

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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1 **Abstract**

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

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

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26 **Introduction**

27 The gastrointestinal tract (GI) is responsible for a wide range of functions, including
28 digestion and absorption of nutrients, water and ions, regulation of host immunity,
29 protection against the ingress of pathogenic microorganism, and the the metabolism and
30 detoxification of xenobiotics. The GI also hosts the largest microbial population of the
31 human body, which works in symbiosis with the host to accomplish these various
32 intestinal functions. Gut bacteria are particularly important for host health, being involved
33 in the synthesis of vitamins, secondary bile acids and neurotransmitters, and playing a
34 direct role in the metabolism and degradation of dietary components and drugs, that can
35 affect their bioavailability and absorption ¹. It has been estimated that over than 1,000
36 different bacterial species populate the intestinal environment, with a genome comprising
37 100-fold more genes than those found in human genome ². The physiological variations
38 in the small intestine and colon, such as the presence of distinct chemical environments,
39 nutrients and host immune activity allow distinct groups of bacterial species to populate
40 the different regions of the lower gastrointestinal tract ^{3,4}, and this variability becomes
41 even more complex considering the inter-individual variations and the influence of host
42 genetics ⁵⁻⁷. Nevertheless, most human gut microbiota share a core set of resident bacteria
43 and related microbial genes ^{8,9}. *Firmicutes*, *Bacteroidetes* and, secondly, *Actinobacteria*
44 are the three most abundant phyla, among the over 50 that have been identified by
45 metagenomic approaches ^{10,11}. A synergistic equilibrium among the different species and
46 the maintenance of a microbial diversity are of crucial importance for health, since the
47 microbiota plays a central role on the proper functioning of the intestinal barrier and
48 maintaining appropriate intestinal permeability (IP), which is directly involved in the
49 development of numerous disorders. In this vein, a low diversity and a scarce abundance
50 of species as *Bifidobacterium* spp. and *Faecalibacterium prausnitzii* have been associated

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

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

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

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

101 ³⁷. Focusing on the application of metabolomics in the study of diet-microbiota
102 interactions and searching for the keywords “metabolomics AND diet AND microbiota”
103 in PubMed, we found that the number of publications almost doubled from 2014 to 2018,
104 as an index of the popularity that metabolomics gained during the recent years (Figure 1).
105 Metabolomic approaches have been widely used to study the transformation of nutrients
106 and xenobiotics by intestinal microbiota ³⁸⁻⁴³, thus allowing the characterization of
107 hundreds of metabolites derived from macro- and micronutrients and polyphenols coming
108 from fruits and vegetables. In 2009, Jacobs published a first review article regarding the
109 role of colonic microbiota in the degradation of non-digestible food ingredients and their
110 impact on gut health and immunity ⁴⁴. For the first time, the importance of metabolomics
111 in the study of the links between the bioconversion of non-digestible food ingredients,
112 their bioavailability and their downstream effects on microbiota composition and host
113 metabolism was recognized ⁴⁴. More recently, the use of integrated multi-omics
114 approaches has facilitated the study of the molecular interactions between diet and
115 microbiota, and has led to the identification of several metabolites that are produced as a
116 result of microbial metabolism of various dietary constituents. Nevertheless, considering
117 the challenges to study the mutual relationship between gut microbiota and the host, its
118 tight connection with diet, environment and lifestyle, and the still incomplete
119 characterization of the huge microbial metabolome, the path to assess precise and
120 validated metabolites to link the microbial activity to specific effects on health is just
121 starting. In a way to find a clinical relevance of metabolomics data and offer to clinicians
122 a robust tool to predict, prevent and treat several diseases, further progress is necessary.
123 The aim of this work was to review the most recent literature regarding the application of
124 metabolomics in the study of the interactions between food components and gut
125 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.

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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

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

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

176 digestible carbohydrates, and alterations in diet lead to variations in the production of
177 intestinal butyrate. In aged mice, the increased butyrate production after the consumption
178 of high doses of soluble fiber was associated with an induced expression of the TJ proteins
179 Tjp2 and Ffar2 and to a counterbalance of the age-related microbiota dysbiosis, with a
180 significant amelioration of the increased IP condition typical of older individuals ⁶⁰.
181 Similar effects of a high fiber diet were also observed in mice affected by autoimmune
182 hepatitis, characterized by an imbalance of Treg/Th17 cells and increased IP ⁶¹.
183 Metabolomics analysis of feces showed increased levels of butyrate after dietary
184 intervention, and the expression of TJ proteins ZO-1, occludin and claudin-1 was induced
185 in the ileum, with consequent increased intestinal barrier function and decreased
186 translocation of bacterial components through the intestinal wall ⁶¹ (Table 1). The same
187 effects were also observed in mice treated with sodium butyrate, indicating a direct
188 involvement of this bacterial metabolite in the regulation of IP ⁶¹. Similar results were
189 recently reported by Fachi and coll., who showed that an inulin-enriched diet protects
190 mice from *Clostridium difficile*-induced colitis through the production of SCFAs ⁶².
191 Metabolomics analysis of feces showed the increased production of butyrate, propionate
192 and acetate after dietary intervention (Table 1). Butyrate reduced the levels of pro-
193 inflammatory cytokines and increased the anti-inflammatory cytokine IL-10 in the colon
194 at the peak of infection, leading to an overall attenuation of the intestinal inflammation
195 ⁶². Butyrate induced the expression of genes associated with claudin-1 and occludin,
196 leading to a reduction of the IP and consequently to a reduction of the microbial
197 translocation in the liver and spleen ⁶².
198 Microbial tryptophan metabolites also play an important role in regulating barrier
199 functions and gut microbiota activity. A metabolomic approach allowed to obtain
200 preliminary elucidations about the role of tryptophan and its microbial and endogenous

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

226 *C. sporogenes* and *Ruminococcus gnavus*)⁶⁸, were also reported to exert anti-
227 inflammatory activity both in the intestinal lumen and in the liver^{68,69}, and to up-regulate
228 the expression of several proteins involved in the trans-epithelial cells linkage on the
229 intestinal wall, such as tight junction proteins TJP1, TJP3, and TJP4, and gap junction
230 proteins GJE1, GJB3, GJB4, and GJA8, among others⁶⁷. A schematic resume of these
231 results is reported in Figure 2.

232 In recent years, polyphenols have been widely considered for their beneficial effects on
233 health and polyphenol-rich diets have been evaluated for the prevention of several chronic
234 diseases, ranging from metabolic disorders to inflammation and cancer. Some studies
235 have also evaluated the consumption of polyphenol-rich food for the prevention of
236 diseases associated to aging, such as cognitive impairment⁷⁰ and depression⁷¹, although
237 up to now the reported effects have been inconsistent. However, numerous *in vitro* and
238 animal studies show that the consumption of polyphenol-rich food could positively affect
239 IP, reinforcing the barrier properties of the intestinal epithelium by direct influence on the
240 synthesis and expression of tight junction proteins^{72,73} or by interaction with gut
241 microbiota. As previously described, this latter is directly involved in the metabolic
242 transformation of plant polyphenols and in the production of smaller MW derivatives⁷⁴,
243 which in turn contribute to the maintenance of barrier function and drives changes in gut
244 microbiome constituents^{75,76}, with important effects for host health. However, although
245 several molecular targets of dietary polyphenols and their metabolites on the intestinal
246 epithelium have been elucidated⁷⁷, it is unclear how the interaction of the same
247 compounds with gut microbiota leads to beneficial effects on the intestinal barrier. In
248 recent studies, through integrated metagenomics-metabolomics analyses of feces and
249 plasma, some authors correlated the variations of the amounts of specific gut-derived
250 metabolites to the effects of polyphenol ingestion on IP (Table 1). It was observed that a

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

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

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

285

286 **Conclusion and future perspective**

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

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

312

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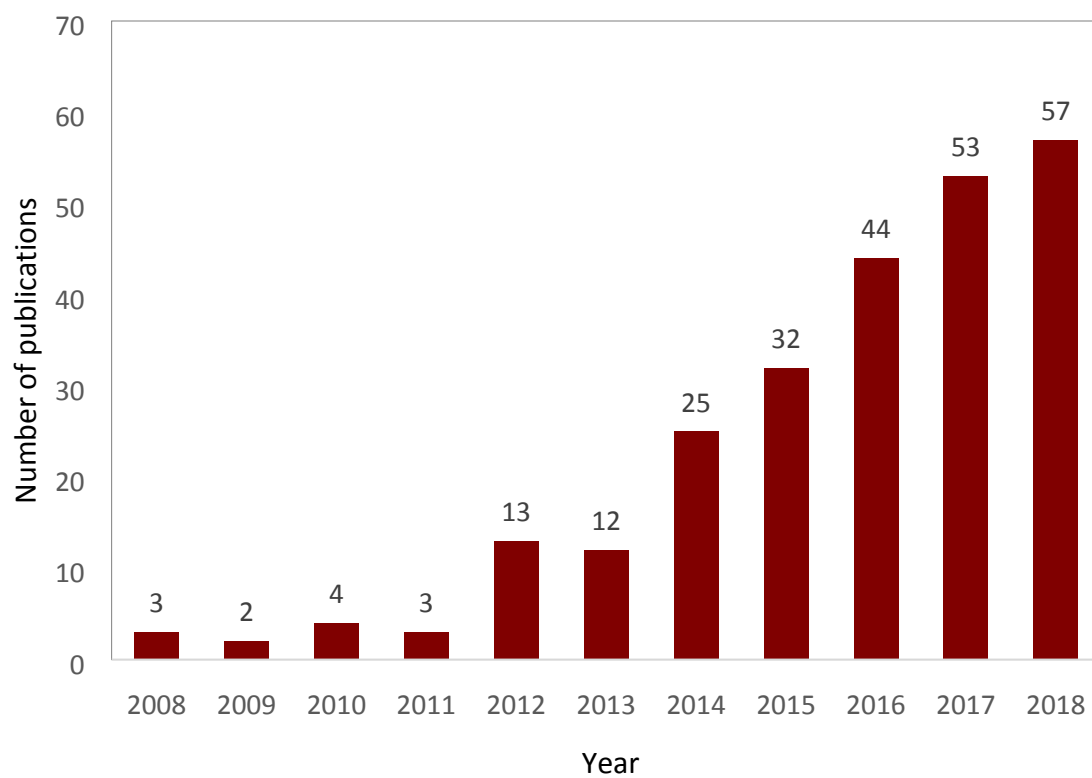
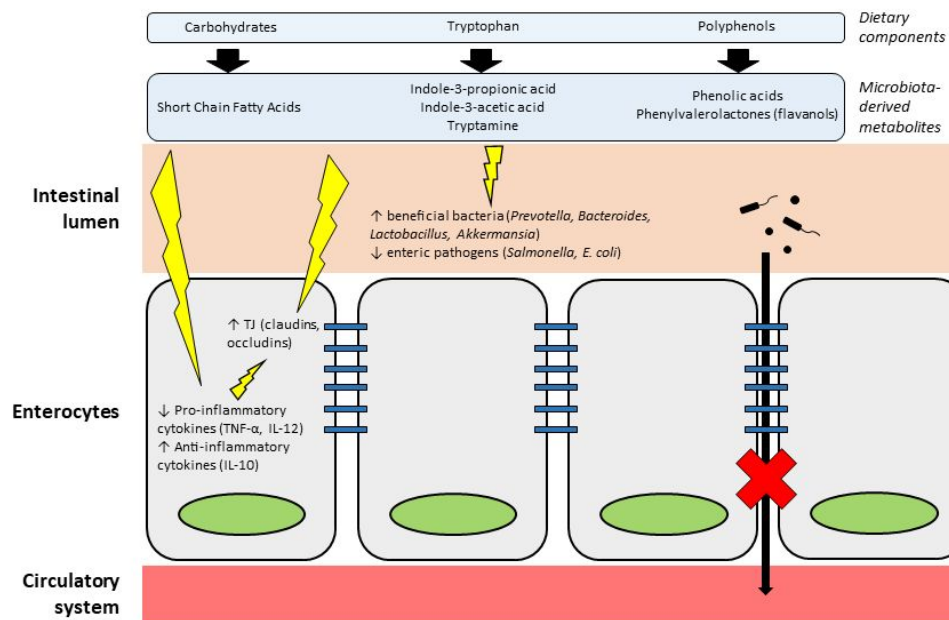
FIGURES**Figure 1**

Figure 2



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	<ul style="list-style-type: none"> • 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 <i>Clostridium difficile</i>	Feces	Targeted GC-MS	Butyrate, propionate, acetate	<ul style="list-style-type: none"> • 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 <i>Clostridium. Sporogenes</i> and standard chow diet	Standard chow (LabDiet 5k67) containing 0.23% tryptophan for 4 weeks	Mouse, germ free colonized with <i>Clostridium. sporogenes</i> by oral gavage (~ 1 $\times 10^7$ CFU)	Serum	Targeted LC-MS	Indole 3-propionic acid (IPA)	<ul style="list-style-type: none"> • IPA produced by <i>C. sporogenes</i>, • Colonization with <i>C. sporogenes</i> ↓ intestinal permeability • IPA signals through PXR to fortify the intestinal barrier 	Dodd et al., 2017 ⁶⁵

	Gavage with probiotics (mice)/ Irritable Bowel Disease (IBD) (human)	Oral gavage with $0.6-2 \times 10^8$ CFU <i>Peptostreptococcus</i> 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)	<ul style="list-style-type: none"> • <i>Peptostreptococcus</i> species \uparrow barrier function through production of IPA and IA • IA \downarrow pro-inflammatory cytokine production • IA \uparrow intestinal epithelial barrier function • Microbes of IBD patients have reduced ability to cleave mucins and metabolize tryptophan • \downarrow mucin utilization by gut bacteria in IBD • \downarrow 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)	<ul style="list-style-type: none"> • IPA \downarrow IP in DIO mice • \downarrow IPA, IAA and ISA in obese subjects • \uparrow IPA, IAA and ISA 3 months after RYGB • IPA \downarrow 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	<ul style="list-style-type: none"> • BA \uparrow starch-utilizing and butyrate-producing bacteria • BA \downarrow 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-	<ul style="list-style-type: none"> • Physical exercise \uparrow absorption of gut-derived flavonoid metabolites • Flavonoid consumption associated to physical exercise \downarrow IP • Flavonoids and their gut-transformed metabolites \uparrow 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)

