [45]	Caco-2	TNF-α	(-)-Epicatechin	↑ERK1/2, AMPK, ↓NF-kB	NOX1/NOX4, FITC-dextran transport, TEER	↑TEER ↓FITC ↓NOX1/NOX4
Vazquez-Olivo et al., 2019 [46]	Caco-2	-	4 polyphenol-rich mango extracts (100 μg/mL) Gallic acid (100 μg/mL)	↑ membrane permeability	Papp	↑Improvement of apparent membrane permeability

Nunes et al., 2019 [59]	HT-29	TNF-α, IL-1, IFN-γ	Non-alcoholic polyphenolic red wine extract (catechin, oligomeric procyanidins, anthocyanin, phenolic acids, ethyl cinnamate, condensed tannin); 200, 400 and 600 µg/mL	↓ paracellular permeability	Occludin, claudin-5, ZO-1	↑ occludin ↑ claudin-5 ↑ ZO-1

Legend:

ACN, anthocyanin; TNF-α, tumor necrosis factor alpha; IL-(1-10), interleukin-(1-10); IFN-γ, interferon gamma; LPS, Lipopolysaccharide; H_2O_2, hydrogen peroxide; NF-kB, nuclear factor-kB; MLCK/MLC, myosin light-chain kinase; ERK1/2, extracellular signal–regulated kinases; AMPK, 5' AMP-activated protein kinase; p38, p38 pathway; JNK, c-Jun N-terminal kinases, p-IKKα, IκB kinase α; TEER, trans-epithelial eletrical resistance; PI3K/Akt, phosphoinositide 3-kinase; PKC, protein kinase C; STAT-1, signal transducer and activator of transcription 1; MAPK, mitogen-activated

protein kinases; NOX, nicotinamide adenine dinucleotide oxidase; FITC, fluorescein; FD4, fluorescein isothiocyanate-labeled dextrans; ZO-1, zonula occludens; JAM-A, junctional adhesion molecule-A; Sp-1, specific protein transcription factor-1.

Table 2- Summary of the main evidence from animal models reporting the effects of polyphenols and polyphenol-rich extracts in the modulation of barrier integrity and function

Reference	Animal model	Diet	Polyphenol source and dose	Signaling Pathway	Response/Marker	Main findings
Gu et al., 2011 [64]	Male C57BL/6 mice	BBR *vs* C LPS-stimulation	BBR: berberine (200 mg/kg) C: control diet 7 days	↓MLCK	Intestinal permeability Claudin-1 Claudin-4 Occludin ZO-1	↑ ZO-1 ↑ occludin ↑Claudin-1 ↑Claudin-4 ↓intestinal permeability
Yang et al., 2014 [65]	C57BL/6 (WT) and IL-10-de□cient (IL-10-/-,	GSE *vs* C dextran sulfate sodium-stimulation	GSE: grape seed extract (0 or 1% GSE C: standard rodent diet 16 weeks	↓NF-kB	Claudin-1 Claudin-2	↑claudin-1 ↓claudin-2

	IL10KO) female mice					
Wang et al., 2013 [66]	IL10-deficient mice (IL10KO)	GSE *vs* C dextran sulfate sodium-stimulation	GSE: grape seed extract (0 or 1% GSE C: standard rodent diet 16 weeks	↓AMPK	Claudin-1 Claudin-2	↑claudin-1 ↓claudin-2
Li et al., 2014 [67]	BALB/c mice	ARF *vs* C dextran sulfate sodium-stimulation	ARF: Anthocyanin-rich raspberry extract (20 mg/kg) C: Saline solution 10 days	↓NF-kB ↓MAPKs	Colonic histological architecture	↑ colonic histological architecture

Wei et al., 2015 [68]	Males Wistar rats	OEO vs C Diquat-stimulation	OEO: oregano essential oil (5 or 20 mg/kg BW) C: saline solution 14 days	↓SOD ↓GSH-Px	ZO-1 occludin	↑ ZO-1 ↑ occludin
Wang et al., 2016 [40]	Male Sprague-Dawley rats	PPE *vs* C 2,4,6-trinitrobenzenesulfonic acid stimulation	PPE: Polyphenol-rich propolis extract (0.3% w/w) C: control diet 14 days	↑AMPK ↑ERK	ZO-1 occludin	↑ ZO-1 ↑ occludin
Bitzer et al 2016 [69]	Male CF-1 mice	DSS treatment D(0.5% citric acid) DE (0.5% citric acid and EGCG)	EGCG: epigallocatechin-3-gallate (3,2 mg/ml) C: control diet	--	GLP-2 LAC/RHA SUC/ERY	↓ GLP-2 ↓ LAC/RHA ↓ SUC/ERY

		C-diet 3 days				
Gil-Cardoso et al 2017 [70]	Female Wistar rats	CAF CAF+GSPE C-group	CAF: cafeteria diet CAF+GSPE: (cafeteria diet + grape seed proanthocyanidin extract 5- 50 mg/kg) C: standard diet 15 weeks CAF 3 weeks CAF+GSPE	--	ZO-1 Occludin Claudin-1 JAM-A	↑ZO-1
Cremonini et al 2018 [45]	C57BL/6J mice	HF *vs* C HFE20 *vs* CE	CE: (-)-epicatechin (2-20 mg/kg) C: control diet 15 weeks	↑ERK1/2 ↑NF-kB (p65) ↑AMPK	p65 GLP-2 NOX1/NOX4	↑ p65 (HF) ↑ GLP-2 (CE and HFE20) ↑ NOX1/NOX4 (HF)

Li et al 2018 [63]	Male Kunming mice	CLP + vehicle CLP+ NG (30) CLP+ NG (60)	Naringin (30 mg/kg and 60 mg/kg) 24 - 72 h	--	TEM FITC-dextrane D-lactate	↑survival CLP +NG (30-60) ↑IM Impairment CLP + Vehicle CLP↑ FITC-dextrane and D-lactate CLP + NG ↓ FITC-dextrane (dose-dependent)

Legend: NF-kB, nuclear factor-kB; MLCK/MLC, myosin light-chain kinase; ERK1/2, extracellular signal–regulated kinases; AMPK, 5' AMP-activated protein kinase; ZO-1, zonula occludens; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; C, control; HF, high fat diet; HFE20, high fat diet + 20 mg/kg epicatechin;

GLP-2, glucagon-like peptide-2; NOX1/NOX 4, NADPH oxidases; CLP, cecal ligation and puncture; NG, naringin; TEM, transmission electron microscopy; CAF, cafeteria diet; GSPE, grape seed proanthocyanidin; ZO, zonula occludens; JAM, junctional adhesion molecule; DSS, dextran sulfate sodium; EGCG, (-)-epigallocatechin-3-gallate; LAC/RHA, lactulose/rhamnose ratio; SUC/ERY, sucralose/erythritol ratio

Table 3- Summary of the ongoing human studies evaluating the effect of polyphenols and polyphenol-rich food on intestinal permeability

Title	**Source**	**Subject number/characterstics Inclusion criteria**	**Study design**	**Intervention**	**Duration of intervention**	**Markers understudy**
Dietary green-tea confection for resolving gut permeability-induced metabolic endotoxemia in obese adults	ClinicalTrials.gov NCT03413735 [71]	40 Overweight/obese (BMI = 28-40 kg/m^2) Fasting glucose < 126 mg/dL Normotensive (blood pressure < 140/90 mmHg) Non-dietary supplement user Non-smoker	Randomized parallel design	Test group: green tea extract (GTE)-rich confection Placebo group: no green tea extract-rich confection Dose: daily (no information about the amount provided in term of polyphenols)	4 weeks	Primary outcome: Endotoxin Secondary outcome: Gut Permeability (Lactulose to Mannitol Ratio, and Sucralose to Erythritol Ratio) Microbiota (Firmicutes to Bacteroidetes Ratio)

						Calprotectin Green tea polyphenol bioavailability
Effect of flavonoids on gut permeability in cyclists	ClinicalTrials.gov NCT03427879 [72]	22 Male or female of any race or ethnicity between 18 to 49 years of age Competed in a road race or triathlon in past 12 months	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%),	2 weeks	Primary outcome: Urinary lactulose:mannitol ratio Plasma intestinal fatty acid binding protein Secondary outcome:

Free of chronic disease and gut inflammation conditions Train at least 3 times per week, 1 hour at a time on average Willing to prepare and consume provided pre-workout beverage daily Maintain weight (no more/less than 5 kg change) Willing to avoid consumption of high flavonoid foods/supplements,	cocoa powder (1.6%), green tea extract (0.1%), whey protein isolate (0.6%) containing approximately 620 mg flavonoids per serving. <u>Placebo group:</u> a low flavonoid, sports nutrition recovery beverage will be prepared from milk (78%), sugar (8.6%),	Fecal calprotectin Urinary sucralose:mannitol ratio Inflammatory markers (TNF-α, IL-10) Endotoxin Other variables related exercise performance

large dose vitamin and mineral supplements, and NSAIDs or other medications known to affect inflammation during study period	maltodextrin (8.6%), placebo blueberry powder (2.4%), alkalized cocoa powder (1.6%), whey protein isolate (0.6%), containing approximately 5mg flavonoids per serving Dose: 330 mL/ day

Effect of dietary flavonoids on intestinal microbiota, intestinal inflammation and metabolic syndrome	ClinicalTrials.gov NCT02728570 [73]	30 Overweight/obese (BMI = 25-35 kg/m^2)	Randomized crossover design	<u>Test group:</u> Prepared diet with diet high levels of dietary flavonoids (340 mg of flavonoids/1000 Kcals) with a macronutrient composition of 17% en from protein, 30% en from fat and 53% energy from carbohydrate <u>Control group:</u>	6 weeks	<u>Primary outcome:</u> Fecal calprotectin Serum PCR Serum TNF-α Serum insulin <u>Secondary outcome:</u> Fecal microbiome composition, short chain fatty acids, eosinophil protein X, myeloperoxidase Intestinal permeability by four

Prepared diet with diet high levels of dietary flavonoids (10 mg of flavonoids/1000 Kcals) with a macronutrient composition of 17% en from protein, 30% en from fat and 53% energy from carbohydrate	sugar differential absorption test
	Serum endotoxin, IL-6, soluble TNFr-2, fasting glucose
	Calculated Homeostatic Model Assessment-Insulin Resistance
	Serum C-peptide
	Plasma lipid profile
	Blood pressure

						Other Outcome Measures: Serum resistin, visfatin, adiponectin, leptin Body weight
Effect of a polyphenol-rich diet on leaky gut in the elderly	ISRCTN registry ISRCTN10214981 [74]	60 healthy older subjects Age > 60 years old Intestinal Permeability evaluated by Zonulin serum level	Randomized crossover design	Test group: habitual diet + polyphenol-rich products (berries and derived products, blood oranges and derived products, pomegranate	8 weeks	Primary outcome: Zonulin serum levels Secondary outcome: Total blood bacterial load

Adequate nutritional status evaluated with Mini Nutritional Assessment (MNA) score ≥24 Good cognitive status tested with Mini Mental State Evaluation (MMSE) score ≥24 Self-sufficiency assessed with validated tests (e.g. Barthel index - activities of daily	juice, Renetta apple and purée, green tea and dark chocolate products) <u>Control group:</u> comparable diet without the polyphenol-rich products Dose: three portion of polyphenol-rich food products daily (about 750	Faecal microbiota composition and metabolism Short chain fatty acids and polyphenol-derived metabolites Inflammatory, oxidative stress and related markers Endotoxin LPS-BP

	living, Tinetti balance assessment)	mg of polyphenols)	Metabolomic markers
			Metabolic and anthropometric markers

Legend: TNF-α, tumor necrosis factor-alpha; TNFr-2: tumor necrosis factor receptor-2; PCR: C-reactive protein; IL-10, interleukin-10; NSAIDs; nonsteroidal anti-inflammatory drugs; BMI, body mass index; LPS-BP, lipopolysaccharide binding protein

References:

(1) Brandtzaeg, P. The Gut as Communicator between Environment and Host: Immunological Consequences. *Eur. J. Pharmacol.* **2011**, *668 Suppl*, S16-32. https://doi.org/10.1016/j.ejphar.2011.07.006.

(2) Bischoff, S. C.; Barbara, G.; Buurman, W.; Ockhuizen, T.; Schulzke, J. D.; Serino, M.; Tilg, H.; Watson, A.; Wells, J. M. Intestinal Permeability - a New Target for Disease Prevention and Therapy. *BMC Gastroenterol.* **2014**, *14* (1), 1–25. https://doi.org/10.1186/s12876-014-0189-7.

(3) Suzuki, T. Regulation of Intestinal Epithelial Permeability by Tight Junctions. *Cell. Mol. Life Sci.* **2013**, *70* (4), 631–659. https://doi.org/10.1007/s00018-012-1070-x.

(4) Lee, S. H. Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. *Intest. Res.* **2015**, *13* (1), 11. https://doi.org/10.5217/ir.2015.13.1.11.

(5) Furuse, M.; Fujita, K.; Hiiragi, T.; Fujimoto, K.; Tsukita, S. Claudin-1 and -2: Novel Integral Membrane Proteins Localizing at Tight Junctions with No Sequence Similarity to Occludin. *J. Cell Biol.* **1998**, *141* (7), 1539–1550.

(6) Furuse, M.; Hirase, T.; Itoh, M.; Nagafuchi, A.; Yonemura, S.; Tsukita, S.; Tsukita, S. Occludin: A Novel Integral Membrane Protein Localizing at Tight Junctions. *J. Cell Biol.* **1993**, *123* (6 Pt 2), 1777–1788.

(7) Ulluwishewa, D.; Anderson, R. C.; Mcnabb, W. C.; Moughan, P. J.; Wells, J. M.; Roy, N. C. Regulation of Tight Junction Permeability by Intestinal Bacteria and Dietary Components. *J. Nutr* **2011**, *141*, 769–776. https://doi.org/10.3945/jn.110.135657.769.

(8) Oda, H.; Takeichi, M. Evolution: Structural and Functional Diversity of Cadherin at the Adherens Junction. *J. Cell Biol.* **2011**, *193* (7), 1137–1146. https://doi.org/10.1083/jcb.201008173.

(9) Hollon, J.; Puppa, E. L.; Greenwald, B.; Goldberg, E.; Guerrerio, A.; Fasano, A. Effect of Gliadin on Permeability of Intestinal Biopsy Explants from Celiac Disease Patients and Patients with Non-Celiac Gluten Sensitivity. *Nutrients* **2015**, *7* (3), 1565–1576. https://doi.org/10.3390/nu7031565.

(10) Dunlop, S. P.; Hebden, J.; Campbell, E.; Naesdal, J.; Olbe, L.; Perkins, A. C.; Spiller, R. C. Abnormal Intestinal Permeability in Subgroups of Diarrhea-Predominant Irritable Bowel Syndromes. *Am. J. Gastroenterol.* **2006**, *101* (6), 1288–1294. https://doi.org/10.1111/j.1572-0241.2006.00672.x.

(11) Melichar, B.; Zezulova, M. The Significance of Altered Gastrointestinal Permeability in Cancer Patients. *Curr. Opin. Support. Palliat. Care* **2011**, *5* (1), 47–54. https://doi.org/10.1097/SPC.0b013e328343a043.

(12) Vancamelbeke, M.; Vermeire, S. The Intestinal Barrier : A Fundamental Role in Health and Disease. *Expert Rev Gastroenterol Hepatol.* **2017**, *11* (9), 821–834. https://doi.org/10.1080/17474124.2017.1343143.The.

(13) Demir, E.; Ozkan, H.; Seckin, K. D.; Sahtiyancı, B.; Demir, B. Plasma Zonulin Levels as a Non-Invasive Biomarker of Intestinal Permeability in Women with Gestational Diabetes Mellitus. 1–8. https://doi.org/10.3390/biom9010024.

(14) Lerner, A.; Matthias, T. Rheumatoid Arthritis-Celiac Disease Relationship: Joints Get That Gut Feeling. *Autoimmun. Rev.* **2015**, *14* (11), 1038–1047. https://doi.org/10.1016/j.autrev.2015.07.007.

(15) Spielman, L. J.; Gibson, D. L.; Klegeris, A. Unhealthy Gut, Unhealthy Brain: The Role of the Intestinal Microbiota in Neurodegenerative Diseases. *Neurochem. Int.* **2018**, *120*, 149–163. https://doi.org/10.1016/j.neuint.2018.08.005.

(16) Buscarinu, M. C.; Romano, S.; Mechelli, R.; Pizzolato Umeton, R.; Ferraldeschi, M.;

Fornasiero, A.; Renie, R.; Cerasoli, B.; Morena, E.; Romano, C.; et al. Intestinal Permeability in Relapsing-Remitting Multiple Sclerosis. *Neurotherapeutics* **2018**, *15* (1), 68–74. https://doi.org/10.1007/s13311-017-0582-3.

(17) Franceschi, C.; Bonafe, M.; Valensin, S.; Olivieri, F.; De Luca, M.; Ottaviani, E.; De Benedictis, G. Inflamm-Aging. An Evolutionary Perspective on Immunosenescence. *Ann. N. Y. Acad. Sci.* **2000**, *908*, 244–254.

(18) Malago, J. Contribution of Microbiota to the Intestinal Physicochemical Barrier. *Benef Microbes.* **2015**, *6(3):295-3*.

(19) Norman, K.; Pirlich, M.; Schulzke, J.; Smoliner, C.; Lochs, H.; Valentini, L.; Bühner, S. Increased Intestinal Permeability in Malnourished Patients with Liver Cirrhosis. *Eur J Clin Nutr*. **2012**.

(20) Guerreiro, C. S.; Calado, Â.; Sousa, J. Diet , Microbiota , and Gut Permeability — The Unknown Triad in Rheumatoid Arthritis. *Front. Med.* **2018**, *5* (December), 1–7. https://doi.org/10.3389/fmed.2018.00349.

(21) De Filippo, C.; Cavalieri, D.; Di Paola, M.; Ramazzotti, M.; Poullet, J.; Massart, S.; Collini, S.; Pieraccini, G.; Lionetti, P. Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children from Europe and Rural Africa. *Proc Natl Acad Sci U S A.* **2010**, *17*, 107(33):14691-6.

(22) Plöger, S.; Stumpff, F.; Penner, G.; Schulzke, J.; Gäbel, G.; Martens, H.; Shen, Z.; Günzel, D.; Aschenbach, J. Microbial Butyrate and Its Role for Barrier Function in the Gastrointestinal Tract. *Ann N Y Acad Sci.* **2012**, 1258:52-9.

(23) Mrduljaš, N.; Krešić, G.; Tea, B. Polyphenols: Food Sources and Health Benefits Nikolina.

(24) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jime, L. Polyphenols: Food Sources and Bioavailability. **2004**.

(25) Fernando Cardonaa; Cristina Andrés-Lacuevac; Sara Tulipania; Francisco J. Tinahonesb; María Isabel Queipo-Ortuño. Benefits of Polyphenols on Gut Microbiota and Implications in Human Health. *J. Nutr. Biochem.* **2013**, *24*, 1415–1422.

(26) Monagas, M.; Urpi-Sarda, M.; Sánchez-Patán, F.; Llorach, R.; Garrido, I.; Gómez-Cordovés, C.; Andres-Lacueva, C.; Bartolomé, B. Insights into the Metabolism and Microbial Biotransformation of Dietary Flavan-3-Ols and the Bioactivity of Their Metabolites. *Food Funct.* **2010**, *1* (3), 233–253. https://doi.org/10.1039/c0fo00132e.

(27) Joseph, S. V; Edirisinghe, I.; Burton-Freeman, B. M. Fruit Polyphenols: A Review of Anti-Inflammatory Effects in Humans. *Crit. Rev. Food Sci. Nutr.* **2016**, *56* (3), 419–444. https://doi.org/10.1080/10408398.2013.767221.

(28) Kaulmann, A.; Bohn, T. Bioactivity of Polyphenols: Preventive and Adjuvant Strategies toward Reducing Inflammatory Bowel Diseases—Promises, Perspectives, and Pitfalls. *Oxid. Med. Cell. Longev.* **2016**, *2016* (c), 1–29. https://doi.org/10.1155/2016/9346470.

(29) Shimizu, M. Multifunctions of Dietary Polyphenols in the Regulation of Intestinal Inflammation. *J. Food Drug Anal.* **2017**, *25* (1), 93–99. https://doi.org/10.1016/j.jfda.2016.12.003.

(30) Mosele, J. I.; Macià, A.; Motilva, M. J. Metabolic and Microbial Modulation of the Large Intestine Ecosystem by Non-Absorbed Diet Phenolic Compounds: A Review. *Molecules* **2015**, *20* (9), 17429–17468. https://doi.org/10.3390/molecules200917429.

(31) Boto-Ordonez, M.; Urpi-Sarda, M.; Queipo-Ortuno, M. I.; Tulipani, S.; Tinahones, F. J.; Andres-Lacueva, C. High Levels of Bifidobacteria Are Associated with Increased Levels of Anthocyanin Microbial Metabolites: A Randomized Clinical Trial. *Food Funct.* **2014**, *5* (8), 1932–1938. https://doi.org/10.1039/c4fo00029c.

(32) Queipo-Ortuno, M. I.; Boto-Ordonez, M.; Murri, M.; Gomez-Zumaquero, J. M.; Clemente-

Postigo, M.; Estruch, R.; Cardona Diaz, F.; Andres-Lacueva, C.; Tinahones, F. J. Influence of Red Wine Polyphenols and Ethanol on the Gut Microbiota Ecology and Biochemical Biomarkers. *Am. J. Clin. Nutr.* **2012**, *95* (6), 1323–1334. https://doi.org/10.3945/ajcn.111.027847.

(33) Guglielmetti, S.; Fracassetti, D.; Taverniti, V.; Del Bo', C.; Vendrame, S.; Klimis-Zacas, D.; Arioli, S.; Riso, P.; Porrini, M. Differential Modulation of Human Intestinal Bifidobacterium Populations after Consumption of a Wild Blueberry (Vaccinium Angustifolium) Drink. *J. Agric. Food Chem.* **2013**, *61* (34), 8134–8140. https://doi.org/10.1021/jf402495k.

(34) Edwards, C. A.; Havlik, J.; Cong, W.; Mullen, W.; Preston, T.; Morrison, D. J.; Combet, E. Polyphenols and Health: Interactions between Fibre, Plant Polyphenols and the Gut Microbiota. *Nutr. Bull.* **2017**, *42* (4), 356–360. https://doi.org/10.1111/nbu.12296.

(35) Luescher, S.; Urmann, C.; Butterweck, V. Effect of Hops Derived Prenylated Phenols on TNF-α Induced Barrier Dysfunction in Intestinal Epithelial Cells. *J. Nat. Prod.* **2017**, *80* (4), 925–931. https://doi.org/10.1021/acs.jnatprod.6b00869.

(36) Atkinson, K. J.; Rao, R. K. Role of Protein Tyrosine Phosphorylation in Acetaldehyde-Induced Disruption of Epithelial Tight Junctions. *Am. J. Physiol. Liver Physiol.* **2001**, *280* (6), G1280–G1288. https://doi.org/10.1152/ajpgi.2001.280.6.g1280.

(37) Park, H.-Y.; Kunitake, Y.; Matsui, T.; Hirasaki, N.; Tanaka, M. Theaflavins Enhance Intestinal Barrier of Caco-2 Cell Monolayers through the Expression of AMP-Activated Protein Kinase-Mediated Occludin, Claudin-1, and ZO-1. *Biosci. Biotechnol. Biochem.* **2015**, *79* (1), 130–137. https://doi.org/10.1080/09168451.2014.951027.

(38) Contreras, T. C.; Ricciardi, E.; Cremonini, E.; Oteiza, P. I. (-)-Epicatechin in the Prevention of Tumor Necrosis Alpha-Induced Loss of Caco-2 Cell Barrier Integrity. *Arch. Biochem. Biophys.* **2015**, *573*, 84–91. https://doi.org/10.1016/j.abb.2015.01.024.

(39) Valenzano, M. C.; Baker, V.; Moore, B. A.; Mixson, B.; Mullin, J. M.; Manley, I.; DiGuilio, K.; To, J.; Ferraro, B.; Wertheimer, J.; et al. Remodeling of Tight Junctions and Enhancement of Barrier Integrity of the CACO-2 Intestinal Epithelial Cell Layer by Micronutrients. *PLoS One* **2015**, *10* (7), e0133926. https://doi.org/10.1371/journal.pone.0133926.

(40) Wang, K.; Hu, F.; Conlon, M.; Song, Z.; Jin, X.; Jiang, X.; Chen, Y.; Topping, D. Polyphenol-Rich Propolis Extracts Strengthen Intestinal Barrier Function by Activating AMPK and ERK Signaling. *Nutrients* **2016**, *8* (5), 272. https://doi.org/10.3390/nu8050272.

(41) Azzini, E.; Venneria, E.; Lombardi-Boccia, G.; Palomba, L.; Garaguso, I.; Intorre, F.; Durazzo, A.; Foddai, M. S.; Polito, A.; Nobili, F.; et al. The Potential Health Benefits of Polyphenol-Rich Extracts from Cichorium Intybus L. Studied on Caco-2 Cells Model. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1–9. https://doi.org/10.1155/2016/1594616.

(42) Cremonini, E.; Mastaloudis, A.; Hester, S. N.; Verstraeten, S. V.; Anderson, M.; Wood, S. M.; Waterhouse, A. L.; Fraga, C. G.; Oteiza, P. I. Anthocyanins Inhibit Tumor Necrosis Alpha-Induced Loss of Caco-2 Cell Barrier Integrity. *Food Funct.* **2017**, *8* (8), 2915–2923. https://doi.org/10.1039/c7fo00625j.

(43) Rybakovsky, E.; Valenzano, M. C.; Deis, R.; Diguilio, K. M.; Thomas, S.; Mullin, J. M. Improvement of Human-Oral-Epithelial-Barrier Function and of Tight Junctions by Micronutrients. *J. Agric. Food Chem.* **2017**, *65* (50), 10950–10958. https://doi.org/10.1021/acs.jafc.7b04203.

(44) Van Buiten, C. B.; Lambert, J. D.; Elias, R. J. Green Tea Polyphenols Mitigate Gliadin-Mediated Inflammation and Permeability in Vitro. *Mol. Nutr. Food Res.* **2018**, *62* (12), 1–8. https://doi.org/10.1002/mnfr.201700879.

(45) Cremonini, E.; Wang, Z.; Bettaieb, A.; Adamo, A. M.; Daveri, E.; Mills, D. A.; Kalanetra, K.

M.; Haj, F. G.; Karakas, S.; Oteiza, P. I. (-)-Epicatechin Protects the Intestinal Barrier from High Fat Diet-Induced Permeabilization: Implications for Steatosis and Insulin Resistance. *Redox Biol.* **2018**, *14* (October 2017), 588–599. https://doi.org/10.1016/j.redox.2017.11.002.

(46) Vazquez-Olivo, G.; Osuna-Enciso, T.; León-Félix, J.; Heredia, J. B.; Antunes-Ricardo, M.; Gutiérrez-Uribe, J. A. Cellular Antioxidant Activity and in Vitro Intestinal Permeability of Phenolic Compounds from Four Varieties of Mango Bark (Mangifera Indica L.). *J. Sci. Food Agric.* **2019**, No. September 2018. https://doi.org/10.1002/jsfa.9567.

(47) Amasheh, M.; Schlichter, S.; Amasheh, S.; Mankertz, J.; Zeitz, M.; Fromm, M.; Schulzke, J. D. Quercetin Enhances Epithelial Barrier Function and Increases Claudin-4 Expression in Caco-2 Cells. *J. Nutr.* **2008**, *138* (6), 1067–1073. https://doi.org/10.1093/jn/138.6.1067.

(48) Suzuki, T.; Hara, H. Quercetin Enhances Intestinal Barrier Function through the Assembly of Zonnula Occludens-2, Occludin, and Claudin-1 and the Expression of Claudin-4 in Caco-2 Cells. *J. Nutr.* **2009**, *139* (5), 965–974. https://doi.org/10.3945/jn.108.100867.

(49) Suzuki, T.; Tanabe, S.; Hara, H. Kaempferol Enhances Intestinal Barrier Function through the Cytoskeletal Association and Expression of Tight Junction Proteins in Caco-2 Cells. *J. Nutr.* **2011**, *141* (1), 87–94. https://doi.org/10.3945/jn.110.125633.

(50) Noda, S.; Tanabe, S.; Suzuki, T. Differential Effects of Flavonoids on Barrier Integrity in Human Intestinal Caco-2 Cells. *J. Agric. Food Chem.* **2012**, *60* (18), 4628–4633. https://doi.org/10.1021/jf300382h.

(51) Noda, S.; Tanabe, S.; Suzuki, T. Naringenin Enhances Intestinal Barrier Function through the Expression and Cytoskeletal Association of Tight Junction Proteins in Caco-2 Cells. *Mol. Nutr. Food Res.* **2013**, *57* (11), 2019–2028. https://doi.org/10.1002/mnfr.201300045.

(52) Cao, M.; Sun, C.; He, W.; Wang, P.; Wang, F. Amelioration of IFN-γ and TNF-α-Induced Intestinal Epithelial Barrier Dysfunction by Berberine via Suppression of MLCK-MLC

Phosphorylation Signaling Pathway. *PLoS One* **2013**, *8* (5), e61944. https://doi.org/10.1371/journal.pone.0061944.

(53) Carrasco-Pozo, C.; Morales, P.; Gotteland, M. Polyphenols Protect the Epithelial Barrier Function of Caco-2 Cells Exposed to Indomethacin through the Modulation of Occludin and Zonula Occludens-1 Expression. *J. Agric. Food Chem.* **2013**, *61* (22), 5291–5297. https://doi.org/10.1021/jf400150p.

(54) Piegholdt, S.; Pallauf, K.; Esatbeyoglu, T.; Speck, N.; Reiss, K.; Ruddigkeit, L.; Stocker, A.; Huebbe, P.; Rimbach, G. Biochanin A and Prunetin Improve Epithelial Barrier Function in Intestinal CaCo-2 Cells via Downregulation of ERK, NF-KB, and Tyrosine Phosphorylation. *Free Radic. Biol. Med.* **2014**, *70*, 255–264. https://doi.org/10.1016/j.freeradbiomed.2014.02.025.

(55) Watson, J. L.; Wang, A.; Ansari, S.; Cameron, H.; McKay, D. M.; Akhtar, M. Green Tea Polyphenol (−)-Epigallocatechin Gallate Blocks Epithelial Barrier Dysfunction Provoked by IFN-γ but Not by IL-4. *Am. J. Physiol. Liver Physiol.* **2004**, *287* (5), G954–G961. https://doi.org/10.1152/ajpgi.00302.2003.

(56) Krug, S. M.; Fromm, M.; Zeitz, M.; Schulzke, J.-D.; Amasheh, S.; Fromm, A.; Amasheh, M.; Andres, S. TNFα-Induced and Berberine-Antagonized Tight Junction Barrier Impairment via Tyrosine Kinase, Akt and NFκB Signaling. *J. Cell Sci.* **2010**, *123* (23), 4145–4155. https://doi.org/10.1242/jcs.070896.

(57) Rogoll, D.; Bergmann, H.; Hellenschmidt, D.; Heinze, J.; Scheppach, W.; Melcher, R.; Richiling, E. Influence of Apple Polyphenols on the Intestinal Barrier in a Colonic Cell Model. *J. Appl. Bot. Food Qual.* **2010**, 83,110-117. https://doi.org/10.1177/0969733010361439.

(58) Amasheh, M.; Fromm, M.; Luettig, J.; Zeitz, M.; Schulzke, J.-D.; Amasheh, S. Effects of

Quercetin Studied in Colonic HT-29/B6 Cells and Rat Intestine in Vitro. *Ann. N. Y. Acad. Sci.* **2012**, *1258* (1), 100–107. https://doi.org/10.1111/j.1749-6632.2012.06609.x.

(59) Nunes, C.; Freitas, V.; Almeida, L.; Laranjinha, J. Red Wine Extract Preserves Tight Junctions in Intestinal Epithelial Cells under Inflammatory Conditions: Implications for Intestinal Inflammation. *Food Funct.* **2019**, 1364–1374. https://doi.org/10.1039/c8fo02469c.

(60) Chuenkitiyanon, S.; Pengsuparp, T.; Jianmongkol, S. Protective Effect of Quercetin on Hydrogen Peroxide-Induced Tight Junction Disruption. *Int. J. Toxicol.* **2010**, *29* (4), 418–424. https://doi.org/10.1177/1091581810366487.

(61) Ling, K. H.; Wan, M. L. Y.; El-Nezami, H.; Wang, M. Protective Capacity of Resveratrol, a Natural Polyphenolic Compound, against Deoxynivalenol-Induced Intestinal Barrier Dysfunction and Bacterial Translocation. *Chem. Res. Toxicol.* **2016**, *29* (5), 823–833. https://doi.org/10.1021/acs.chemrestox.6b00001.

(62) Shin, D. Y.; Lu, J. N.; Kim, G. Y.; Jung, J. M.; Kang, H. S.; Lee, W. S.; Choi, Y. H. Anti-Invasive Activities of Anthocyanins through Modulation of Tight Junctions and Suppression of Matrix Metalloproteinase Activities in HCT-116 Human Colon Carcinoma Cells. *Oncol. Rep.* **2011**, *25* (2), 567–572. https://doi.org/10.3892/or.2010.1104.

(63) Li, Z.; Gao, M.; Yang, B.; Zhang, H.; Wang, K.; Liu, Z.; Xiao, X.; Yang, M. Naringin Attenuates MLC Phosphorylation and NF-KB Activation to Protect Sepsis-Induced Intestinal Injury via RhoA/ROCK Pathway. *Biomed. Pharmacother.* **2018**, *103* (September 2017), 50–58. https://doi.org/10.1016/j.biopha.2018.03.163.

(64) Gu, L.; Li, N.; Gong, J.; Li, Q.; Zhu, W.; Li, J. Berberine Ameliorates Intestinal Epithelial Tight-Junction Damage and down-Regulates Myosin Light Chain Kinase Pathways in a Mouse Model of Endotoxinemia. *J. Infect. Dis.* **2011**, *203* (11), 1602–1612. https://doi.org/10.1093/infdis/jir147.

(65) Yang, G.; Wang, H.; Kang, Y.; Zhu, M. J. Grape Seed Extract Improves Epithelial Structure and Suppresses Inflammation in Ileum of IL-10-Deficient Mice. *Food Funct.* **2014**, *5* (10), 2558–2563. https://doi.org/10.1039/c4fo00451e.

(66) Wang, H.; Xue, Y.; Zhang, H.; Huang, Y.; Yang, G.; Du, M.; Zhu, M.-J. Dietary Grape Seed Extract Ameliorates Symptoms of Inflammatory Bowel Disease in Interleukin-10 Deficient Mice Hui. **2013**, *71* (11), 3831–3840. https://doi.org/10.1158/0008-5472.CAN-10-4002.BONE.

(67) Li, L.; Wang, L.; Wu, Z.; Yao, L.; Wu, Y.; Huang, L.; Liu, K.; Zhou, X.; Gou, D. Anthocyanin-Rich Fractions from Red Raspberries Attenuate Inflammation in Both RAW264.7 Macrophages and a Mouse Model of Colitis. *Sci. Rep.* **2014**, *4*, 1–11. https://doi.org/10.1038/srep06234.

(68) Wei, H. kui; Chen, G.; Wang, R. J.; Peng, J. Oregano Essential Oil Decreased Susceptibility to Oxidative Stress-Induced Dysfunction of Intestinal Epithelial Barrier in Rats. *J. Funct. Foods* **2015**, *18*, 1191–1199. https://doi.org/10.1016/j.jff.2015.02.035.

(69) Bitzer, Z. T.; Elias, R. J.; Vijay-Kumar, M.; Lambert, J. D. (-)-Epigallocatechin-3-Gallate Decreases Colonic Inflammation and Permeability in a Mouse Model of Colitis, but Reduces Macronutrient Digestion and Exacerbates Weight Loss. *Mol. Nutr. Food Res.* **2016**, *60* (10), 2267–2274. https://doi.org/10.1002/mnfr.201501042.

(70) Gil-Cardoso, K.; Ginés, I.; Pinent, M.; Ardévol, A.; Arola, L.; Blay, M.; Terra, X. Chronic Supplementation with Dietary Proanthocyanidins Protects from Diet-Induced Intestinal Alterations in Obese Rats. *Mol. Nutr. Food Res.* **2017**, *61* (8), 1–12. https://doi.org/10.1002/mnfr.201601039.

(71) https://clinicaltrials.gov/ct2/show/NCT03413735.

(72) https://clinicaltrials.gov/ct2/show/NCT03427879.

(73) https://clinicaltrials.gov/ct2/show/NCT02728570.

(74) http://www.isrctn.com/ISRCTN10214981.

(75) Arrieta, M. C.; Bistritz, L.; Meddings, J. B. Alteration in Intestinal Permeability. *Gut* **2006**, *55*, 1512–1520. https://doi.org/10.1136/gut.2005.085373.

(76) Wang, W.; Uzzau, S.; Goldblum, S. E.; Fasano, A. Human Zonulin , a Potential Modulator of Intestinal Tight Junctions. **2000**, *4440*, 4435–4440.

(77) Qi, Y.; Goel, R.; Kim, S.; Richards, E. M.; Carter, C. S.; Pepine, C. J.; Raizada, M. K.; and Buford, T. W. Intestinal Permeability Biomarker Zonulin Is Elevated in Healthy Aging. **2017**, *18(9)*, 810.e1-810.e4. https://doi.org/10.1002/cncr.27633.Percutaneous.

(78) Sapone, A.; De Magistris, L.; Pietzak, M.; Clemente, M. G.; Tripathi, A.; Cucca, F.; Lampis, R.; Kryszak, D.; Cartenì, M.; Generoso, M.; et al. Zonulin Upregulation Is Associated with Increased Gut Permeability in Subjects with Type 1 Diabetes and Their Relatives. *Diabetes* **2006**, *55* (5), 1443–1449. https://doi.org/10.2337/db05-1593.

(79) Zak-Golab, A.; Kocelak, P.; Aptekorz, M.; Zientara, M.; Juszczyk, L.; Martirosian, G.; Chudek, J.; Olszanecka-Glinianowicz, M. Gut Microbiota, Microinflammation, Metabolic Profile, and Zonulin Concentration in Obese and Normal Weight Subjects. *Int. J. Endocrinol.* **2013**, *2013*, 674106. https://doi.org/10.1155/2013/674106.

(80) Grootjans, J.; Thuijls, G.; Verdam, F.; Derikx, J. P.; Lenaerts, K.; A, W.; Joep, B. Non-Invasive Assessment of Barrier Integrity and Function of the Human Gut. *World J. Gastrointest. Surg.* **2010**, *2* (3), 61. https://doi.org/10.4240/wjgs.v2.i3.61.