Exploring the molecular pathways behind the effects of nutrients and dietary polyphenols on gut microbiota and intestinal permeability: a perspective on the potential of metabolomics and future clinical applications

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Abstract

The gut microbiota is involved in the regulation of the intestinal permeability (IP), whose disruption is a frequent condition in older people and is associated to the development of several diseases. The diet can affect the gut microbiota and IP, although the molecular mechanisms involved are unclear. Metabolomics is one of the suitable approaches to study the effects of diet on gut microbiota and IP, although up to now the research has focused only on few dietary components. The aim here was to review the most recent literature concerning the application of metabolomics to the study of the diet-induced alterations of gut microbiota and the effects on IP, with a particular focus on the molecular pathways involved. An additional aim was to give a perspective on the future research involving dietary polyphenols, because despite their potential use in the management of increased IP, few studies have been reported to date.

Keywords: metabolomics, gut microbiota, intestinal permeability, nutrients, polyphenols

Introduction

The gastrointestinal tract (GI) is responsible for a wide range of functions, including digestion and absorption of nutrients, water and ions, regulation of host immunity, protection against the ingress of pathogenic microorganism, and the the metabolism and detoxification of xenobiotics. The GI also hosts the largest microbial population of the human body, which works in symbiosis with the host to accomplish these various intestinal functions. Gut bacteria are particularly important for host health, being involved in the synthesis of vitamins, secondary bile acids and neurotransmitters, and playing a direct role in the metabolism and degradation of dietary components and drugs, that can affect their bioavailability and absorption [1]. It has been estimated that over than 1,000 different bacterial species populate the intestinal environment, with a genome comprising 100-fold more genes than those found in human genome [2]. The physiological variations in the small intestine and colon, such as the presence of distinct chemical environments, nutrients and host immune activity allow distinct groups of bacterial species to populate the different regions of the lower gastrointestinal tract [3,4], and this variability becomes even more complex considering the inter-individual variations and the influence of host genetics [5-7]. Nevertheless, most human gut microbiota share a core set of resident bacteria and related microbial genes [8,9]. *Firmicutes, Bacteroidetes* and, secondly, *Actinobacteria* are the three most abundant phyla, among the over 50 that have been identified by metagenomic approaches [10,11]. A synergistic equilibrium among the different species and the maintenance of a microbial diversity are of crucial importance for health, since the microbiota plays a central role on the proper functioning of the intestinal barrier and maintaining appropriate intestinal permeability (IP), which is directly involved in the development of numerous disorders. In this vein, a low diversity and a scarce abundance of species as *Bifidobacterium* spp. and *Faecalibacterium prausnitzii* have been associated

with gut disease states, e.g. Crohn's disease [12], type 1, type 2 and gestational diabetes [13-15], celiac disease [16] and obesity [17].

Diet, as a source of macro- and micro-nutrients and other bioactive components, is one of the factors that most can affect the microbiota. Among the dietary constituents, polyphenols have been in the spotlight in recent years, due to their particular physicochemical properties and their potential to directly affect microbiota activity and host health. Polyphenols are secondary metabolites of plants, fruits and vegetables, and major components of commonly consumed foods and beverages such as chocolate, tea and coffee [18-20] which, due to their characteristic (poly)hydroxylated phenyl moieties and the presence of ionizable functional groups on their scaffolds, have a low bioavailability and are scarcely absorbed by the intestine [21,22]. Consequently, they are prone to catabolism by the gut microbiota, which leads to the production of smaller molecular weight (MW) compounds that can be absorbed across the intestinal wall, enter the bloodstream and eventually, undergo further transformation and conjugation in the liver [23,24]. It has been estimated that total polyphenol absorption in the small intestine is around 5%–10%, while the remaining 90%–95% transits to the large intestinal lumen and accumulates in the millimolar range [25]. Hence, microbial polyphenol derivatives could be responsible for the biological effects attributed to their parent compounds, or at least contribute to the overall activity. Catechins from green tea, for example, have been reported to exert antioxidant, anti-inflammatory and anti-tumorigenic activities [26-28]. However, the most representative green tea catechin, (−)-epigallocatechin gallate, is scarcely absorbed from the intestine and is extensively metabolized by gut microbiota [29] to form smaller MW derivatives that not only contribute to the observed bioactivities of green tea, but can also exert higher activity than the parent compound [30]. Polyphenols and their microbial metabolites could also exert antimicrobial and bacteriostatic activities, hence regulating the overgrowth of

harmful bacteria on the intestinal and urinary tract epithelia [20,31]. As an example, cranberry (*Vaccinium macrocarpon* Ait.) fruits, rich sources of type-A procyanidins (PAC-A), are known to exert anti-adhesive activity against the uropathogenic bacteria responsible for most of the lower urinary tract infections, although the mechanisms of action are still unknown and the outcomes of in vitro assays and *in vivo* clinical trials aimed at reducing urinary tract infections are frequently inconsistent [32]. Recent studies conducted in both rats and human volunteers show that, after supplementation with dry cranberry extracts, urine samples exert effective anti-adhesive activity against uropathogenic *E. coli*, despite their negligible contents of intact PAC-A [33,34]. However, the same urine samples were characterized by high amounts of hydroxyphenyl-valeric acid and hydroxyphenyl-valerolactone derivatives, previously reported as end-products of microbial degradation of flavan-3-ols [35], indicating the important contribution of the microbial metabolites of procyanidins to the observed bioactivity [33,34]. Finally, the effects of polyphenols on microbiota, inflammation and oxidative stress and their capacity to regulate the synthesis and expression of specific proteins on the intestinal epithelium seem to be part of the mechanisms by which these compounds can regulate the permeability of the intestinal barrier [36], whose alterations are related to the development of several diseases, especially in older subjects.

Many efforts have been made to characterize the microbial community colonizing the human intestine, for which the widespread use of metataxonomics based on 16S rRNA gene profiling and metagenomics (microbiomics) has been particularly important. However, although representing powerful tools for bacterial identification and classification, microbiomics does not allow to obtain information about fluctuations in metabolic activities [1]. To this purpose, metabolomics is the most suitable approach, and numerous reports based on metabolomic analysis have been reported over the last decade

[37]. Focusing on the application of metabolomics in the study of diet-microbiota interactions and searching for the keywords "metabolomics AND diet AND microbiota" in PubMed, we found that the number of publications almost doubled from 2014 to 2018, as an index of the popularity that metabolomics gained during the recent years (Figure 1). Metabolomic approaches have been widely used to study the transformation of nutrients and xenobiotics by intestinal microbiota [38-43], thus allowing the characterization of hundreds of metabolites derived from macro- and micronutrients and polyphenols coming from fruits and vegetables. In 2009, Jacobs published a first review article regarding the role of colonic microbiota in the degradation of non-digestible food ingredients and their impact on gut health and immunity [44]. For the first time, the importance of metabolomics in the study of the links between the bioconversion of non-digestible food ingredients, their bioavailability and their downstream effects on microbiota composition and host metabolism was recognized [44]. More recently, the use of integrated multi-omics approaches has facilitated the study of the molecular interactions between diet and microbiota, and has led to the identification of several metabolites that are produced as a result of microbial metabolism of various dietary constituents. Nevertheless, considering the challenges to study the mutual relationship between gut microbiota and the host, its tight connection with diet, environment and lifestyle, and the still incomplete characterization of the huge microbial metabolome, the path to assess precise and validated metabolites to link the microbial activity to specific effects on health is just starting. In a way to find a clinical relevance of metabolomics data and offer to clinicians a robust tool to predict, prevent and treat several diseases, further progress is necessary.

The aim of this work was to review the most recent literature regarding the application of metabolomics in the study of the interactions between food components and gut microbiota and the effects on IP, with a particular focus on the elucidation of the

126 molecular pathways involved. Since to date the research has mainly focused on the
127 degradation of non-digestible fibers and tryptophan and on the bioactivity of their
128 metabolites, a major part of the work will be dedicated to these important dietary
129 components. Additionally, a perspective on the future research involving the role of
130 dietary polyphenols in modulating the activity and composition of gut microbiota and the
131 effects on IP will be discussed, given that, despite their potential implication in the
132 prevention and treatment of several diseases, few clinical studies have been performed up
133 to now.
134

135 **The role of microbiota and microbiota-derived dietary metabolites in regulating**
136 **intestinal permeability: the application of metabolomics for the discovery of new**
137 **biomarkers**

138 The intestinal wall represents a barrier that selectively transports nutrients, ions and water
139 from the lumen to the bloodstream, via passive and active mechanisms. A layer of
140 epithelial cells constitutes the main physical barrier between the intestinal lumen and the
141 mucosal tissues [45]. Tight junctions (TJ), composed of transmembrane proteins and
142 junctional adhesion molecules that regulate the flow of water, ions and small molecules,
143 seal the paracellular spaces [46]. Several distinct proteins contribute to form the TJ,
144 including mainly occludins and claudins, depending on the tissue and location that
145 interlink within the paracellular space [47]. Although highly cross-linked, the structure of
146 TJ is dynamic, so that it can be 'opened' and 'closed' following specific stimuli [48].
147 Physiological stimuli could shrink the TJ to prevent the diffusion of toxins, viruses or
148 bacterial fragments to the mucosal layer, while they can open the paracellular space to
149 allow the diffusion of nutrients [49]. For instance, the activation of the sodium dependent
150 glucose transporter led to the opening of TJ and allowed the diffusion of small molecules

and peptides with MW < 40,000 Da [50]. On the other hand, the physiological structure and dynamism of TJ could be altered due to pathological states [51], leading to a condition of increased IP, also known as "leaky gut". Celiac disease, inflammatory bowel disease and type I diabetes are three of the principal pathological causes of leaky gut [52], which leads to the permeation of potentially harmful molecules, organisms or microbial fragments from the intestinal lumen to the mucosal layer, inducing a cascade of events that result in immune activation and local or systemic inflammation. Older people are frequently affected by decreased intestinal barrier function and consequently leaky gut [53]. Among the causes, the aging-related decline of immune function (namely immune-senescence), the remodeling of intestinal epithelium and the alterations of gut microbiota composition are thought to be the most important ones [53-55]. As observed in disease-associated increased IP, the dysfunction of the intestinal barrier in older subjects facilitates the diffusion of toxic substances or peptides and microbial fragments to the mucosal layer and to the bloodstream and the triggering of a systemic inflammatory response [56].

As previously stated, diet plays an important role in the maintenance of the gut barrier integrity and is hence determinant for IP. The short-chain fatty acids (SCFAs), produced by the degradation of dietary fibers by several bacteria in the gut (including *Clostridium, Eubacterium*, and *Butyrivibrio*), have been the most studied microbial catabolites involved in the regulation of IP to date. Among them, butyrate has been identified as a marker of the positive effects of non-digestible dietary fiber consumption on microbiota composition and intestinal permeability. It exerts several activities on the intestinal wall, such as controlling inflammation by altering the expression of pro-inflammatory cytokines [57], preserving the intestinal barrier function by inducing the expression of TJ proteins claudin-1 and claudin-2 [58], and modulating composition of gut microbiota by inhibiting the growth of pathogenic bacteria [59] (Figure 2). Food is the only source of non-

digestible carbohydrates, and alterations in diet lead to variations in the production of intestinal butyrate. In aged mice, the increased butyrate production after the consumption of high doses of soluble fiber was associated with an induced expression of the TJ proteins Tjp2 and Ffar2 and to a counterbalance of the age-related microbiota dysbiosis, with a significant amelioration of the increased IP condition typical of older individuals [60]. Similar effects of a high fiber diet were also observed in mice affected by autoimmune hepatitis, characterized by an imbalance of Treg/Th17 cells and increased IP [61]. Metabolomics analysis of feces showed increased levels of butyrate after dietary intervention, and the expression of TJ proteins ZO-1, occludin and claudin-1 was induced in the ileum, with consequent increased intestinal barrier function and decreased translocation of bacterial components through the intestinal wall [61] (Table 1). The same effects were also observed in mice treated with sodium butyrate, indicating a direct involvement of this bacterial metabolite in the regulation of IP [61]. Similar results were recently reported by Fachi and coll., who showed that an inulin-enriched diet protects mice from *Clostridium difficile*-induced colitis through the production of SCFAs [62]. Metabolomics analysis of feces showed the increased production of butyrate, propionate and acetate after dietary intervention (Table 1). Butyrate reduced the levels of pro-inflammatory cytokines and increased the anti-inflammatory cytokine IL-10 in the colon at the peak of infection, leading to an overall attenuation of the intestinal inflammation [62]. Butyrate induced the expression of genes associated with claudin-1 and occludin, leading to a reduction of the IP and consequently to a reduction of the microbial translocation in the liver and spleen [62].

Microbial tryptophan metabolites also play an important role in regulating barrier functions and gut microbiota activity. A metabolomic approach allowed to obtain preliminary elucidations about the role of tryptophan and its microbial and endogenous

derivatives in the regulation of immune tolerance toward intestinal microbiota [63]. Starting from these findings, further research has elucidated the role of other microbial-derived tryptophan metabolites in the regulation of gut permeability, by direct effects on epithelial cells. Venkatesh et al. showed that indole-3-propionic acid (IPA), produced by the firmicute *Clostridium sporogenes*, regulates mucosal integrity and intestinal barrier function by activating the pregnane X receptor (PXR) and upregulating junctional protein-coding mRNAs [64]. More recently, Dodd et al. used an integrated targeted-untargeted approach to identify 12 microbial metabolites derived from the reductive activity of *C. sporogenes* on aromatic amino acids (phenylalanine, tyrosyne and tryptophan), of which nine (lactate, acrylate and propionate derivatives) were reported to accumulate in host plasma [65]. The authors particularly focused on IPA and its effects on gut barrier and the mucosal immune system, and their results supported the findings of Venkatesh and coll. about the PXR-mediated effect on gut permeability [64,65] (Table 1). A treatment with 20 mg kg^{-1} IPA for four consecutive days was shown to significantly decrease the IP in HFD-fed obese T2D mice [66], which, prior to treatment, were characterized by higher IP and lower circulating IPA levels compared to lean animals. Plasma IPA amounts were also reported to increase in obese subjects 3 months after Roux-en-Y gastric bypass (RYGB) surgery [66], indicating, once again, the direct involvement of gut microbiota in the maintenance of the intestinal barrier functions. Furthermore, results from *in vitro* assays reported by the same authors showed that IPA could reduce the permeability of T84 cell monolayer compromised by pro-inflammatory cytokines [66]. Other metabolites derived from the same degradation pathway of tryptophan, i.e. indole (produced by *Escherichia coli, Clostridium bifermentans, Proteus vulgaris, Paracolobactrum coliforme, Achromobacter liquefaciens*, and *Bacteroides* spp.) [67], indole-3-acetic acid (produced by *C. sporogenes*) and tryptamine (produced by

C. sporogenes and *Ruminococcus gnavus*) [68], were also reported to exert anti-inflammatory activity both in the intestinal lumen and in the liver [68,69], and to up-regulate the expression of several proteins involved in the trans-epithelial cells linkage on the intestinal wall, such as tight junction proteins TJP1, TJP3, and TJP4, and gap junction proteins GJE1, GJB3, GJB4, and GJA8, among others [67]. A schematic resume of these results is reported in Figure 2.

In recent years, polyphenols have been widely considered for their beneficial effects on health and polyphenol-rich diets have been evaluated for the prevention of several chronic diseases, ranging from metabolic disorders to inflammation and cancer. Some studies have also evaluated the consumption of polyphenol-rich food for the prevention of diseases associated to aging, such as cognitive impairment [70] and depression [71], although up to now the reported effects have been inconsistent. However, numerous *in vitro* and animal studies show that the consumption of polyphenol-rich food could positively affect IP, reinforcing the barrier properties of the intestinal epithelium by direct influence on the synthesis and expression of tight junction proteins [72,73] or by interaction with gut microbiota. As previously described, this latter is directly involved in the metabolic transformation of plant polyphenols and in the production of smaller MW derivatives [74], which in turn contribute to the maintenance of barrier function and drives changes in gut microbiome constituents [75,76], with important effects for host health. However, although several molecular targets of dietary polyphenols and their metabolites on the intestinal epithelium have been elucidated [77], it is unclear how the interaction of the same compounds with gut microbiota leads to beneficial effects on the intestinal barrier. In recent studies, through integrated metagenomics-metabolomics analyses of feces and plasma, some authors correlated the variations of the amounts of specific gut-derived metabolites to the effects of polyphenol ingestion on IP (Table 1). It was observed that a

high-fat diet supplemented with 4% *w/w* powdered green tea leaves rich in flavanols leads to an increased intestinal population of *Akkermansia* spp. after 22 weeks [78], a bacterium that has been implied in the maintenance of a functional intestinal barrier through the preservation of mucus layer thickness [79]. Li et al. reported that the consumption of a medium-dose (20 mg/kg per day) of bilberry anthocyanin extract (BAE) promoted the generation of SCFAs (acetic acid, propionic acid and butyric acid) in aging rats, through the regulation of the intestinal microbiota [80]. Specifically, several starch-utilizing and butyrate-producing bacteria (among whom *Lactobacillus* and *Bacteroides*) were induced by BAE, while harmful species such as *Verrucomicrobia* and *Euryarchaeota* where inhibited. These variations, associated with decreased levels of TNF-α and IL-6 in the colon induced by BAE consumption, contributed to the restoring of the intestinal barrier function typically altered in older individual [80]. In a more recent work by Nieman and coll., the authors observed the effects of the association of acute moderate physical activity (sustained walking for 45 min and moderate-intensity running for 2.5 h) and a two-week flavonoid supplementation on the IP in healthy volunteers [81]. The results, obtained using a targeted metabolomics approach, showed that acute moderate exercise leads to higher circulating amounts of 15 metabolites derived from flavonoids metabolism by gut microbiota (mainly hippuric acid, methoxybenzoic acid and benzaldehyde derivatives; Table 1). The increased levels of these compounds were correlated to the significant decrease of IP observed in both "walking" and "running" groups of volunteers, although information about the mechanism(s) of action involved are lacking [81].

Overall, the data published up to now indicate that the effects of polyphenols on IP are related to both direct activity on the expression of TJ proteins and to changes induced to the intestinal microbiota, with an increase in the prevalence of species that can preserve barrier functions through the production of active metabolites or by direct action on the

mucous layer (Figure 2). On the other hand, the data supporting these observations are still scarce, and up to now only few compounds (e.g. butyrate and some gut-derived polyphenol metabolites) correlating the polyphenol-induced modifications of gut microbiota to the effects on the intestinal integrity and permeability have been discovered. Nevertheless, as demonstrated by the works of Li [80] and Nieman [81], the integration of metagenomics and metabolomics approaches for the study of the bacterial and metabolic composition of feces and biological fluids represents one of the most suitable approaches for the identification of the pathways leading to the effects of polyphenols on gut microbiota and IP, as well as for the assessment of the key metabolites involved.

Conclusion and future perspective

Although the study of the effects of dietary interventions on gut microbiota and IP and investigations of the mechanisms of action have begun only recently, it appears clear that appropriate dietary habits and the regular consumption of vegetables and fruits rich in fibers and polyphenols play an important role in the maintenance of proper intestinal functions. The precursors of SCFAs and of several indole or phenolic derivatives produced by bacterial catabolism in the intestinal lumen, for example, are abundant constituents of both plant-derived foods, as cereals, nuts, fruits and vegetables rich in non-digestible fibers [82], and animal-based foods such as dairy products, eggs and meat, which are rich sources of tryptophan [83]. Thanks to the employment of integrated multi-omics approaches, the involvement of several partners (food components, microbiota and microbial-derived compounds) in the maintenance of the intestinal barrier function and the molecular pathways behind this activity are being gradually elucidated, although further efforts are required to link specific food components and their metabolites to specific mechanisms of action. Nevertheless, the increasing amounts of data regarding

specific metabolites (e.g. physicochemical properties, spectroscopic properties, location in biofluids, involvement in metabolic pathways) stored in freely available databases and the affordability of even more sensitive and robust instrumentations will allow, in the near future, to obtain further biological information to better understand the molecular mechanisms behind the effects of diet on gut microbiota and IP. Once that both metabolites and molecular pathways will be assessed and validated for clinical relevance, they will represent novel instruments available to clinicians for the assessment of the "intestinal health" and for the development of dietary plans aimed at managing and preventing diseases directly linked to increased IP, as chronic inflammation and immunological disorders, which are determinant for the gradual decline of health in older subjects.

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FIGURES

Figure 1

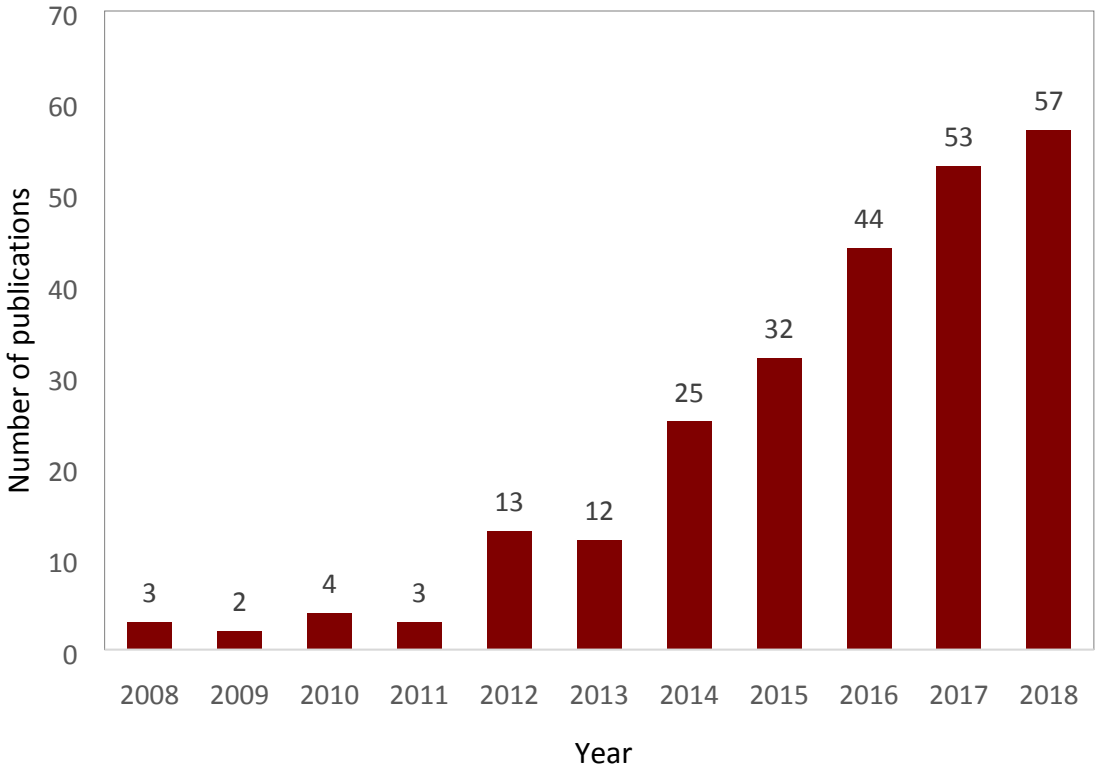
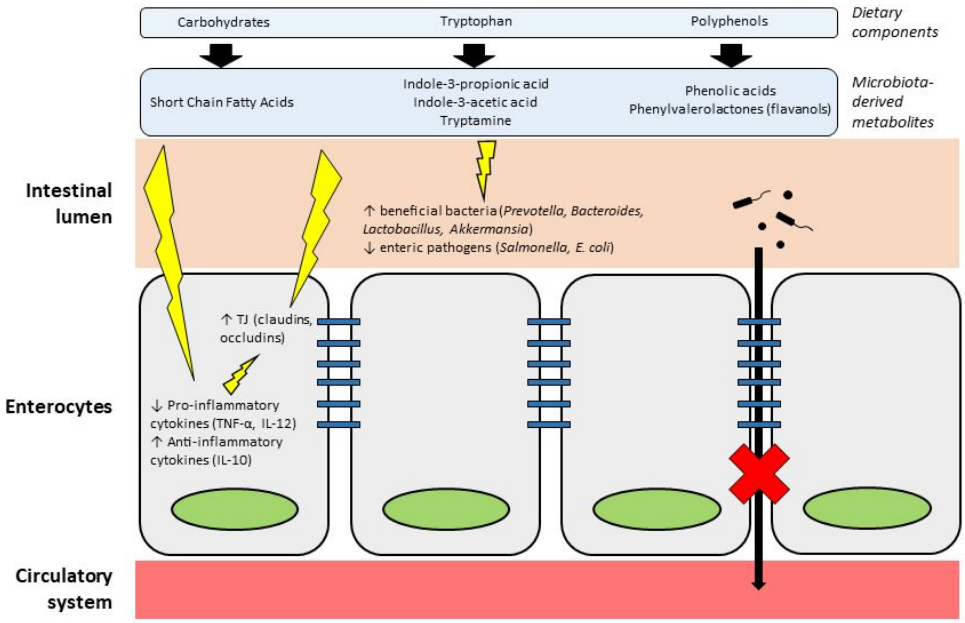


Figure 2


Carbohydrates
Tryptophan
Polyphenols
Dietary components
Short Chain Fatty Acids
Indole-3-propionic acid
Indole-3-acetic acid
Tryptamine
Phenolic acids
Phenylvalerolactones (flavanols)
Microbiota-derived metabolites
Intestinal lumen
↑ beneficial bacteria (Prevotella, Bacteroides, Lactobacillus, Akkermansia)
↓ enteric pathogens (Salmonella, E. coli)
↑ TJ (claudins, occludins)
Enterocytes
↓ Pro-inflammatory cytokines (TNF-α, IL-12)
↑ Anti-inflammatory cytokines (IL-10)
Circulatory system

TABLE

Table 1

	Intervention/ Condition	Source, dose and length of treatment	Model	Biofluid/ biomatrix analyzed	Metabolomic approach *	Gut-derived metabolites correlated to effects on IP	Main outcomes of the study **	Reference
Dietary fibers	High-fiber diet	Laboratory diet composed of 30% barley and 70% standard AIN-93 for 28 days	Mouse, healthy	Feces	Targeted GC-MS	Butyrate, propionate, acetate	• Butyrate from fiber ↓ pro-inflammatory cytokines (IL-17A, IL-6, Cxcl-1) • ↑ IL-10 and TGF-β mRNA expression • ↓ intestinal tract lesions • ↑ Claudin-1, occludin and ZO-1 • ↓ bacterial translocation	Hu et al., 2018 [61]
	Inulin-enriched diet	Laboratory diet supplemented with 5% cellulose and 25% inulin for 7 days	Mouse, healthy and colonized with 1×10^8 CFU *Clostridium difficile*	Feces	Targeted GC-MS	Butyrate, propionate, acetate	• Butyrate from fiber ↓ pro-inflammatory cytokines (IL-6, IL-1b, Cxcl-1) • ↑ anti-inflammatory cytokine IL-10 • ↓ intestinal tract lesions • ↑ Claudin-1 and occludin • ↓ bacterial translocation • ↑ intestinal barrier integrity	Fachi et al., 2019 [62]
Tryptophan	Gavage with *Clostridium. Sporogenes* and standard chow diet	Standard chow (LabDiet 5k67) containing 0.23% tryptophan for 4 weeks	Mouse, germ free colonized with *Clostridium. sporogenes* by oral gavage (~ 1×10^7 CFU)	Serum	Targeted LC-MS	Indole 3-propionic acid (IPA)	• IPA produced by *C. sporogens*, • Colonization with *C. sporogenes* ↓ intestinal permeability • IPA signals through PXR to fortify the intestinal barrier	Dodd eta al., 2017 [65]

	Gavage with probiotics (mice)/ Irritable Bowel Disease (IBD) (human)	Oral gavage with 0.6-2 × 10^8 CFU *Peptostreptococcus* species every other day, for 2 weeks (mice)	Mouse, dextran sodium sulfate-induced colitis/ Human, ulcerative colitis and Crohn's disease	Feces	Untargeted LC-MS	IPA, indoleacrylic acid (IA)	• *Peptostreptococcus* species ↑ barrier function through production of IPA and IA • IA ↓ pro-inflammatory cytokine production • IA ↑ intestinal epithelial barrier function • Microbes of IBD patients have reduced ability to cleave mucins and metabolize tryptophan • ↓ mucin utilization by gut bacteria in IBD • ↓ colonization of microbes that metabolize tryptophan in the intestine of IBD	Wlodarska et al., 2017 [69]
	High-fat diet (mice) supplemented with IPA/ Obese T2D subjects before and after RYGB (human)	Daily oral gavage with 20 mg/kg IPA for 4 consecutive days (mice)	Mouse, diet-induced obese (DIO)/ Human, obese with type-2 diabetes	Plasma	Targeted and Untargeted LC-MS, GC-MS	IPA, indoxyl 3-sulfuric acid (ISA), indole 3-acetic acid (IAA)	• IPA ↓ IP in DIO mice • ↓ IPA, IAA and ISA in obese subjects • ↑ IPA, IAA and ISA 3 months after RYGB • IPA ↓ IP in obese subjects	Jennis et al., 2018 [66]
Dietary polyphenols	Oral bilberry anthocyanin (BA) consumption in aging model	Old and young animals treated with 3 different BA doses (6 animal groups) for 10 weeks: LBA group: 10 mg/kg/dia; MBA group: 20 mg/kg/dia; HBA group: 40 mg/kg/dia	Rat, young (4 months) and old (12 months), healthy	Cecal content	Targeted GC-FID	Butyrate, propionate, acetate	• BA ↑ starch-utilizing and butyrate-producing bacteria • BA ↓ inflammatory factors (TNF-α, IL-6) and mucosa damages in the colon	Li et al., 2019 [80]
	Combination of flavonoid supplementation and moderate physical exercise (45 min walking and 2.5 h running)	Capsule containing 329 mg total flavonoids: bilberry fruit extract (64 mg anthocyanins), green tea leaf extract (184 mg total flavan-3-ols), 104 mg quercetin aglycone.	Human, healthy	Plasma	Targeted LC-MS	Hippuric acid, 3-hydroxyhippuric acid, quercetin-3-O-glucuronide, delphinidin-3-O-glucoside, 4-hydroxycinnamic acid, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, 3-(3-hydroxy-4-	• Physical exercise ↑ absorption of gut-derived flavonoid metabolites • Flavonoid consumption associated to physical exercise ↓ IP • Flavonoids and their gut-transformed metabolites ↑ intestinal barrier integrity	Nieman et al., 2019 [81]

	1 capsule/dia for “walking” group; 2 capsules/dia for “running” group. Supplementation time: 2 weeks	methoxyphenyl)propanoic acid-3-O-glucuronide, methoxybenzoic acid derivatives, benzaldehyde derivatives

* LC-MS: Liquid Chromatography coupled to Mass Spectrometry; GC-MS: Gas Chromatography coupled to Mass Spectrometry; GC-FID: Gas Chromatography coupled to Flame Ionization Detector.
** ↓ indicates “decrease”; ↑ indicates “increase”.

FIGURE CAPTIONS

Figure 1. The increase of the scientific literature regarding the use of metabolomics in the study of the interactions between diet and gut microbiota during the last 11 years. Source: PubMed (https://www.ncbi.nlm.nih.gov/pubmed/).

Figure 2. Schematic representation of the mechanisms of action responsible for the effects of microbiota-derived dietary metabolites on intestinal permeability.

TABLE CAPTION

Table 1. Summary of the studies involving the application of metabolomics to the study of the effects of diet-gut microbiota interactions on intestinal permeability *in vivo*.

GRAPHICAL ABSTRACT (TOC)