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Flavonoids and the gastrointestinal tract: Local and systemic effects

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ABSTRACT

The gastrointestinal (GI) tract plays a central role in the absorption, distribution, metabolism, and excretion of flavonoids, which ultimately define the health effects of these bioactives. These aspects are modulated by the interactions of flavonoids with other dietary components, environmental factors, the host, and the GI microbiota. Flavonoid can target molecules in the luminal content, the different GI tract cell types, and the microbiota. Importantly, flavonoid actions at the GI tract can have an impact systemically, e.g. on glucose homeostasis, lipid and energy metabolism, or cardiovascular risk factors. The beneficial actions of flavonoids at the GI include their capacity to: i) protect the intestinal epithelium against pharmacological insults and food toxins; ii) modulate the activity of enzymes involved in lipid and carbohydrate absorption; iii) maintain the intestinal barrier integrity; iv) modulate the secretion of gut hormones; v) modulate the GI tract immune system; vi) exert potential anti-colorectal cancer activity; and vii) shape microbiota composition and function. Further understanding of the mechanisms mediating the effects of flavonoids on the intestine (and its microbiota) is of critical importance given the relevance of the GI tract on sustaining overall health and of the widespread recommendations of increasing the intake of plant bioactives.

1. Introduction

The gastrointestinal (GI) tract, and particularly the intestinal barrier (Wells et al., 2017), plays a central role in sustaining health. The multiple functions of the intestinal epithelium include: i) nutrient absorption; ii) to act as a barrier providing a line of defense against the entrance of bacteria, bacterial toxins and byproducts, and toxins present in ingested foods; iii) to maintain the GI tract immune homeostasis; and iv) to regulate important aspects of body energy metabolism including satiety and energy expenditure. Furthermore, the microbiota present in the intestinal lumen is a key contributor to whole body physiology.

The cross talk among host, diet, environmental factors, and the microbiota has been found to either sustain health or to underlie the pathophysiology of common diseases and unwanted conditions, e.g., inflammatory bowel diseases (IBD), celiac disease, diabetes, obesity-associated insulin resistance, and non-alcoholic fatty liver disease (NAFLD) (Fasano and Shea-Donohue, 2005; Odenwald and Turner, 2017).

The provision of nutrients and bioactives that can optimize GI functions is a theme of current relevance. In this direction, this review addresses current knowledge of the actions of dietary flavonoids regulating GI tract physiology, including their interactions with the gut microbiota. Chemically, flavonoids are constituted by two aromatic rings (A and B) linked through three carbons that usually form an oxygenated heterocycle (C-ring). This C ring is characteristic of each flavonoid subfamily. Among the subfamilies, monomers differ in the type, location and number of substituents (e.g. hydroxyl groups), and some flavonoids can also form oligomers. We will focus on three major flavonoid subfamilies, i.e. flavanols and their polymers (proanthocyanidins), flavonols and

Abbreviations: CRC, colorectal cancer; DPP-IV, dipeptidyl peptidase IV; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin gallate; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GI, gastrointestinal; IBD, inflammatory bowel diseases; LPS, lipopolysaccharides; NAFLD, nonalcoholic fatty liver disease; T2D, type 2 diabetes; TJ, tight junction; TNFα, tumor necrosis factor alpha.

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anthocyanins. These three families were selected based on their abundance in the diet, and the growing body of experimental evidence pointing to their beneficial actions at the GI tract.

2. The GI tract and flavonoid metabolism

We will only discuss general aspects of the role of the GI tract on flavonoid absorption and metabolism; this review will not address specific and chemical aspects of flavonoid metabolism given that they have been recently and extensively reviewed (Crozier et al., 2010; Kay et al., 2017; Williamson and Clifford, 2017). It has been well established that flavonoid bioavailability at the GI tract is determined by flavonoid structure, interactions with the food matrix, the activity of GI hydrolytic enzymes, the composition of the microbiota, and intestinal epithelial cell transporters (reviewed in (Gonzales et al., 2015). Flavonoids can be absorbed as parent compounds, after conjugation (e.g. methylation, sulfation) and/or after other chemical modifications. Flavonoids can be metabolized both by enzymes present in intestinal epithelial cells and to a large extent by the microbiota. Overall, after ingestion, flavonoids have three main potential metabolic fates, which define their molecular targets and their biological activities (Fig. 1). The first is to exert direct effects at the GI tract, which can be mediated by parent compounds or metabolites. Such effects can be of considerable importance given the high concentrations flavonoids can reach in the stomach and in the upper part of the intestine. Examples of these actions are those exerted on GI tract epithelial, endocrine and immune cells, and those exerted on the gut lumen content, e.g. direct antioxidant actions. Local actions can also in part underlie flavonoid systemic actions, e.g. changes in GI tract hormone release, which affects systemic glucose homeostasis. Secondly, flavonoids can interact with the microbiota leading to changes in microbiota profiles, e.g. benefiting the growth of beneficial bacteria. This metabolism of flavonoids by the microbiota leads to the production of smaller molecules that can be absorbed, entering the circulation where they are able to reach distal organs (Williamson and Clifford, 2017) (Fig. 1). For example, the flavan-3-ol (−)-epicatechin (EC) is metabolized by the microbiota to 5C-ring fission metabolites, i.e. 5-(hydroxyphenyl)-γ-valerolactones and 5-(hydroxyphenyl)−γ-hydroxyvaleric acids, which are absorbed and, as evaluated through urinary metabolites, account for 42% of the ingested EC (Borges et al., 2017). Importantly, while flavonoid polymers (e.g. proanthocyanidins) are poorly or not absorbed at the GI tract, their metabolism by the microbiota leads to compounds, e.g. valerolactones and phenolic acids, that are absorbed and can have systemic effects (Appeldoorn et al., 2009; Zhang et al., 2016).

The third potential fate is the biotransformation of flavonoids by intestinal epithelial cells. In this case, conjugates formed in the intestinal epithelium can either be transported into the bloodstream or alternatively, secreted back into the gut lumen, e.g. secretion to the lumen of EC sulfated by epithelial cells (Actis-Goretta et al., 2013), where they may be further metabolized and/or exert local biological actions.

Thus, the initial metabolism of flavonoids at the GI tract is highly relevant to flavonoid health effects as metabolism can modify flavonoid absorption and flavonoid metabolites can be biologically more or less active than the parent compounds. In summary, flavonoids and their metabolites can exert their biological actions both locally and systemically.

3. Flavonoids and the GI tract in health and disease

Flavonoids' actions at the GI tract can target the lumen content and/or the different cell types that are involved in sustaining GI tract physiology. The intestinal monolayer is mainly composed of: i) intestinal epithelial cells involved in nutrient absorption, immunoglobulin transcytosis, preservation of barrier integrity and regulation of trans- and paracellular transport; ii) goblet cells which produce the mucins that act as the first physical barrier against bacteria and toxins and iii) Paneth cells which secret bactericidal peptides. Immune cells located at the lamina propria act in conjunction with the above mucosal cells to integrate the GI

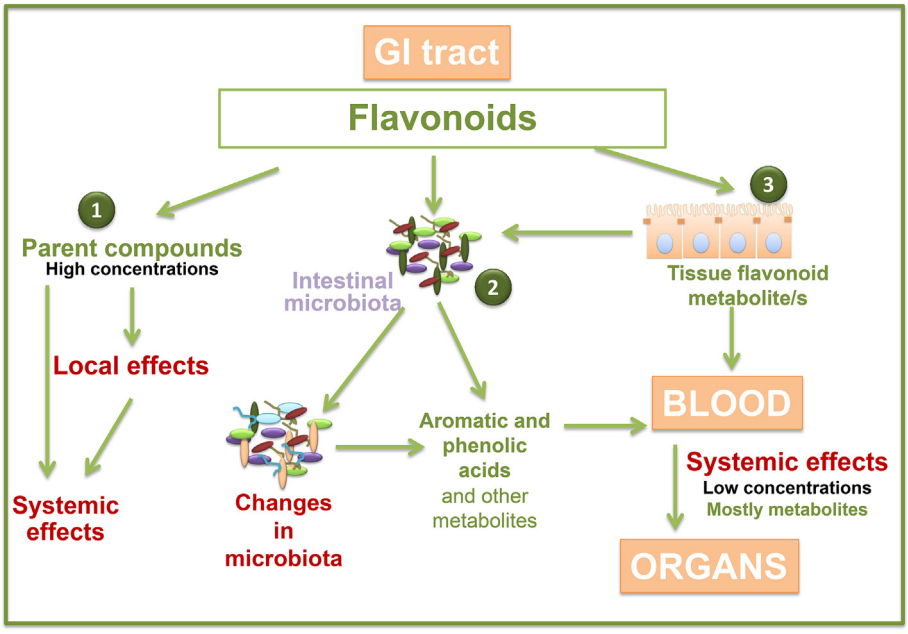

Fig. 1. Fate of dietary flavonoids at the GI tract. At the GI tract dietary flavonoids can (**1**) as parent compounds exert local effects favored by their high concentrations, (**2**) interact with the microbiota changing its profile and/or be metabolized mainly to aromatic and phenolic acids which can be absorbed (systemic effects) and/or exert local effects, and (**3**) be metabolized, usually to conjugates of the parent compounds by intestinal epithelial cells; subsequently metabolites may be absorbed (systemic effects) or excreted to the gut lumen (local effects). Local effects can also extend systemically, e.g. when they lead to the secretion of gut hormones with systemic actions.

tract immune system (Brown et al., 2013).

Among the beneficial actions at the GI tract, flavonoids have been found to: i) protect the intestinal wall against pharmacological insults and food toxins; ii) maintain the intestinal barrier integrity; iii) modulate the secretion of gut hormones; iv) modulate the GI tract immune system; v) have potential anti-colorectal cancer activity; and vi) shape microbiota profiles (Fig. 2).

3.1. Flavonoids and protection of the GI tract against luminal insults

Some frequently used therapies, such as nonsteroidal anti-inflammatory drugs, have been found to cause damage to the intestinal epithelium which can be mitigated by flavonoids. For example, the flavonol quercetin inhibited diclofenac-induced and ranitidine aggravated GI toxicity, including bleeding, loss of serum proteins, intestinal permeabilization, and restoring luminal pH in rats (Singh et al., 2017). *In vitro*, quercetin inhibited indomethacin-induced Caco-2 monolayer permeabilization (measured as trans-epithelial electrical resistance), restoring the expression of the tight junction (TJ) proteins ZO-1 and occludin (Carrasco-Pozo et al., 2013). In fact, quercetin *per se*, and to a lesser extent, the related flavanol myricetin, enhanced transepithelial electrical resistance and decrease paracellular transport in Caco-2 monolayers. Quercetin appears to act in part by modulating ZO-2, occludin, and claudin-1 assembly at the TJ via protein kinase C inhibition (Suzuki and Hara, 2009).

The intestinal epithelium is also exposed to toxins present in the diet (either in foods or generated in the lumen by interactions among dietary components), and to toxins, known as endotoxins, locally produced by the microbiota. As an example of the former, consumption by rats of polyunsaturated fatty acids in conjunction with heme iron has been demonstrated to lead to the generation of lipid oxidation products, e.g., malondialdehyde and 4-hydroxynonenal, which can exert negative effects within the colon or be absorbed where they can have systemic pro-oxidant effects (Gueraud et al., 2015). Sterol oxidation products constitute other major absorbable food toxins and these compounds can also potentially exert local adverse effects, e.g., meat consumption and potential promotion of GI cancers (Biasi et al., 2008), or after absorption, lead to systemic adverse effects. In Caco-2 cells, oxysterols and particularly 7β-hydroxycholesterol, promoted NADPH oxidase activation and inflammatory responses which were inhibited by the flavanol-3-ol (−)-epigallocatechin gallate (EGCG) (Mascia et al., 2010).

While a direct antioxidant action of flavonoids in most tissues and compartments are highly unlikely (Fraga, 2007; Galleano et al., 2010), the GI tract is the only part of the body in which the biological relevance of scavenging of free radicals is realistic. Both thermodynamic and kinetic considerations stress that only in the GI tract flavonoids reach high enough concentrations to inhibit free radical reactions by scavenging radicals and chelating redox-active metals (Galleano et al., 2010).

3.2. Flavonoids and the GI tract in inflammatory/metabolic diseases

3.2.1. Flavonoids and intestinal permeability

The function of the intestinal epithelium as a barrier against the entrance of bacterial and food toxins is largely achieved by the TJ (Odenwald and Turner, 2017). TJs constitute a complex of proteins located close to the apical side of adjacent epithelial cells that physiologically regulate the paracellular transport of water and ions. Structurally, TJ proteins, e.g. claudins, occludin, ZO-1, and

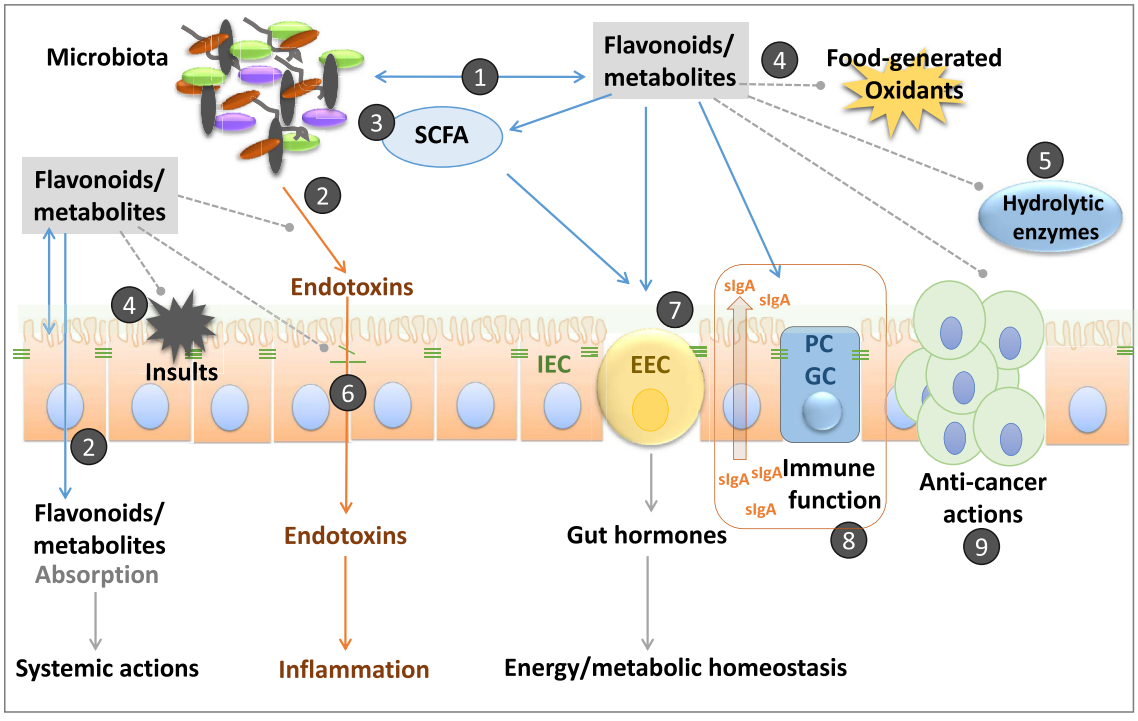

Fig. 2. Mechanisms of action of dietary flavonoids at the GI tract. At the GI tract flavonoids/metabolites can (**1**) positively shape the microbiota, (**2**) inhibit the production of deleterious endotoxins; and (**3**) positively modulate the production of beneficial short chain fatty acids (SCFA). Flavonoids/metabolites can exert other beneficial effects at the GI tract by: (**4**) preventing the harmful effects of food, e.g. oxidants and pharmacological insults; (**5**) inhibiting the activity of hydrolytic enzymes, e.g. pancreatic lipase, amylase; (**6**) mitigating intestinal permeabilization and the associated paracellular transport of endotoxins that can initiate local/systemic inflammation; (**7**) modulating the secretion of gut hormones by enteroendocrine cells (EEC) which have both local effects and can systemically modulate energy and metabolic homeostasis; (**8**) modulating the GI tract immune system, e.g. Paneth cells (PC); and (**9**), exerting anti-cancer actions.

junctional adhesion molecules (JAMs), associate with actin filaments. TJ disruption can occur as a result of chronic inflammation, autoimmune disorders, diet components, (e.g. fat), toxins, obesity, and several diseases, e.g. type 2 diabetes (T2D) and IBD. Alterations in TJ lead to increased intestinal permeability which can allow the passage of bacteria, bacterial wall endotoxins (lipopolysaccharides (LPS)), and other toxins which can initiate local, and systemic inflammation if they reach the blood. Increased blood LPS, i.e. endotoxemia, exposes different organs to LPS, which upon binding to the toll-like receptor 4 (TLR4) initiates processes of inflammation and subsequent tissue damage. Endotoxemia is proposed as an underlying mechanism of the development of NAFLD and hepatic insulin resistance, cardiovascular disease, adipose inflammation, and other pathologies observed in obese individuals and in animal models of obesity and T2D (Cani et al., 2008). Significantly, fat and energy intake are associated with endotoxemia both in healthy (Amar et al., 2008; Laugerette et al., 2014) and in T2D individuals (Harte et al., 2012). In high fat-fed and obese mice, increased intestinal permeability and endotoxemia is proposed to be secondary to the dietary fat and associated increase in luminal bile acids rather than to obesity *per se* (Suzuki and Hara, 2010). Dysbiosis of the microbiota can be another source of chronic endotoxemia contributing to the pathophysiology of T2D and obesity (Cani et al., 2008; Shen et al., 2013).

A link between the protection of the intestinal barrier by flavonoids and the onset of metabolic/systemic diseases has been observed in experimental animals. For example, both the flavanol EC and the flavonol quercetin have been demonstrated to improve insulin sensitivity and modulate systemic inflammation in rodent models of overfeeding (high fructose and high fat diets) (Bettaieb et al., 2014; Gutierrez-Salmean et al., 2014; Vazquez Prieto et al., 2015). Furthermore, EC and EC-rich foods have been consistently shown to improve insulin sensitivity in healthy, obese and glucose-intolerant humans (Bettaieb et al., 2016; Cremonini et al., 2016; Davison et al., 2008; Dower et al., 2015; Grassi et al., 2005, 2008; Shrime et al., 2011). These beneficial effects of EC and quercetin could be due, in part, through their actions at the GI tract. In fact, we recently observed that EC capacity to mitigate insulin resistance and inflammation in high fat diet-fed C57BL/6 J mice is associated with its ability to mitigate intestinal permeabilization and endotoxemia (Cremonini et al., 2018). Mechanistically, high levels of fat intake cause disruptions of TJ structure (decreased expression of TJ proteins) and increase paracellular transport (Suzuki and Hara, 2010). EC supplementation prevents high fat consumption-associated intestinal permeability by regulating upstream events that modulate TJ structure/function: i) inhibition of barrier disrupting signals (NF-κB, ERK1/2); ii) decreased NADPH oxidase overexpression and oxidative damage; and iii) upregulation of the gut hormone glucagon-like peptide-2 (GLP-2), which stabilizes the TJ. In Caco-2 cell monolayers, an *in vitro* model of intestinal epithelial cell monolayers, EC has also been reported to prevent tumor necrosis factor alpha (TNFα)-induced permeabilization (Contreras et al., 2015). This effect was mediated by EC capacity to inhibit the NADPH oxidase-dependent activation of ERK1/2 and NF-κB (Contreras et al., 2015). Consistent with a potential effect of flavanols and related proanthocyanidins on maintenance of intestinal permeability, supplementation with a grape seed extract rich in proanthocyanidins, EC, catechin and EC gallate prevented the downregulation of the TJ protein ZO-1 induced by a cafeteria-type diet in rats (Gil-Cardoso et al., 2017). EGCG assayed at pharmacological doses (100 μM) protected T84 cell monolayers from permeabilization induced by interferon gamma, but not that triggered by interleukin-4 or enteropathogenic *Escherichia coli* (Watson et al., 2004). These findings stress the selectivity of different flavonoids towards particular mechanisms, especially inflammatory targets, underlying intestinal barrier permeabilization.

Pure anthocyanins and plant extracts rich in have also been shown to protect Caco-2 cell monolayers from inflammation-induced permeabilization (Cremonini et al., 2017, 2018; Olejnik et al., 2016). A differential capacity to prevent TNFα-mediated permeabilization is observed among different anthocyanins. For example, O-glucosides of cyanidin and delphinidin were more efficient than the O-glucosides of malvidin, peonidin and petunidin in the mitigation of TNFα-induced increase in paracellular transport and loss of monolayer electrical resistance (Cremonini et al., 2017). These results stress the concept that there is a chemical structure- and conformation-related specificity of the biological effects of flavonoids in intestinal cells.

The observed capacity of select flavonoids to mitigate the adverse effects of metabolic diseases may also occur through their actions on different organs, e.g. liver, pancreas, adipose tissue, and brain, beyond their initial effects at the GI tract. However, the fact that non-absorbable proanthocyanidins can mitigate obesity in high fat-high fructose-fed mice provides evidence that some polyphenols could mitigate the pathophysiology of metabolic diseases directly at the GI tract (Masumoto et al., 2016). While this can in part occur through procyanidin-induced shaping of the microbiota (Masumoto et al., 2016) and their breakdown to absorbable metabolites, proanthocyanidins could also act by directly modulating intestinal cell signaling cascades through interactions with the cell membrane (Verstraeten et al., 2003, 2013), protecting the intestinal barrier from permeabilization (Erlejman et al., 2006) and/or regulating the secretion of gut hormones (section 3.2.).

Finally, several flavonoids have been reported to inhibit the activity of gut metabolizing enzymes including pancreatic lipoprotein lipase, alpha glucosidase, and amylase. As an example, anthocyanidins from muscadine grapes have been reported to inhibit both lipoprotein lipase and alpha glucosidase (You et al., 2011). It is well established that the chemical structure of flavonoids is important for these inhibitory actions, with the presence of galloyl moieties and degree of procyanidin polymerization enhancing the *in vitro* inhibition of lipoprotein lipase activity (Nakai et al., 2005). *In vivo*, proanthocyanidins also have been demonstrated to inhibit triglyceride absorption in mice and in humans (Sugiyama et al., 2007). Both EGCG and (−)-epigallocatechin 3-O-(3-O-methyl) gallate present in oolong tea inhibited α-amylase (Fei et al., 2014). In the GI tract lumen, the inhibition of the above enzymes would result in a decreased absorption of fatty acids from triglycerides and glucose from complex carbohydrates, which would be beneficial in the framework of obesity and metabolic diseases.

3.2.2. Flavonoids and the GI tract endocrine system

Enteroendocrine cells distributed along the length of the GI tract secrete several hormones, e.g. GLP-2, gastrin, somatostatin, ghrelin, and cholecystokinin, and incretins, e.g. glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Gribble and Reimann, 2016). These peptides respond to food intake and play major roles, regulating satiety and metabolic responses, particularly glucose metabolism. Thus, the modulation of their tissue levels (e.g. by flavonoids) can be of relevance in the mitigation of obesity and Western style diet-induced metabolic disorders. We will particularly discuss the actions of flavonoids on GLP-1 and GLP-2, given their critical local and systemic effects.

GLP-1 and GLP-2 are secreted by intestinal L cells, and are originated from the cleavage of proglucagon by prohormone convertase. Once released into the bloodstream, they are rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP-IV) (Deacon et al., 1995). GLP-1 binds to a G-coupled receptor, which is present in large amounts in the pancreas (both in alpha and beta cells) and

in the brain (Campbell and Drucker, 2013). GLP-1 regulates satiety and improves glucose homeostasis, lipid metabolism, and gut motility (Campbell and Drucker, 2013). The capacity of GLP-1 to improve glycemic control and decrease body weight is so relevant and recognized, that several agonists of its receptor have been developed and are currently used for the management of T2D (Rajeev and Wilding, 2016). In the case of GLP-2, its main functions are to modulate gut physiology to increase nutrient absorption, improve barrier function, and exert trophic and protective actions in the small intestine (Drucker and Yusta, 2014). Recent research has shown that GLP-2 is also relevant in modulating energy balance (Baldassano et al., 2016).

Flavonoids may increase circulating GLP-1 and GLP-2 by acting at different levels, including increases in: i) expression of proglucagon; ii) prohormone convertase activity; iii) hormone release by enteroendocrine cells; and iv) number of enteroendocrine cells. Flavonoids may also inhibit DPP-IV, increasing the half-life of GLP-1 and GLP-2 in the circulation. The effects of pure flavanols and of plant extracts containing flavanols and proanthocyanidins on GLP-1 have been recently reviewed (Pinent et al., 2017). Overall, an increase in plasma GLP-1 has been observed upon acute and chronic consumption of proanthocyanidins, flavanols and other monomeric polyphenols. Gavage of glucose and a grape seed extract containing a mixture of these compounds caused a rapid increase in GLP-1 plasma levels which was associated with a trend for lower activity of intestinal, but not plasma, DPP-IV activity (Gonzalez-Abuin et al., 2014). The grape seed extract also caused an increase in plasma GLP-1 which was proposed to be due to increased GLP-1 secretion (Gonzalez-Abuin et al., 2014). In fact, the anthocyanin delphinidin 3-rutinoside present in grapes has been demonstrated to promote *in vitro* GLP-1 secretion from GLUTag cells, a model of enteroendocrine cells, through the activation of Ca^{2+}/calmodulin-dependent kinase II (Kato et al., 2015). Regarding the effects of flavonoids on GLP-2, published evidence is almost nonexistent. However, since GLP-2 originates from the same precursor and shares a similar processing mechanism with GLP-1, it is likely that GLP-2 can also be affected by flavonoids. In fact, we recently observed that mice supplemented with EC have 1-fold higher plasma levels of GLP-2 than controls (Cremonini et al., 2017b).

Select components of the microbiota can also regulate gut hormone production and/or secretion. For example, prebiotics have been reported to increase plasma GLP-2 concentrations and decrease intestinal permeability in ob/ob mice (Cani et al., 2009). Thus, flavonoids that have the capacity to shape the microbiota towards profiles that promote GI tract hormone release could be beneficial in the mitigation of metabolic diseases.

Overall, and although further research is needed, the modulation of gut hormone production by flavonoids can in part explain the beneficial actions of dietary flavonoids on the mitigation of metabolic disorders.

3.3. Flavonoids and the GI tract immune system

Emerging evidence points to potential benefits of flavonoids on the GI tract immune system. For example, in a mouse model of elemental enteral nutrition, cranberry proanthocyanidins prevented lamina propria Th2 cytokine IL-4 and IL-13 decreases which were dependent on the degree of procyanidin polymerization (Pierre et al., 2013). Proanthocyanidins also mitigated elemental enteral nutrition-induced decreases in luminal small IgAs (Pierre et al., 2014). The latter is particularly relevant as small IgAs are the first barrier against pathogenic microorganisms.

IBD is a group of GI conditions with significant involvement of the immune system. The beneficial actions of flavonoids on IBD has been extensively studied in animal models and in cells in culture; a critical review on this topic has been recently published (Salaritabar et al., 2017). Many flavonoids have been found to have potent anti-inflammatory actions in different experimental designs, and given the high concentrations that they reach at the GI tract; it is possible that these effects could be beneficial in IBD. However, the relevance of those findings for patients is still very limited given the scarce number and pilot nature of the available studies in humans.

3.4. Flavonoids and colorectal cancer

Colorectal cancer (CRC) is the fourth most common cause of cancer mortality worldwide and the third most frequent cancer diagnosed in the United States. Although there is a genetic component, environmental factors are major contributors to the accumulation of mutations throughout life that finally trigger CRC development. Given the direct exposure of the GI tract to food, diet constitutes one of the main environmental factors affecting the risk for CRC. In fact, flavonoids have been found to modulate cell signaling cascades that are targeted by conventional anti-CRC therapeutic strategies (Waldner and Neurath, 2010).

A large body of *in vitro* experimental evidence has shown the capacity of flavonoids to exert anti-CRC actions in cell cultures. For example, proanthocyanidins, a group of compounds with consistent epidemiological evidence of anti-CRC actions in human populations (Rossi et al., 2010a, 2010b) have been shown to inhibit cell proliferation and promote apoptosis (Choy et al., 2016; Gosse et al., 2005). In support of a potential action directly at the colon, after ingestion flavonoids are known to be present throughout the GI tract (Tsang et al., 2005) and to reach the colon (Choy et al., 2013, 2014). The high molecular weight proanthocyanidins are not absorbed or taken up by cells, instead, they interact with model and CRC cell membranes (Erlejman et al., 2004; Verstraeten et al., 2013) and inhibit pro-oncogenic signaling pathways (Choy et al., 2016; Da Silva et al., 2012).

Although *in vitro* and animal study evidence are relevant to understand mechanisms underlying flavonoid anti-CRC action, it is important that this research is supported by solid epidemiological evidence. Diets rich in fruits and vegetables, and consequently in flavonoids and fiber, have been linked to a lower risk for CRC (Aune et al., 2011; Ben et al., 2014; Forester and Waterhouse; Terry et al., 2001). Of note, epidemiological evidence points to beneficial effects for particular flavonoid families rather than to total flavonoid content in the diet. For example, a meta-analysis of studies up to 2012, showed no association between total flavonoid intake and gastric cancers or CRC (Woo and Kim, 2013). However, an inverse association was observed between CRC risk and the dietary intake of specific subgroups including, flavonols, flavanols, anthocyanidins, and proanthocyanidins (Woo and Kim, 2013). Another recent meta-analysis reported protective effects on CRC risk for proanthocyanidins and isoflavones, but only in case control studies for anthocyanins and flavonols (He and Sun, 2016). In a third meta-analysis, only flavonols, flavanols, flavones, and proanthocyanidins were found to be protective (Grosso et al., 2017). Notably, when considering monomers and the proanthocyanidins, the risk for CRC decreased with increasing degree of polymerization (1–10 monomers) (Rossi et al., 2010b). A low CRC risk was also observed for high intakes of anthocyanidins, flavonols, flavones, and isoflavones (Rossi et al., 2010a). While several epidemiological studies have reported protective effects, others have found no association between the intake of total flavonoid or of the different families and CRC risk (Nimptsch et al., 2016; Zamora-Ros et al., 2017).

Overall, while evidence is somewhat inconsistent, there is a clear positive trend for a decreased CRC risk with consumption of select flavonoid families. However, the evidence to date has

limitations as it is based primarily on case-control studies and the methods used to evaluate flavonoid intake vary in their validity and reliability. Future prospective cohort studies are needed that measure biochemical parameters of flavonoid consumption, which can provide information on both intake and the capacity of the individual to absorb and metabolize flavonoids.

4. Flavonoid-microbiota interactions: relevance to flavonoid metabolism and health benefits

A large body of research in the past 20 years has revealed the tremendous importance of the gut microbiome in the digestion of different food classes. Indeed, only recently has the role of specific food structures been robustly linked to changes in the gut microbial communities (David et al., 2014) and even specific bacterial taxa (De Leoz et al., 2015). Most flavonoids (except flavanols) are naturally bound to sugars as β-glycosides, which prevent the easy absorption of flavonoids in the small intestine (Hollman, 2004; Kumar and Pandey, 2013). This means a large proportion of glycosylated flavonoids reach the colon (Cassidy and Minihane, 2017) where the colonic microbiota break down the flavonoids into phenolic acids and other metabolites, which can then be absorbed (Hollman, 2004; Williamson and Clifford, 2017). As a result: i) flavonoids are present in the colon where they can influence the gut microbiome; ii) microbes metabolize flavonoids and change their bioavailability and activity; and iii) both changes in the gut microbiome and changes in flavonoid activity and bioavailability may influence health (Fig. 1).

4.1. Flavonoid influence on microbiota

Various foods are known to drive changes in both gut microbiome constituents and their function. The antimicrobial activity of flavonoids is well known and many studies have demonstrated inhibition of specific microbes, including commensal and pathogenic microorganisms. To name only a few of many examples: quercetin inhibits the growth of *Ruminococcus gauvreauii*, *Bacteroides galacturonicus*, and *Lactobacillus* sp. (Duda-Chodak, 2012). One proposed mechanism of action for quercetin's antimicrobial activity is through its ability to inhibit DNA gyrase, a topoisomerase involved in bacterial DNA replication, recombination, and transcription (Cushnie and Lamb, 2005). The polyphenols present in cloudberry also exhibited antimicrobial activity against *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfingens*, *Helicobacter pylori*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans* (Nohynek et al., 2006). The antimicrobial activity of flavonoids is tied to different structural features in different classes (reviewed in (Xie et al., 2015)). In brief, these authors concluded that hydroxyl groups on aromatic rings improve antimicrobial function while methylation decreases such activity. Furthermore, prenyl groups, alkylamino chains, alkyl chains, and heterocyclic moieties containing nitrogen or oxygen usually increase the antimicrobial activity of flavonoids (Xie et al., 2015). More recent work in legumes has confirmed the antimicrobial activity of prenylated isoflavonoids against *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*, and further found that prenylation at position β was more predictive of antimicrobial activity than prenylation at other sites (Araya-Cloutier et al., 2017).

In addition to their antimicrobial activity, flavonoids can increase the presence of specific bacteria within the gut community. For example, mice fed a diet high in apple flavonoids exhibited higher levels of *Bifidobacterium* spp. and bacteria belonging to a combined group of *Bacteroides-Prevotella-Poryphyromonas,* but significantly lower levels of *Lactobacillus* spp. (Espley et al., 2014). The stimulatory effect of flavonoids on bacteria is dependent on both the specific flavonoid structure and dose, and specific microbial species and strains. For example, both rutin and quercetin stimulated the growth of *Bifidobacterium bifidum in vitro*, but quercetin at high doses also inhibited the growth of *Bifidobacterium adolescentis* (Gwiazdowska et al., 2015). Other organisms may also exhibit growth enhancement in the presence of flavonoids. For example, *Lactobacillus plantarum* and *Lactobacillus casei* are particularly efficient at growing on flavanol rich grape seed extract (Tabasco et al., 2011). Flavonoid-induced changes of microbiota composition can have multiple impacts on health through the different processes that the microbiota regulates. Thus, flavonoids can modulate microbial populations and these microbial populations in turn: i) generate endotoxins (Section 3.1.); ii) convert primary into secondary bile acids, which play major roles in health and disease (Ridlon et al., 2013; Long et al., 2017; Martinot et al., 2017); iii) may influence epigenetic modifications (Remely and Haslberger, 2017); iv) are involved in sustaining immune homeostasis (Maynard et al., 2012); and v) are involved in nutrient and bioactive absorption and metabolism, including the formation of short chain fatty acids (Shortt et al., 2017).

Whether flavonoids should be considered a prebiotic depends on the exact definition of a prebiotic — a subject of some recent controversy (Bindels et al., 2015; Gibson et al., 2017; Hutkins et al., 2016). The latest consensus statement from the International Scientific Association for Prebiotics and Probiotics indicates that a prebiotic is “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al., 2017). These authors acknowledge the demonstration of microbial specificity and causality linked to a health benefit will require convincing evidence in the target host. Importantly, this new definition appears to be more inclusive than older definitions, which focused on *fermentation*, as opposed to *utilization*, as the mode of selective microbial modification of prebiotic substrates (mostly carbohydrate based). This sets the stage for the classification of flavonoids as prebiotics if research demonstrates that targeted microbial utilization is concretely linked to a health benefit.

4.2. Microbiota metabolism of flavonoids

As mentioned before and shown in Fig. 1, the relationship between flavonoids and microbiota is bidirectional. In addition to flavonoids inhibiting and promoting growth of specific microbes, microbes metabolize flavonoids. These metabolites may in turn affect the host organism and/or other microbes differently than the parent compound. Except for flavanols, most flavonoids are present as O- or C-glycosides and usually undergo deglycosylation prior to absorption or further conversion; as human enzymes are incapable of hydrolyzing C-glycosides, humans are dependent on bacteria for cleavage of C-glycosides (Braune and Blaut, 2016).

Differences in biological activity between flavonoid glycosides, aglycones, and phenolic acid metabolites have long been observed. For example, the phenolic acid metabolites 3,4-dihydroxyphenylacetic acid and 4-hydroxylphenylacetic acid have greater anti-platelet aggregation activity (a measure relevant to the anti-cardiovascular disease properties of flavonoids) than the flavonoid glycosides and aglycones they are derived from (Kim et al., 1998). The metabolism of flavonoids may also alter their antiproliferative activity towards cancer cells, for example, *in vitro* assays found that rutin did not inhibit cancer cell proliferation, but rutin hydrolyzed by the microbial enzyme hesperidinase displayed moderate antiproliferative activity against select cell lines (de Araújo et al., 2013).

4.3. Microbiota, flavonoids, and health

The interaction of flavonoids and the microbiome offer enormous promise for human health, both locally to the gut and systemically. The ability of flavonoids to shape the microbiome offers the promise of diet based therapies for a wide array of conditions associated with dysbiosis. Orange and apple polyphenols are able to alter the microbiome of patients with systemic lupus erythematosus, with flavanones increasing *Lactobacillus* and dihydroflavonols increasing *Bifidobacterium* levels in patients, suggesting it may be possible to correct the dysbiosis associated with systemic lupus erythematosus through flavonoid focused dietary changes (Cuervo et al., 2015). Another condition potentially improved by flavonoid influence on the microbiome is NAFLD. This disease is associated with intestinal microbiota dysbiosis and supplementing a high fat diet with quercetin in a mouse model effectively decreased the severity of NAFLD and reduced dysbiosis (Porras et al., 2017).

Flavonoid-microbiome interactions may also prove helpful in the treatment of infectious disease. Recent work in mice found that *Clostridium orbiscindens* is capable of processing flavonoids into desaminotyrosine, which augments type 1 interferon signaling and reduces influenza associated mortality (Steed et al., 2017). The flavone baiclin also appears to inhibit influenza plaque assay, but must be converted into its aglycone baicalein to be absorbed (Xu et al., 2010) which would be dependent on the microbiota.

More work is needed to understand the connection between microbes, flavonoids, and health, especially as not all effects of flavonoids are positive. For example, flavonoids led to a delay in recovery in an experimental autoimmune encephalomyelitis mouse model despite *in vitro* evidence that flavonoids would reduce T cell proliferation, leading the authors to speculate that the metabolites produced *in vivo* may result in very different effects than the parent compounds (Verbeek et al., 2005). The bidirectional connection between microbes and flavonoids, combined with the potential to both benefit and hinder health, highlights the need to study both flavonoids and the microbiome in concert. Understanding how the microbiome changes in response to flavonoids and how flavonoids are in turn altered by the microbiome may clarify seemingly contradictory *in vitro* and *in vivo* results. Future work is needed to better understand how the bidirectional interaction of flavonoids and the microbiome affect health.

5. Conclusions

Knowledge on the actions of flavonoids at the GI tract is essential to design dietary and pharmacological approaches for the prevention and/or treatment of disease. Although some evidence exists for different aspects of gut physiology that can be regulated by flavonoids, there is still limited information on the targets and mechanisms of flavonoid actions, microbiota/flavonoids crosstalk, and ultimate health effects. Further understanding of the mechanisms mediating the effects of flavonoids on the intestine (and its microbiota) is of critical importance given the relevance of the GI tract on sustaining overall health and of the widespread recommendations of increasing the intake of plant bioactives.

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