Received: 25 April 2020 | Revised: 19 August 2020 | Accepted: 21 September 2020

DOI: 10.1111/1541-4337.12652

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive REVIEWS in Food Science and Food Safety WILEY

Citrus flavonoids and the intestinal barrier: Interactions and effects

Meiyan Wang[1] | **Hui Zhao**[1] | **Xiang Wen**[1,2] | **Chi-Tang Ho**[3] | **Shiming Li**[2,3]

[1] Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin, China

[2] Hubei Key Laboratory for EFGIR, Huanggang Normal University, Hubei, China

[3] Department of Food Science, Rutgers University, New Brunswick, New Jersey

Correspondence
Meiyan Wang, Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, No. 409 Guangrong Rd., Building IV 718, Tianjin 300134, China.
Email: wangmeiyan@tjcu.edu.cn
Shiming Li, Department of Food Science, Rutgers University, New Brunswick, NJ 08901.
Email: shiming@rutgers.edu

Funding information
Hubei Province Technical Innovation Special Project, Grant/Award Number: 2019ABA100; National Natural Science Foundation of China, Grant/Award Number: 81803548; Natural Science Foundation of Tianjin City, Grant/Award Number: 19JCQNJC12400; Tianjin Innovative Research Team Grant, Grant/Award Number: TD13-5087

Abstract

The intestinal barrier plays a central role in sustaining gut homeostasis and, when dysfunctional, may contribute to diseases. Dietary flavonoids derived from *Citrus* genus represent one of the main naturally occurring phytochemicals with multiple potential benefits for the intestinal barrier function. In the intestine, citrus flavonoids (CFs) undergo ingestion from the lumen, biotransformation in the epithelial cells and/or crosstalk with luminal microbiota to afford various metabolites that may in turn exert protective actions on gut barrier along with their parental compounds. Specifically, the health-promoting properties of CFs and their metabolic bioactives for the intestinal barrier include their capacity to (a) modulate barrier permeability; (b) protect mucus layer; (c) regulate intestinal immune system; (d) fight against oxidative stress; and (e) positively shape microbiome and metabolome. Notably, local effects of CFs can also generate systemic benefits, for instance, improvement of gut microbial dysbiosis helpful to orchestrate gut homeostasis and leading to alleviation of systemic dysmetabolism. Given the important role of the intestinal barrier in overall health, further understanding of underlying action mechanisms and ultimate health effects of CFs as well as their metabolites on the intestine is of great significance to future application of citrus plants and their bioactives as dietary supplements and/or functional ingredients in medical foods.

KEYWORDS
citrus flavonoids, gut microbiota, health-promoting effect, intestinal barrier, metabolic fate

1 | INTRODUCTION

The intestinal tract serves an essential function of digestion and absorption of foods to generate calories and provide nutrients to sustain life. However, the intestinal mucosa surface (~400 m^2) is in continuous contact with luminal bacteria, bacterial products, and dietary antigens, which enhances the need for a highly selective barrier. The intestinal barrier, constructed by epithelial cells, mucus, commensal microbiota, and the intestinal immune system, as well as some biochemical components, tackles the daunting task to maintain gut homeostasis. Hence, an integrated intestinal barrier plays a significant role in supporting human health (Choi, Yeruva, & Turner, 2017; Groschwitz & Hogan, 2009). The loss of the barrier is related to a range of gastrointestinal diseases such as coeliac disease, inflammatory bowel disease (IBD), and even colorectal cancer (Lavoie et al., 2020; Lee et al.,

2020; Lissner et al., 2015; Sukkar, Schenone, Foppiani, & Nobile, 2004). Apart from gut symptoms, dysfunction of the barrier has been reported to underlie the pathophysiology of systemic diseases such as obesity, diabetes, and nonalcoholic fatty liver disease (NAFLD) among others (Clemente-Postigo et al., 2019; Odenwald & Turner, 2017; Thaiss et al., 2018). More interestingly, recent investigations in *Drosophila* suggest intestinal barrier loss involved in age-associated metabolic and inflammatory pattern as a reliable marker of "impending" death (Gervais & Bardin, 2017; Rera, Clark, & Walker, 2012).

Citrus flavonoids (CFs) are literally group of dietary flavonoids derived from the *Citrus* genus (Rutaceae, subfam. Aurantioideae), including lemon, lime, sweet orange, sour orange, admixture mandarin, and grapefruit among others (Wu et al., 2018). Chemically, CFs are diversified polyphenolic molecules. More than 80 natural flavonoids have been identified in citrus plants (Cirmi et al., 2016; Zheng, Zeng, Peng, Wu, & Su, 2019). In traditional Mediterranean diet, an average intake of flavonoids is up to 670 mg/day and citrus fruits contribute significantly to the flavonoids ingestion, indicating a high intake of CFs in human diet (Davis, Bryan, Hodgson, & Murphy, 2015; Tresserra-Rimbau et al., 2014). Upon ingestion, depending on their chemical structures, CFs may undergo a complex metabolic process in the intestine, such as biotransformation in the epithelial cells and interplay with local microbiota and conversion into conjugated derivatives, phenolic and aromatic acids, and/or other catabolites (Stevens et al., 2019).

The provision of dietary phytochemicals that can optimize the intestinal barrier functions is a theme of current relevance. In this direction, a growing body of evidence points to the health-promoting properties of CFs and their metabolites for intestinal barrier function, including protection of barrier permeability, positive modification of gut microbiota, and immunomodulation, as well as suppression of oxidative stress and inflammation in gut lumen (Pei, Liu, & Bolling, 2020; Wang et al., 2018). These protective actions of CFs exerted on the intestinal barrier are not only beneficial for the prevention and/or treatment of local diseases within gut, but also helpful for improving barrier loss-related systemic disorders such as diet-induced obesity (Gil-Cardoso et al., 2016).

There is a plethora of published research in the field of CFs, mainly concerning the various biological properties and potential application as functional foods or dietary supplements in preventing and combating diseases. However, the metabolic fate of CFs after ingestion and prior to blood circulation, in particular, the node of action of CFs to the intestine and gut, and vice versa, has not been discussed mechanistically in a great detail. This review aims to provide a comprehensive summary of the metabolic fate of CFs in gut, the key roles of CFs in the interplay with gut microbiota, and the protective effects of CFs and their metabolites on the intestinal barrier function, thus to convey evidence based findings from both *in vitro* and *in vivo* that CFs may be promising efficacious candidates in effectively preventing and treating of the intestinal barrier dysfunction-associated diseases.

2 | BRIEF INTRODUCTION OF CFS

Citrus fruits and juices represent one of the main sources of various dietary flavonoids (Chun, Chung, & Song, 2007; Liu, Heying, & Tanumihardjo, 2012). The basic skeleton of flavonoids is composed of two benzene rings (A and B) linked through a 3-carbon unit, with the carbon structure abbreviated as C6-C3-C6. Based on the status of oxidation at C4, saturation between C2 and C3, and substitution in C3 unit, CFs can be classified into six subgroups, that is, flavanones, flavonols, flavones, anthocyanins, flavanonols, and chalcones (Figure 1).

CFs can present as the free form of aglycones such as hesperetin and naringenin, or alternatively as their *O*/*C*-glycosidic forms with monosaccharide, for example, glucose, galactose, rhamnose, and arabinose, or disaccharide, for example, rutinose (rhamnosyl-α-1,6 glucose) and neohesperidose (rhamnosyl-α-1,2 glucose), such as hesperidin (hesperetin-7-*O*-rutinoside), neohesperidin (hesperetin-7-*O*-neohesperidoside), naringin (naringenin-7-*O*-neohesperidoside) and narirutin (naringenin-7-*O*-rutinoside) (Merken & Beecher, 2000). Compared with free aglycones, glycosylated derivatives are the predominant form of naturally occurring CFs, as glycosylation renders the flavonoids less reactive and more water-soluble, allowing for better storage in the cell vacuole (Cuyckens & Claeys, 2005). The *O*-glycoside forms of flavonol, flavanone, flavone, flavanonol, and anthocyanidin commonly exist in the citrus species. Most *O*-glycosylated substitution for CFs occur at the C-3 or C-7 position (Figure 1), although 4′-*O*-glycosides were also reported in some cases, for example, bergamot Juice (Gattuso et al., 2006). The isolation of a uridine diphosphate-sugar dependent glycosyltransferases (UGT) with a high expression level from the sweet orange, flavonoid 7-*O*-UGT, marked the accumulations of multiple CF 7-*O*-glycosides (Liu et al., 2018). *C*-glycosides of CFs are less abundant and only *C*-glycosylated at the C-6 and/or C-8 position of the flavone nucleus were reported, such as diosmetin-6-*C*-glucoside, luteolin-8-*C*-glucoside, and luteolin-6,8-di-*C*-glucoside. (Zheng et al., 2019).

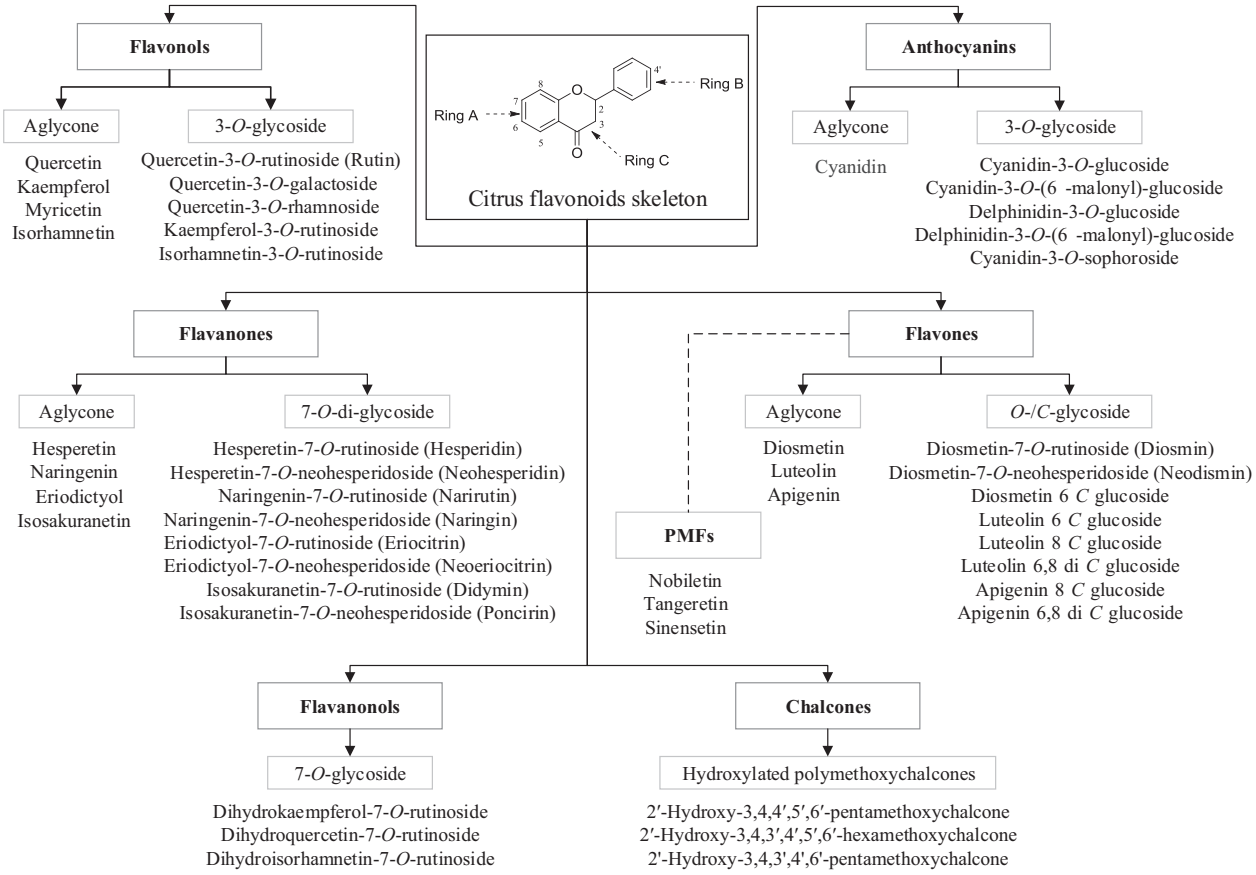

FIGURE 1 Classification of representative flavonoids identified in the *Citrus* genus

3 | METABOLIC FATES OF CFS IN THE INTESTINE

After ingestion, CFs would follow three potential intestinal fates as illustrated in Figure 2 and detailed below, which defines the molecular targets and bioactivities of CFs.

3.1 | Absorption from the intestinal lumen

The bioactivities of CFs are significantly influenced by their absorption and metabolism in the intestine, which, in turn, depends on their chemical structures (Thilakarathna & Rupasinghe, 2013). CFs can be classified into three groups based on their *O*-substitution, that is, aglycones, flavonoid glycosides (FG) and polymethoxyflavones (PMF). Flavonoid aglycones are moderately hydrophobic and relatively easier and faster to be absorbed from lumen than their FG counterparts. This is well-established, as for example with hesperetin and hesperidin. In healthy human subjects, the peak concentration occurred in plasma at 4 hr postconsumption of hesperetin compared with 7 hr for hesperidin-rich juices (Kanaze, Bounartzi, Georgarakis, & Niopas, 2007; Nielsen et al., 2006). With regard to PMFs, the presence of multimethoxyl groups on the phenyl ring of the flavonoids improves their hydrophobic properties, thereby exhibiting a better metabolic stability and intestinal absorption. This is supported by an absorption investigation *in vivo* with nobiletin, a citrus peel PMF. After ingestion, nobiletin was rapidly absorbed (within 20 min), then predominantly accumulated in the intestinal mucosa and in muscularis of rats at 1 to 4 hr (Murakami et al., 2002).

Compared with PMFs and aglycones, FGs principally exhibit a slower and more complicated absorption pathway. Generally, FGs can be metabolized into corresponding aglycones in gut by hydrolases and/or by microbiota. Interactions between CFs and gut microbiota primarily occur in the large intestine, which will be discussed in Section 3.3. Herein, we focus on the metabolism of flavonoid glycosides by hydrolases in the small intestine, of which the two most proposed are cytosolic broad-specificity β-glucosidase (CBG) and lactase phloridzin hydrolase (LPH).

CBG is a β-glucoside hydrolase abundant in IECs, as well as in liver and kidney of mammals (Daniels, Coyle, Chiao, Glew, & Labow, 1981; Mellor & Layne, 1971), which is capable of hydrolyzing the β-glucoside moiety,

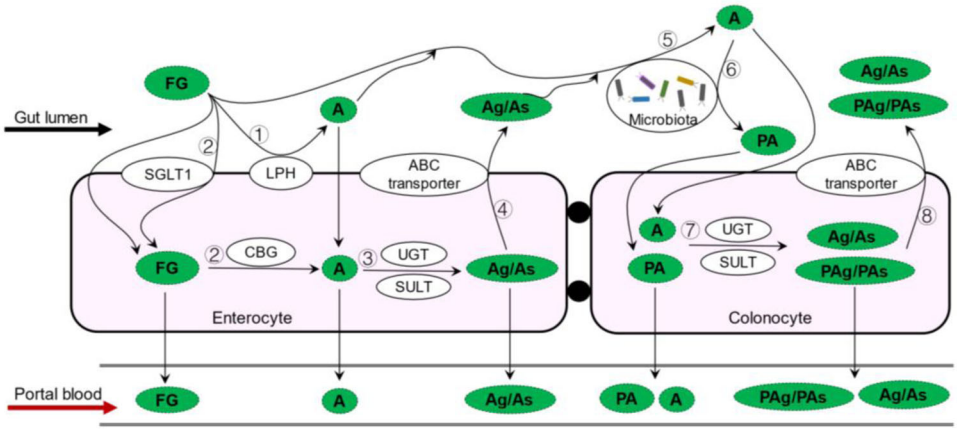

FIGURE 2 A model for the potential intestinal fates of citrus flavonoid glycosides (FG). After ingestion, FG resistant to stomach digestive enzymes can reach the small intestine, of which a small percentage possibly penetrate through the intestinal membrane and be absorbed intact into the portal blood, whereas majority would: (1) undergo direct hydrolysis by lactase phloridzin hydrolase (LPH) to release aglycones (A), and/or (2) transport into the enterocytes via sodium-glucose cotransporter-1 (SGLT1) prior to hydrolysis by cytosolic broad-specificity β-glucosidase (CBG) to give A, which can either enter into the portal blood, or (3) undergo further phase II conjugation in the enterocytes by UDP-glucuronosyltransferases (UGT)/sulfotransferases (SULT) to afford glucuronidated/sulfated metabolites (Ag/As), which can be reabsorbed into the portal blood, or (4) alternatively, be secreted back into the lumen by ATP-binding cassette (ABC) transporters. Even after this complex sequence, there are still some intractable FG/metabolites, for example, *C*-glycosides and some *O*-glycosides, aglycones, and conjugates, will be subjected to the large intestine, where they can (5) be broken down by local microbiota to release A. Some aglycones can be directly ingested by colonocytes, but most of these aglycones will (6) suffer a subsequent ring fission by microbiota to produce small phenolic acids (PA) and other catabolites, and then enter the colonocytes through passive diffusion. In the colonocytes, some A and PA can (7) be converted into conjugates, that is, glucuronidated/sulfated aglycones (Ag/As) and glucuronidated/sulfated phenolic acids (PAg/PAs) by UGT/SULT. Then, A and PA as well as their conjugated derivatives will enter into the portal blood. Also, some conjugates including Ag/As and PAg/PAs can (8) be pumped back to the lumen by ABC transporters, as in the small intestine

including various natural FGs (Day et al., 1998). Prior to deglycosylation by CBG in IECs, FGs are transferred from the intestinal lumen to epithelial cells, probably via sodium-glucose cotransporter-1 (SGLT1) (Hollman, de Vries, van Leeuwen, Mengelers, & Katan, 1995; Walgren, Lin, Kinne, & Walle, 2000). As the resulting metabolites, flavonoids in aglycone forms or further conjugated derivatives can enter into the portal blood (Gil-Cardoso et al., 2016). LPH is a membrane-bound enzyme found in the small intestine, which is primarily responsible for hydrolysis of lactose (Auricchio, Rubino, Landolt, Semenza, & Prader, 1963). Interestingly, the second hydrophobic domain of LPH is found to be capable of hydrolyzing FGs (Leese & Semenza, 1973). This is supported by an *in vivo* study based on a rat perfusion model, which displayed that 27.6% of quercetin-3-glucoside was hydrolyzed during intestinal passage and LPH was a major determinant of the absorption process (Sesink, Arts, Faassen-Peters, & Hollman, 2003). As the LPH hydrolysates, aglycones can be absorbed into the intestinal mucosa. Of note, hydrolysis pathways of both CBG and LPH bear substrate-selectivity, which are tied to the chemical structures of CFs and the position/nature of the sugar substitutions (Cermak, Landgraf, & Wolffram, 2004; Day, Gee, DuPont, Johnson, & Williamson, 2003; Walgren, Karnaky, Lindenmayer, & Walle, 2000; Wolffram, Block, & Ader, 2002).

Although glycosidic hydrolysis to aglycones is an inevitable fate for majority of CF glycosides, some can directly penetrate through IECs and then be absorbed intact into the portal blood, which is attested by the detection of both hesperidin and hesperetin in the portal blood at 1 and 4 hr after oral administration of hesperidin at a dose of 10 mg/kg to rats, with the peak concentrations of 0.06 and 0.13 nmol/mL, respectively (Nectoux et al., 2019). The absorption delay for hesperetin can be explained by the time needed for hesperidin hydrolysis to release hesperetin. This clear and convincing evidence supports that flavanone rutinosides and their aglycone counterparts can enter into the portal vein as their intact forms. However, there was no measurable intact hesperidin or hesperetin in the circulating blood that contained sulfates and glucuronides of hesperetin instead, which agreed with multiple previous reports (Ameer, Weintraub, Johnson, Yost, & Rouseff, 1996; Matsumoto, Ikoma, Sugiura, Yano, & Hasegawa, 2004).

In brief, the absorption of CFs from the intestinal lumen is closely associated with *O*-substitution of the CF ring, namely, aglycones and PMFs are easily absorbed intact,

whereas FGs are hardly and slowly absorbed prior to hydrolysis. The memberane surface of the intestinal lumen is lipophilic by nature. Thus, the lipophilicity of the CF molecules is a significant element for the absorption process, as the *O*-glycosylation drives FGs more hydrophilic than aglycones and PMFs.

3.2 | Biotransformation in IECs

The gut barrier, especially the intestinal epithelial cells (IECs), plays a dominant role in the biotransformation of flavonoids prior to a liver detoxification process (Wong, Zhang, Lin, & Zuo, 2009). The abundant and diversified metabolic enzymes in IECs such as UDP-glucuronosyltransferases, sulfotransferases and catechol-*O*-methyltransferases are involved in the phase II conjugation reactions of aglycones ingested into IECs to obtain glucuronidated, sulfated and/or methylated metabolites (Del Rio et al., 2013). In an investigation carried out among 10 male endurance athletes, the metabolites of hesperetin, naringenin, and eriodictyol were quantitatively assessed after ingestion of 500 mL orange juice which contained 250 μmol hesperetin-*O*-glycosides, 76 μmol naringenin-*O*-glycosides, and 4 μmol eriodictyol-7-*O*-rutinoside (Pereira-Caro et al., 2017). In spite of inter-individual differences, the urinary excretion of CF metabolites 0 to 24 hr after orange juice consumption corresponded to 16% intake, approximately one-third of which was detected as glucuronidated/sulfated conjugates (Pereira-Caro et al., 2017).

CFs conjugates formed in IECs can either be absorbed into the bloodstream or alternatively, secreted back into the intestinal lumen, where they may exert local biological actions and/or undergo further metabolism (for example, rehydrolysis) (Aura et al., 2002). In a rat in situ intestinal perfusion model, conjugates pumped back to the lumen represented 25.5 and 20% of the net transfer into the enterocytes for genistein and hesperetin, respectively (Silberberg et al., 2006). Such efflux actions of flavonoid conjugates are considered as an ATP-binding cassette (ABC) transporters-mediated result (Chambers et al., 2020). In Caco-2 cell culture model, hesperetin conjugates including hesperetin 7-*O*-glucuronide and hesperetin 7-*O*-sulfate, were predominantly transported to the apical side of the cell monolayer. This apical efflux of conjugated hesperetin was 1.9-fold reduced upon the presence of an inhibitor of the breast cancer resistance protein (BCRP/ABCG2), implying that hesperetin conjugates are a high affinity substrate of BCRP (Brand et al., 2011). In this context, the aborption of CFs by the intestinal lumen seems like a balance between the permeability of structure-dependent lipophilicity and the efflux of the conjugated CFs. However, secretion of the conjugates back to the intestinal lumen might lower the bioavailability of CFs (Liu & Hu, 2007).

3.3 | Metabolism via the intestinal microbiota

After sequential path in the stomach and small intestine, CFs resistant to permeation and enzymatic digestion and conjugates pumped back into the lumen will be subjected to the large intestine, where they can be broken down by local microbiota (Stevens et al., 2019). The distal intestine harbors an enormous microbial community, with an estimated load of up to 10^{10} to 10^{12} CFUs/mL (O'Hara & Shanahan, 2006). This microbial population plays an essential and irreplaceable role in the *in vivo* metabolism of CFs. The bacteria-mediated metabolic reactions include deglycosylation of *C*-/*O*-glycosides, double reduction of bond and cleavage of the *C*-ring, hydrolysis of esters, and demethylation of PMFs (Bang, Hyun, Shim, Hong, & Kim, 2015; Rowland et al., 2018). For example, CFs in *C*-glycoside forms are dependent on microflora for cleavage as human enzymes are unable to hydrolyze them (Braune & Blaut, 2016). Microbes metabolize CFs occurring in a common procedure which includes hydrolysis to release aglycones first and then ring fission to generate small phenolic and aryl acids and other catabolites.

A surprising number of anaerobic bacteria in the large intestine are able to produce various hydrolytic enzymes such as α-rhamnosidase, β-glucosidase and β-glucuronidase among others. These enzymes are important glycosidic bond-hydrolases, which contribute to the hydrolysis of flavonoid glucosides to give aglycones. For example, naringenin-7-*O*-rutinoside and hesperetin-7-*O*-rutinoside were converted to their aglycone counterparts after a coincubation with *Bifidobacterium* longum R0175 (Pereira-Caro et al., 2018).

Aglycones of CFs obtained from enzymatic hydrolysis can be absorbed directly by the large intestine on one side and on the other can undergo further ring fission. Metabonomics analyses of samples from plasma, urine, and/or feces suggest that main catabolites from ring fission of CFs are simple phenolic acids. For example, a human study has investigated the colonic microbiota-mediated breakdown of CFs-rich orange juice and quantified a total of 11 phenolic acids in urine, which account for 25% of the intake of total CFs (Pereira-Caro et al., 2014). After administration of hesperidin to rats, eriodictyol and their glucuronides, and together with hesperetin glucuronides were detected in the urine as hesperidin metabolites (Jin et al., 2010). As a comparison, germ-free rats exhibited a significantly smaller quantity of metabolites compared with the normal group (Jin et al., 2010), indicating that individual

variability in gut microbiome affected the metabolic process of CFs and led to the difference in the content of metabolites. Regardless of the variation among different individuals, CFs are metabolized extensively by the gut microbiota and yields multiple catabolites.

3.4 | Metabolic pathways and metabolites of representative CFs

To further depict CF metabolic pathways, we choose hesperidin and narirutin, the most abundant CFs in *Citrus* genus, and three major PMFs from citrus peels, that is, nobiletin, tangeretin and sinensetin, because of the growing body of evidence pointing to their beneficial effects on the intestinal barrier and human health (Guirro et al., 2020; Lai et al., 2011; Shen, Wan, Wang, & Jiang, 2019).

3.4.1 | Hesperidin and narirutin

Hesperidin and narirutin are two predominant flavanone rutinosides identified in citrus fruits. It is considered that less than 5% of flavanone glycosides can penetrate through the IECs and enter into the portal blood in their intact forms, whereas the remaining majority would undergo deglycosylation in the distal part of the small intestine and in the colon by local microflora to release corresponding aglycones prior to absorption (Cao, Chen, Jassbi, & Xiao, 2015; Nectoux et al., 2019; Orrego-Lagarón, Vallverdú-Queralt, Martínez-Huélamo, Lamuela-Raventos, & Escribano-Ferrer, 2016). Supporting evidence on the absorption site of flavanone rutinosides is from a comparative study preformed with 16 human subjects. This study found that the aglycone hesperetin was much more bioavailable for the group consuming α-rhamnosidase-treated juice, showing significantly higher area under the curve and the peak plasma concentration, as well as much shorter time to reach peak plasma concentration of hesperetin, than that for the group with untreated hesperidin juice intake (Nielsen et al., 2006). Another piece of convincing evidence is from Borges et al., who investigated the absorption and elimination of hesperidin between volunteers with a functioning colon and ileostomists. Results from metabolites detection showed that 12.0% of ingested hesperidin was recovered in the urine of subjects with an intact colon, whereas in ileostomists consumed an equal amount of hesperetin, only 3.5% of intake was recovered, which indicated approximately two-third of hesperetin was absorbed in the lower bowel absorption while one-third in the upper gastrointestinal tract (Borges et al., 2010; Borges, Lean, Roberts, & Crozier, 2013). Notably, a further ileal fluid analysis revealed that even when absorption occurred in the small intestine, in subjects with a functioning colon a substantial proportion of the ingested flavanone rutinosides still pass from the small into the large intestine, where allows catabolism by colonic bacteria (Borges et al., 2013). An experiment conducted in rats with naringenin-7-*O*-glucoside and narirutin was in line with this observation, as indicated by the result that glucosidic naringenin was efficiently hydrolyzed into aglycone in the upper part of the gut, whereas rutinosidic naringenin was hydrolyzed in the colon (Felgines et al., 2000). In brief, the authors concluded that CBG/LPH hydrolases in the small intestine are capable of hydrolyzing 7-*O*-glucoside flavanones, but appear ineffective for flavonoids linked to a rutinose. In contrast, flavanone rutinosides are preferentially subjected to microbial hydrolysis in the distal gastrointestinal tract. *Blautia* sp. MRG-PMF1 and *Bifidobacterium dentium* K13 respectively contribute to the deglycosylation of hesperidin and naringin, while *Parabacteroides distasonis* JCM 5825T and *Bacteroides uniformis* JCM 5828 are involved in hydrolysis of eriocitrin (Bang et al., 2015; Kim, Kim, & Han, 2014; Miyake, Yamamoto, & Osawa, 1997).

Hesperetin and naringenin released from deglycosylation process might be incepted by IECs and undergo phase II metabolism, for example, glucuronidation, sulfation, and methylation, locally or in the liver. After ingestion of 500 mL of orange juice containing 444 mg/L hesperidin and 96.4 mg/L narirutin by human volunteers, their metabolites were detected in plasma of subjects at 3 hr, with 87% of glucuronides and 13% of sulphoglucuronides (Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Remesy, 2003). These conjugates excreted in urine contributed for 4.1 and 7.1% of the intake of hesperidin and narirutin, respectively. Another human trial identified hesperetin-3′-*O*-glucuronide, hesperetin-7-*O*-glucuronide, hesperetin-3′-*O*-sulfate, naringenin-4′-*O*-glucuronide, and naringenin-7-*O*-glucuronide were the major conjugates in plasma and urine after orange fruit (150 g) or juice (300 g) consumption (Brett et al., 2009). These glucuronided/sulfated metabolites of hesperidin and narirutin were reaffirmed in other human trials, which also characterized hesperetin-7-*O*-sulfate, hesperetin-*O*-diglucuronide-*O*-sulfate, hesperetin-5,7-*O*-diglucuronide, and methylnaringenin-*O*-glucuronide among others (see Figure 3 for details of phase II metabolites of hesperidin and narirutin) (Bredsdorff et al., 2010; Zeng et al., 2017). Interestingly, eriodictyol could be metabolized directly from eriocitrin or generated as an intermediate derived from the hydroxylation of naringenin and demethylation of hesperetin, which was subsequently submitted to phase II conjugation to release corresponding glucuronide/sulfate metabolites (Miyake et al., 2000; Zeng et al., 2017). Human gut bacterium *Blautia* sp. MRG-PMF1

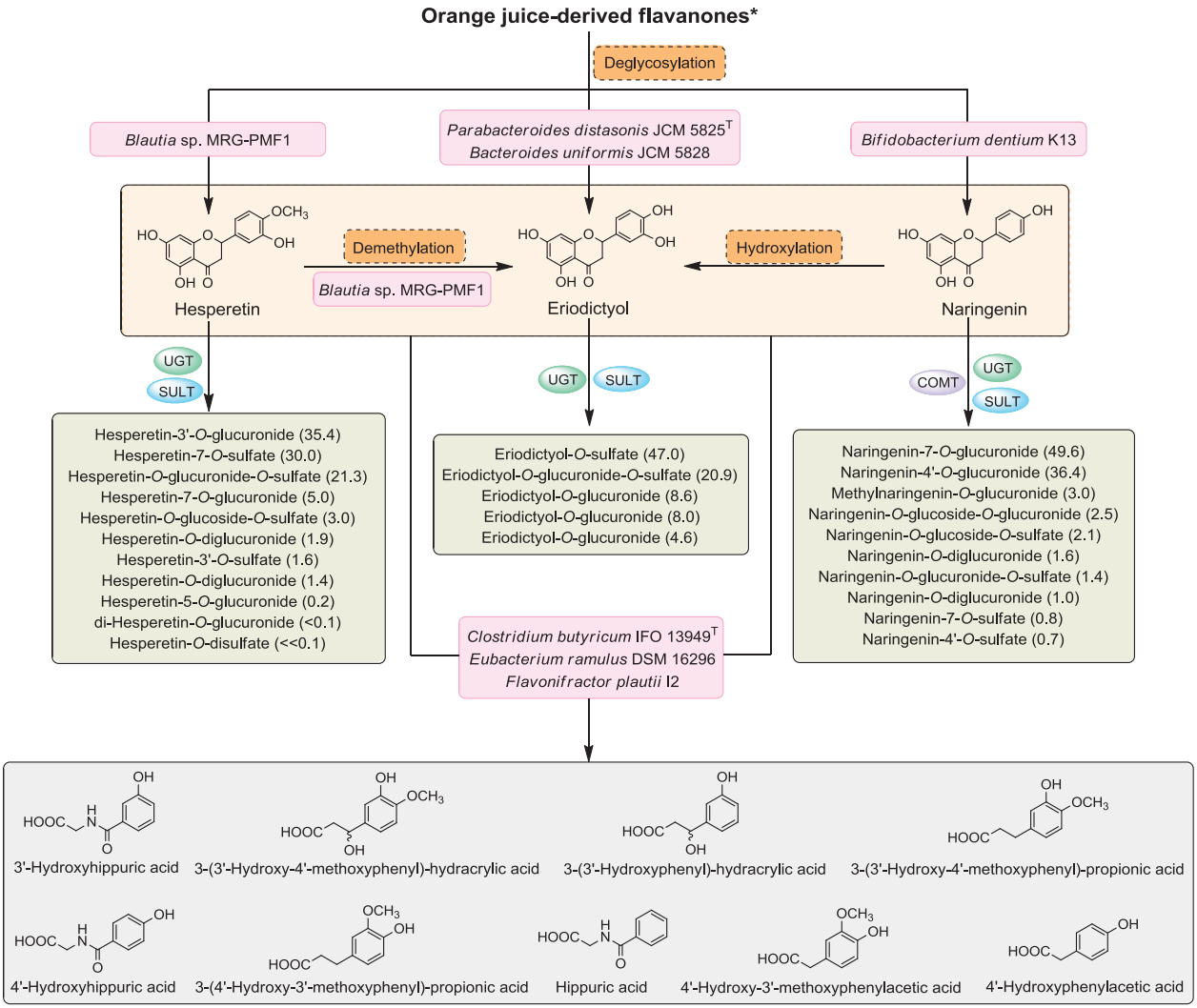

FIGURE 3 Proposed metabolic pathways for orange juice-derived flavanones, based on metabolites identified in the urine of human after ingestion of orange juices. *Major flavanones in orange juice contain hesperetin-*O*-glycosides, naringenin-*O*-glycosides, and eriodictyol-7-*O*-rutinoside. Numbers in parenthesis represent the proportion of a metabolite in total metabolites in the corresponding column, expressed as a percentage. Pink rectangles mark microbiota involved in the biotransformation of citrus flavanones. Ovals mark mammalian enzymes involved in phase II conjugation of CFs. UGT, UDP-glucuronosyltransferases; SULT, sulfotransferases; COMT, catechol-*O*-methyltransferases. Based on data from Braune et al. (2001), Bredsdorff et al. (2010), Brett et al. (2009), Manach et al. (2003), Schoefer et al. (2003), Zeng et al. (2017), Yoshiaki et al. (1997), Bang et al. (2015), and Kim et al. (2014)

was found to show demethylation activity, and might participate in the biotransformation from hesperetin to eriodictyol (Kim et al., 2014). Proposed pathways for the metabolism of orange juice-derived flavanones are illustrated in Figure 3.

In addition to conjugation, another possible metabolic fate of flavonoid aglycones is catabolism by intestinal microflora and host enzymes into phenolic and/or aryl acids, as illustrated in Figure 3. In a feeding study with orange juice that contained 518 μmol flavanones, specifically 348 μmol of hesperetin-*O*-glycosides, 165 μmol of naringenin-*O*-glycosides and 5 μmol of eriodictyol-7-*O*-rutinoside, a total of 217 μmol metabolites were detected in urine from human subjects (Pereira-Caro et al., 2014). Of those metabolites, 83 μmol was glucuronides/sulfates, contributing to 16% of the flavanone intake, and 134 μmol was phenolic catabolites, accounting for 23%. These phenolic catabolites included 3-(3′-hydroxy-4′-methoxyphenyl)-hydracrylic acid (approximately 54% of total phenolic catabolites), 3-(3′-hydroxyphenyl)-hydracrylic acid (21%), 3-(3′-methoxy-4′-hydroxyphenyl)-propionic acid (5%) and 3-(3′-hydroxy-4′-methoxyphenyl)-propionic acid (4%), as well as low levels of 3′-methoxy-4′-hydroxyphenylacetic acid, 4′-hydroxyphenylacetic acid, 3′-hydroxyhippuric acid, and 4′-hydroxyhippuric acid (shown in Figure 3). *Clostridium butyricum* IFO 13949^T, *Eubacterium ramulus*

DSM 16296, and *Flavonifractor plautii* I2 were reported to be involved in the C-ring cleavage and subsequent reactions of flavanone aglycones (Braune, Gütschow, Engst, & Blaut, 2001; Miyake et al., 1997; Schneider & Blaut, 2000; Schoefer, Mohan, Schwiertz, Braune, & Blaut, 2003). Some of these small phenolic catabolites can be further conjugated into corresponding glucuronide/sulfate derivatives (Pereira-Caro et al., 2016). Williamson et al. proposed detailed metabolic pathways combining the microbiota-mediated conversion with mammalian enzymes for the catabolism of hesperetin and naringenin released from orange juice intake by humans (Williamson, Kay, & Crozier, 2018). In addition, significant amounts of hippuric acid presented in the urine of orange juice treated group, which was 2.6-fold higher than that of the placebo group (Pereira-Caro et al., 2014). However, given the fact that hippuric acid can be produced endogenously from catecholamine and as a normal metabolite in human urine (Pero, 2010), exactly how much of the urinary hippuric acid excretion is related to metabolism of consumed citrus flavanones remains to be ascertained.

Overall, assessment of the urinary metabolome in humans after orange juice consumption defines the common metabolites of hesperidin and narirutin as glucuronides/sulfates and phenolic catabolites as well as their conjugates. However, there are some inter-individual variations in the amounts and composition of metabolites, as well as in the excretion time among different human subjects. A large-sample research, feeding orange juice to 129 human subjects aged 18 to 80 years, revealed an inter-individual variation for the excretion recovery, ranging from 1.6% to 59% of intake of naringenin and 0% to 25% for hesperetin (Brett et al., 2009). Furthermore, the excretion of hesperetin exhibited a negative correlation with age, indicating that age might be a factor interfering with the flavanone metabolism (Brett et al., 2009). Consistent with this finding, the urinary excretion rate of naringenin was observed to show a reduction in aged rats compared with adult ones (Zeng et al., 2019). Although age and factors like analytical methodology, juice processing technique and others, could affect detectable metabolites of hesperidin and narirutin, the intestinal bacteria might be a non-negligible factor that contributes to their metabolism variability among humans. This assumption is not surprising given the intestinal microflora playing an indispensable role in the metabolism of flavanone rutinosides, and particularly their characteristic of person-to-person differences. For example, after ingestion of flavanone-rich orange juice by 12 volunteers (6 men and 6 women aged 23 to 60 years), the number of volunteers who showed a significant increase in excretion of any of ten detected catabolites ranged from 4 to 11, which presumably was a reflection of the varying microflora of volunteers (Pereira-Caro et al., 2014). However, how and how much the gut microbiota involved in the metabolism of flavanones in host deserves in-depth studies.

3.4.2 | Nobiletin, tangeretin, and sinensetin

Nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone), tangeretin (5,6,7,8,4′-pentamethoxyflavone) and sinensetin (5,6,7,3′,4′-pentamethoxyflavone) are three representative PMFs identified in the peels of citrus species. With the presence of multiple methoxyl groups, they exhibit significantly improved intestinal absorption in comparison to their nonmethylated counterparts. In human Caco-2 intestinal cells, the permeability of methoxylated flavones was approximately five- to eightfold higher than unmethylated flavones (Wen & Walle, 2006). Metabolically, once consumed, citrus PMFs have been found to undergo phase I metabolism to give demethylated metabolites and phase II metabolism to form sulfate/glucoronidate conjugates.

Demethylation is considered as the major metabolic pathway of citrus PMFs *in vivo*. For nobiletin, sinensetin, and tangeretin, 4′-position on the B-ring seems like a more preferable site for demethylation, and the main metabolites are their 4′-demethylated counterparts. In mice, for instance, 4′-demethylnobiletin (4′-hydroxy-5,6,7,8,3′-pentamethoxyflavone) was identified as the major urinary metabolite of nobiletin, with a concentration of 28.9 μg/mL (Li, Wang, Sang, Huang, & Ho, 2006). Also, seven demethylated metabolites of nobiletin were detected and quantified in murine urine, of which 4′-demethylnobiletin was the most plentiful, accounting for up to 84.2% (Zheng et al., 2015). The other six metabolites were 3′,4′-didemethylnobiletin (12.3%), 3′-demethylnobiletin (1.3%), 5-demethylnobiletin (1.0%), 5,4′-didemethylnobiletin (0.8%), 5,3′,4′-tridemethylnobiletin (0.3%), and 5,3′-didemethylnobiletin (<0.1%). Hence, 4′-position of nobiletin is the dominant site of demethylation. Potential metabolic pathways for the demethylation of nobiletin *in vivo* is illustrated in Figure 4. By employing an isotope-labeling method, four demethylated derivatives were identified in mouse urine as sinensetin metabolites, that is, 4′-, 5-, and 6-demethylsinensetin, and sulfate of 7-demethylsinensetin (Wei et al., 2013). Results seem to indicate that demethylated metabolism of sinensetin possibly occurs at all positions but the C-3′ position. In contrast, C-4′ seems more likely to be demethylated during sinensetin metabolism compared with other positions. Main metabolites of sinensetin were shown in Figure 5. With respect to tangeretin, its metabolites were evaluated in both the urine and the fecal samples of rats at 24 hr

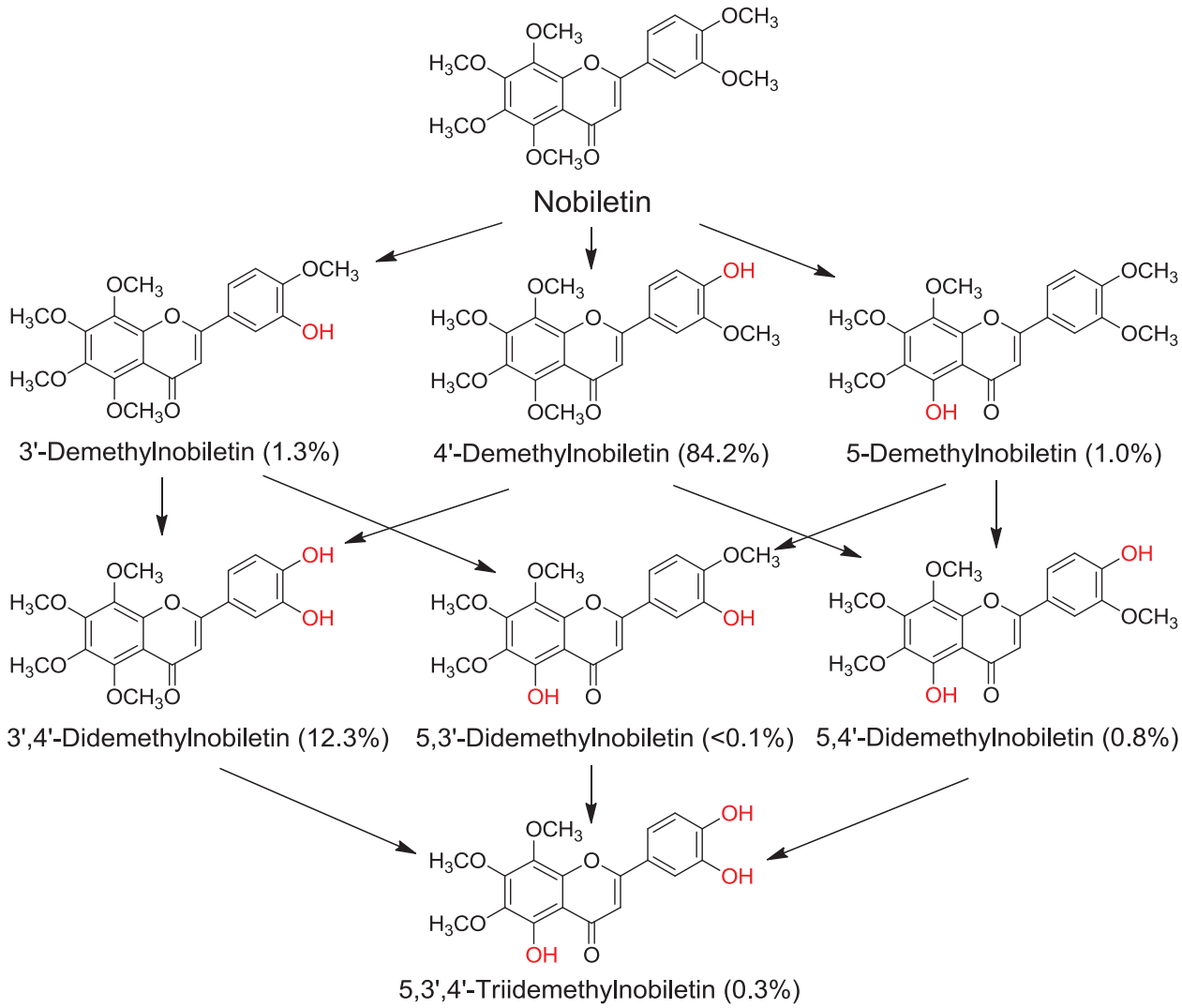

FIGURE 4 Proposed metabolic pathways for the demethylation of nobiletin *in vivo*. Based on metabolites identified in murine urine, collected at 12 h after oral administration of nobiletin. Values in parentheses are percentages of detected total metabolites of nobiletin. Based on data from Li et al. (2006) and Wei et al. (2013)

after oral administration (39.65 mmol/rat/day for 12 days) (Nielsen, Breinholt, Cornett, & Dragsted, 2000). Results showed that tangeretin metabolites amounted to as much as 18.48 mmol (approximately 47% of the daily dose), more than 75% of which were excreted in faeces, and the rest was in the urine. Either in faeces or in urine, the most abundant metabolite was identified as 4′-demethyltangeretin, contributing 26.6% to the total excretion. Of note, tangeretin in the intact form was detected in faeces, over 7% of the daily dose, but the intact form was not found in urine. Additionally, the metabolites of tangeretin included five demethylated ones, that is, 4′,6-, 4′,7-, and 5,6-didemethyltangeretin, 6-demethyltangeretin, and 4′,6,7-tridemethyltangeretin, and one hydroxylated derivative, that is, 3′-hydroxy-4′-demethyltangeretin (3′,4′-dihydroxy-5,6,7,8-tetramethoxyflavone), as shown in Figure 6.

In addition, phase II sulfation/glucuronidation is a common metabolic pathway of PMFs *in vivo*. A study found that 62% of tangeretin urinary metabolites in rats were demethylated derivatives, while the remaining 38% identified as glucuronic acid or sulfate conjugates (Nielsen et al., 2000).

The hepatic cytochrome P450 enzymes (CYPs) might involve in the metabolic process of PMFs. By employing rat liver CYPs, tangeretin was metabolized to 4′-demethyltangeretin and 3′,4′-didemethylnobiletin (Nielsen, Breinholt, Justesen, Cornett, & Dragsted, 1998). Mechanistically, the CYP3A, CYP1A2, and CYP2B were proposed to be responsible for flavonoid demethylation, whereas CYP1A was involved in hydroxylation. In human liver CYPs, nobiletin was demethylated to yield 4′-, 7-, and 6-demethylnobiletin with a relative ratio of 1:4.1:0.5, respectively (Koga et al., 2011). Furthermore,

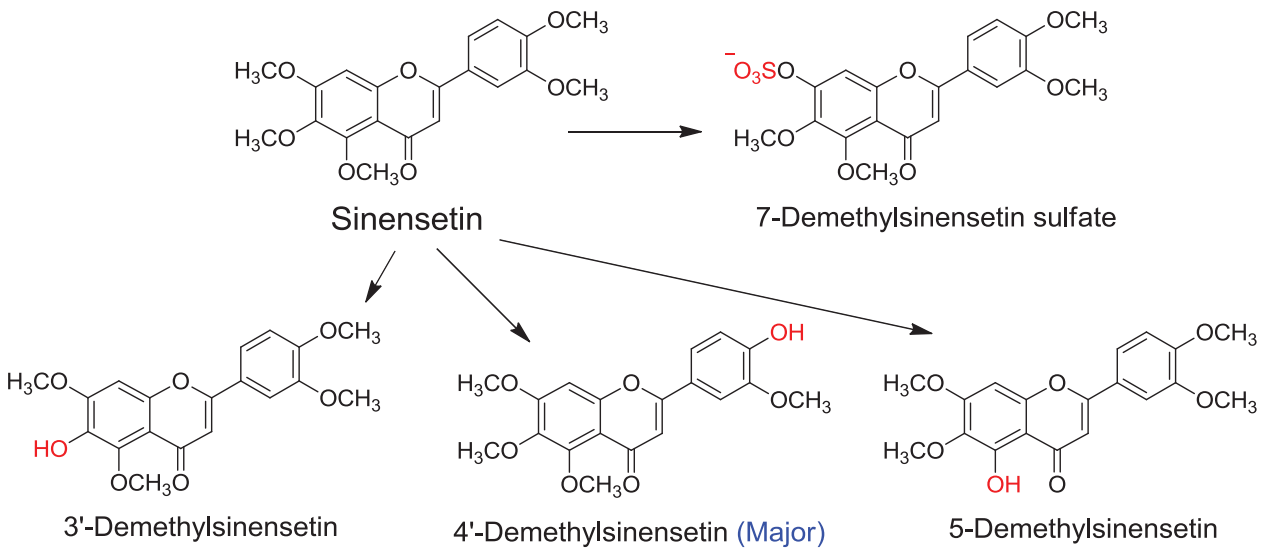

FIGURE 5 Main metabolites of sinensetin identified in rat urine, including three demethylated derivatives and one demethylated and sulfated conjugate, with 4′-demethylsinensetin (marked with "major" in blue parentheses) most predominant. Based on data from Wei et al. (2013)

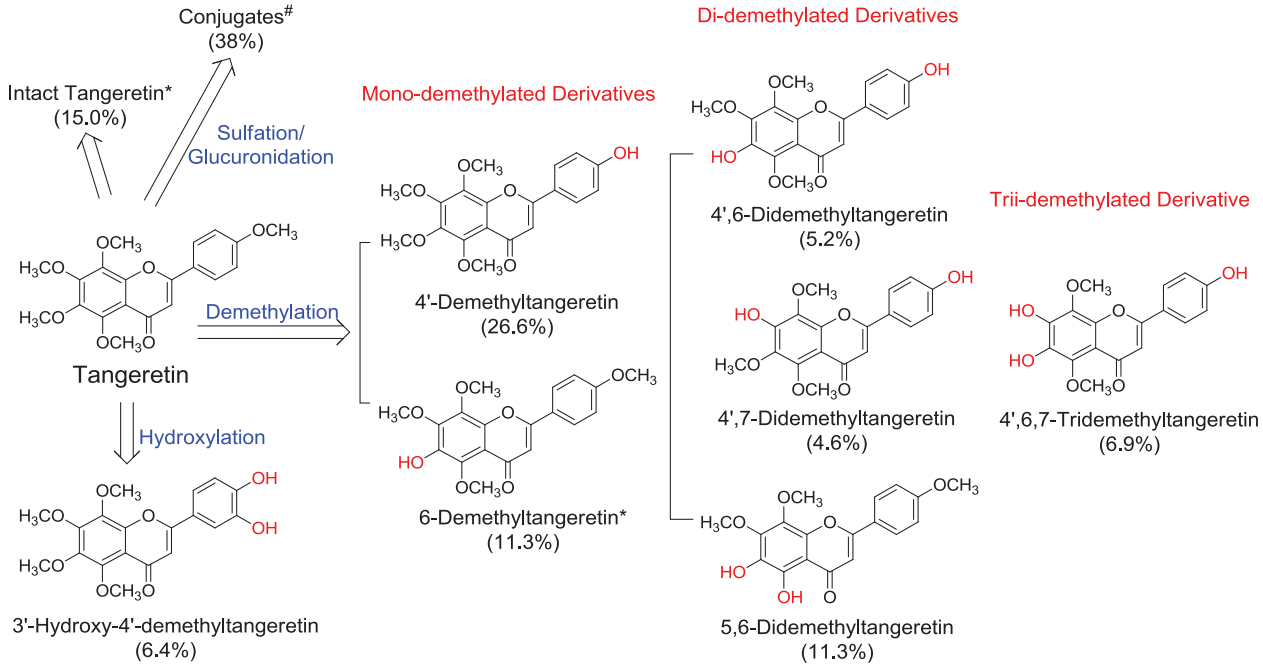

FIGURE 6 Main metabolites of tangeretin identified in rat urine and faeces, including two mono-, three di-, and one tri-demethylated derivatives, one hydroxylated derivative, and tangeretin in its intact form, as well as glucuronidated/sulfated conjugates, with 4′-demethyltangeretin most predominant. Values in parentheses are percentages of detected total urinary and faecal metabolites of tangeretin. *Metabolites detected only in the faeces. #Structures undefined and not shown here. Based on data from Nielsen et al. (2000)

CYP1A1, CYP1A2 and CYP1B1 showed high activity for the formation of 4′-demethylnobiletin, while CYP3A4 and CYP3A5 tended to catalyze the formation of 7-, and 6-demethylnobiletin. Different P-450 isozymes appear to be responsible for specific metabolic reactions (e.g., demethylation and hydroxylation) and specific reactive sites, and thereby afford different metabolites. Additionally, gut bacteria were reported to be implicated in the biotransformation of PMFs. *Blautia* sp. MRG-PMF1, isolated from human gut bacterium, was stated to completely demethylate 12 PMFs *in vitro* (Burapan, Kim, & Han, 2017). Despite many efforts aiming to determine the

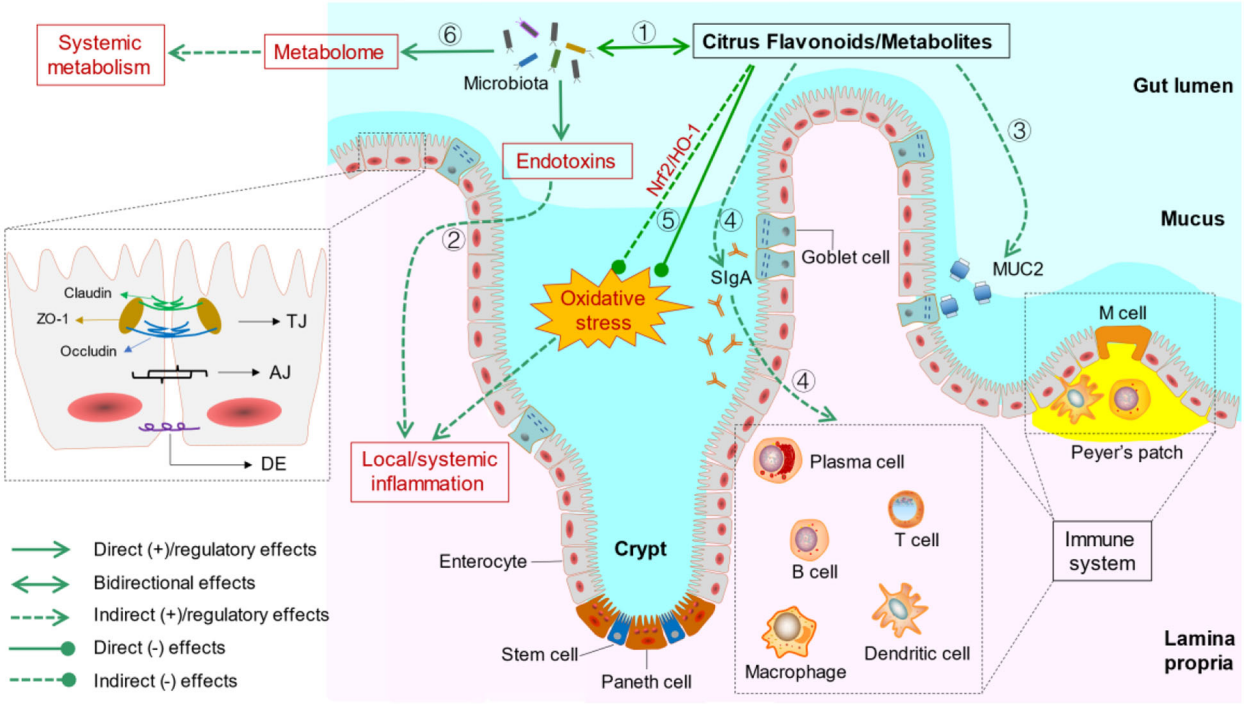

FIGURE 7 Beneficial actions of CFs as well as their metabolic bioactives on the intestinal barrier. (1) Positive modification of microbiota profiles, and in turn, microbiota metabolizing CFs; (2) protection of the intestinal permeability against damage from luminal insults (e.g., endotoxins) by shaping TJs to mitigate paracellular permeabilization, and further improvement of local/systemic inflammatory condition; (3) maintenance on the mucus layer by promoting MUC2 production; (4) modulation of the intestinal immunity, for example, regulating the differentiation and proliferation of immune cells, and increasing sIgA secretion; (5) inhibition of oxidative damage through direct free radicals scavenging and/or indirect antioxidant action based on Nrf2/HO-1 pathway; (6) remodeling of systemic metabolism (e.g., glucose metabolism and lipid metabolism) homeostasis via regulation of microbiome and metabolome. TJ, tight junction; AJ, adherens junction; DE, desmosome; MUC2, mucin 2; SIgA, secretory immunoglobulin A

metabolites of PMFs, the underlying molecular mechanism whereby these PMFs are metabolized as well as the associated metabolic pathway should be drawn further attention, which is very important to evaluate the safety and biofunctionality of PMFs.

In summary, as a result of the intestinal metabolism, the bioavailable fraction of CFs is comprised of a mixture of the parent compound, aglycones, sulfated/glucuronidated/methylated conjugates, and/or phenolic catabolites. The initial metabolism in gut is highly associated with CFs' health-promoting properties as this metabolic process can modify absorption of the parental compounds and metabolites might exhibit more or similar, or perhaps less bioactivities. Actually, emerging evidence suggests that *in vivo* metabolic processes, particularly transformation by gut microbiota, may elicit or enhance the intrinsic bioactivities of CFs, and have a positive impact on health (Chiou et al., 2014; Luca et al., 2020; Shakour, Fayek, & Farag, 2020; Wu, Song, Rakariyatham, Zheng, Wang, et al., 2015). In this context, it might not be difficult to understand the paradox of why CFs show a low oral bioavailability but afford a myriad of bioactivities. CFs and their intestinal metabolites can exert their biological actions locally, for example, direct effects on the structural elements of gut barrier including tight junctions, mucus layer, immune cells, and particularly gut microbiota, and also can enter into the bloodstream to exhibit systemic actions. Notably, local actions of CFs can also in part underlie systemic actions, for instance, modulation of microbiota profiles benefiting intestinal barrier and remodeling of glucose and lipid homeostasis (Gil-Cardoso et al., 2016; Thevaranjan et al., 2017; Tung et al., 2018; Zhang, Zhu, et al., 2020).

4 | PROTECTIVE EFFECTS OF CFS/METABOLITES ON THE INTESTINAL BARRIER: LOCALLY AND SYSTEMICALLY

The intestinal barrier is a dynamic functional entity. It is integrated by the physical barrier (e.g., IECs, intercellular junction and mucus layer), gut microbiota, the intestinal immunity, and related biochemical components (e.g., secretory immunoglobulin A) (Figure 7). Protective actions of CFs exerted on the intestinal barrier can target the

luminal microbiota, and/or the different pathways as well as molecules that are involved in sustaining the barrier physiology (Figure 7). On the basis of the structural elements of the intestinal barrier, health-promoting effects of CFs on the barrier function can be summarized as following: (a) maintenance of the intestinal permeability; (b) protection of mucus layer; (c) regulation of intestinal immune system; d) defense against oxidative stress; and e) interaction with intestinal microbiota.

4.1 | Regulation of the intestinal permeability

The intestinal permeability, contributing to the regulation of solute and fluid exchange between the lumen and tissues, is a key criterion to evaluate the intestinal barrier function (Camilleri, 2019). Impaired intestinal permeability might enable an invasion of luminal insults (e.g., from endotoxins and dietary antigens), and trigger local injuries such as barrier dysfunction, diarrhea, and IBD (Chang et al., 2017). Furthermore, if these luminal insults reach other tissues and organs along with the blood, it can lead to a cascade response mediated by cytokines, which might trigger systemic disorders, such as insulin resistance, type 2 diabetes mellitus (T2DM), cardiovascular disease, adipose inflammation, and NAFLD (Cani et al., 2008; Clemente-Postigo et al., 2019; Li, Lin, Vanhoutte, Woo, & Xu, 2016; Nagata et al., 2017). Hence, it is of critical importance to maintain the intestinal permeability for health.

4.1.1 | Modulation of the intestinal permeability by protecting TJs

As depicted in Figure 7, tight junctions (TJs) is a highly integrated complex between adjoining enterocytes, which are constructed by claudins, zonula occludens 1 (ZO-1), and occludins. TJs are directly responsible for the regulation of intestinal permeability (Pearce et al., 2018).

Multiple evidence (Table 1) indicates that citrus PMFs are beneficial for shaping TJs and preserving the intestinal permeability. The mechanism of recent discovery that nobiletin-based dietary intervention significantly attenuated dextran sulfate sodium-induced intestinal barrier dysfunction in mice has demonstrated the association with the up-regulation of claudin-7 expression in intestinal epithelial tissue, leading to a protective restoration of TJs and barrier permeability (Wen et al., 2020). Mechanism investigation indicated that the TJs protective action of nobiletin is mediated by a nuclear transcription factor—hepatocyte nuclear factor 4α (HNF4α). Chen's group found that nobiletin protected mice from diet-induced metabolic dysfunction by directly activating retinoid acid receptor-related orphan receptors (RORs) to enhance circadian rhythms (He et al., 2016). Both HNF4α and RORs are nuclear receptors, which are usually characterized *in vivo* by steroid hormones-dependent activation of transcription (Mangelsdorf et al., 1995). Interestingly, nobiletin is similar to endogenous steroid molecules in both chemical structure and polarity. Moreover, an *in vitro* cell culture experiment discovered the anti-inflammatory action of nobiletin was very similar to dexamethasone, an anti-inflammatory steroid drug (Lin et al., 2003). Considering all these studies, we postulate that HNF4α is another direct *in vivo* target of nobiletin, by which nobiletin initiated the HNF4α-claudin-7 signaling pathway to recover the damaged intestinal barrier.

Additionally, the intestinal permeability protective action of CFs can be mediated by multiple TJs expression regulation-associated upstream events: (a) facilitation of TJs assembly signals, for example, AMP-activated protein kinase (AMPK); (b) inhibition of TJs disassembly signals, for example, nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPK), and phosphoinositide-3-kinases (PI3K)/Akt, and (c) mitigation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-related oxidative damage to TJs (Cao et al., 2018; Cremonini et al., 2019; Junyuan et al., 2018; Walker et al., 2000; Yang, Bibi, Du, Suzuki, & Zhu, 2017).

4.1.2 | Improvement of local/systemic inflammatory status

Alterations in TJs resulting in intestinal permeabilization can allow the passage of luminal insults which can initiate local, and even systemic inflammation (Awad, Hess, & Hess, 2017). Some CFs/metabolites can confer protective actions onto the intestinal permeability by modulating TJs, which is conducive to improving local/systemic inflammatory status (Table 1).

A 0.3% (wt/wt) dietary supplementation with naringenin prevented intestinal barrier defects and inflammation, and thus significantly attenuated colitis-related symptoms (evaluated as body weight, disease activity index score and colon length) in colitic mice (Azuma, Shigeshiro, Kodama, Tanabe, & Suzuki, 2013). This effect of naringenin was mediated, at least partially through recovery of the intestinal permeability (Azuma et al., 2013). Also, improvement of local inflammatory status within gut has been observed for metabolites of nobiletin. After oral administration, nobiletin and its demethylated metabolites concentrated in the colonic tissue of mice, suppressed local inflammatory reactions, and ultimately contributed to the inhibition of colitis-related colon carcinogenesis

TABLE 1 Overview of CFs/metabolites that protect the intestinal permeability to confer local/systemic effects

Bioactive compound/component	Model	Treatment	Outcome vs. control[Proposal mechanism]	Reference
Nobiletin	DSS-induced intestinal barrier damage mice; inflammatory stimulation in HT-29 cells and IEC-6 cells	0.01% (wt/wt) in diet, along with 3% (wt/vol) DSS in drinking water for 1 week; TNF-α, IL-1β, and LPS	↓ Body weight loss, DAI score, colon shortness, villi shortness, fluorescein isothiocyanate-dextran permeability; ↑ claudin-7 and hepatocyte nuclear factor 4α expression (in vivo) ↑ TER, claudin-7 mRNA level, claudin-7 fluorescence signaling; → claudin-7 level upon HNF-4α knockdown; ↓ luciferase activity of claudin-7 reporter construct in HNF-4α mutants (in vitro) [Attenuation of the intestinal barrier damage by activating HNF4α-claudin-7 signaling pathway]	Wen et al. (2020)
Nobiletin	TNBS-induced colitis in Sprague–Dawley male rats; LPS-induced barrier dysfunction model in Caco-2 cell	20 and 40 mg/kg b.w., intragastric, after 1 day of TNBS induction for 7 days	↑ Body weight and food intake; ↓ macroscopically visible damage, DAI, colon weight-to-length ratio (in vivo) ↑ TER; ↓ inulin flux (in vitro) ↓ Akt, NF-κB p65 and MLCK level (in vivo & in vitro) [Restoration of gut barrier permeability through inhibition of Akt/NF-κB/MLCK pathway]	Xiong et al. (2015)
Naringenin	DSS-induced colitis in male BALB/c mice	0.3% (wt/wt) dietary supplementation for 9 days	↓ Colitis-related symptoms by improving body weight, DAI score and colon length; ↓inflammatory cytokines level; ↑ TJs expression [Anticolitic effect and barrier protection based on anti-inflammation]	Azuma et al. (2013)
Nobiletin and its demethylated metabolites	LPS-stimulated RAW 264.7 cells	20.7, 41.4, and 82.8 μM mixture with nobiletin, and 3′-, 4′-, and 3′,4′-demethylnobiletin at a ratio of 1: 1.65: 12.05: 6	↓ Proinflammatory enzyme level; ↑ antioxidative enzymes expression; ↓ cell cycle progression	Wu et al. (2017)
Cyanidin and delphinidin	High fat-fed C57BL/6J mice	anthocyanins containing 66% cyanidin, 28% delphinidin and 5.6% peonidin, 40 mg/kg b.w., dietary supplementation for 14 weeks	↓ Obesity, dyslipidemia, insulin resistance, intestinal permeability, endotoxemia, NADPH oxidase overexpression, oxidative stress; ↑ TJs expression [Systemically metabolic protective effects partly due to redox signaling-mediated modulation of TJs expression and intestinal permeability improvement]	Daveri et al. (2018); Cremonini et al. (2019)

(Continues)

TABLE 1 (Continued)

Bioactive compound/component	Model	Treatment	Outcome vs. control[Proposal mechanism]	Reference
Quercetin	High-fat diet fed C57BL/6J mice	0.05% (wt/wt) mixed in diet, roughly equal to 80 mg/kg b.w. per day, for 16 weeks	↓ Body weight gain, epididymal fat accumulation, hepatic triglyceride concentration; ↑ insulin sensitivity; ↓ endotoxemia [Protection from developing NAFLD by rebuilding gut homeostasis]	Porras et al. (2019)

Abbreviations: Akt, protein kinase B; b.w., body weight; DAI, disease activity index; DSS, dextran sulfate sodium; HNF-4α, hepatocyte nuclear factor 4α; IL-1β, interleukin-1β; LPS, lipopolysaccharide; MLCK, myosin light chain kinase; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, nonalcoholic fatty liver disease.; NF-κB, nuclear factor-kappa B; TER, transepithelial electric resistance; TJs, tight junctions; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF-α, Tumor Necrosis Factor Alpha.
Mark caption: ↑ increased/active effect; ↓ decreased/inhibitory effect; → no significant change.

(Wen et al., 2020; Wu et al., 2017). HPLC analysis identified 4′-demethylnobiletin, 3′-demethylnobiletin and 3′,4′-didemethylnobiletin as the major metabolites of nobiletin in the colon. In contrast, the colonic level of nobiletin was about 20-fold lower than that of its three metabolites (Wu, Song, Wang, et al., 2015).

Dietary intervention-based improvement of the intestinal permeability to mitigate systemically inflammatory status and associated diseases is one of the research focuses currently (Cox, West, & Cripps, 2015). For instance, anthocyanins such as cyanidin and delphinidin have been demonstrated to alleviate high fat-stimulated obesity, liver steatosis, and insulin resistance in mice (Cremonini et al., 2019; Daveri et al., 2018). Quercetin consumption was found to protect obese mice from developing NAFLD (Porras et al., 2019). These beneficial effects of CFs could be due, in part, through their anti-inflammatory actions in the intestine. Gil-Cardoso et al. reviewed *in vivo* and *in vitro* evidence to support that dietary flavonoids prevent systemic inflammation by means of reducing the intestinal permeability and improving local inflammatory condition to remodel systemically metabolic homeostasis (Gil-Cardoso et al., 2016). In brief, these authors concluded that selected CFs can mitigate systemic inflammation and associated metabolic disorders through their actions on different organs such as pancreas, liver, and adipose tissue, beyond their initial effects in gut.

4.2 | Protective actions on the intestinal mucus layer

The mucus layer overlies the IECs as the first barrier preventing large particles, including commensal bacteria, from directly contacting the IECs layer (De Lisle, Roach, & Jansson, 2007; Martens, Neumann, & Desai, 2018). Defects in the intestinal mucus layer such as reduced mucins production and damaged mucin polymers connection might be a risk factor for dozens of diseases, such as cystic fibrosis, ulcer colitis (UC), and IBD (Fridge, Conrad, Gerson, Castillo, & Cox, 2007; Heazlewood et al., 2008; Ijssennagger, van der Meer, & van Mil, 2016). Take UC as an example, one suggested pathogenesis is that some bacteria cleave mucins and/or dissolve the mucus by proteases to make the inner mucus layer permeate, and enable bacteria to reach the epithelium, which is when the immune system is activated and inflammation is triggered (Johansson, Sjovall, & Hansson, 2013).

Some CFs exhibit potential mucus protective effect, particularly on regulation of the expression and secretion of mucins. In human intestinal goblet cell-like LS174T, quercetin induced the secretion of two main intestinal gel-forming mucins, that is, Muc2 and Muc5AC, via phospholipase C (PLC)/protein kinase C (PKC)/kinase (ERK)1-2 pathway (Damiano et al., 2018). The mucins production-promoting effect of some CFs was further observed in animal experiments. For example, hesperetin significantly relieved the colitis symptoms in mice, which was partly due to up-regulation of Muc2 expression and maintenance of the epithelial barrier function (Zhang, Lei, Hu, & Dong, 2020). Mucin-depleted foci refers to the foci of crypts with scarce or absent mucins. It is a type of premalignant lesions that could predict colon carcinogenesis (Caderni et al., 2003). Naringenin and a CFs-rich extract derived from bergamot juice were observed to effectively limit the deterioration of precancerous lesions (measured as the numbers of mucin-depleted foci) in rats, respectively (Navarra et al., 2020; Rehman et al., 2018).

The Muc2 mucin forms the skeleton of the intestinal mucus, which is well documented as an essential role in maintaining homeostasis in the gut (Cornick, Kumar, Moreau, Gaisano, & Chadee, 2019). A study from Klepsch et al. (2018) discovered that nuclear orphan receptor NR2F6 was a upstream regulator of Muc2, which can

directly transactivate Muc2 expression via binding to Muc2 promoter. *Nr2f6*-deficient mice showed a reduced Muc2 expression and altered intestinal permeability, which could result in spontaneous late-onset colitis (Klepsch et al., 2018). Given less attention to the role of CFs in the intestinal mucus regulation, and particularly of the resulting effects on UC and related diseases, CFs-based dietary intervention to regulate Muc2 expression by targeting NR2F6 might be a worthwhile research topic to treat mucus damage-related diseases.

4.3 | Modulatory effects on intestinal immunity

Emerging evidence points to the regulatory property of hesperidin for intestinal immunity, including modulation of T cells differentiation and the subsequent activation and infiltration of effector cells, as well as the secretory immunoglobulin A (SIgA) level in the intestine. In a Th2 responses-bearable mouse model, hesperidin administration increased regulatory T cell differentiation in mesenteric lymph nodes and also changed the lymphocyte compositions in both intraepithelial lymphocytes and lamina propria lymphocytes (Camps-Bossacoma, Franch, Perez-Cano, & Castell, 2017). Further ELISA quantitative analyses detected an increasing secretion of SIgA in gut after the hesperidin treatment. In healthy rats, hesperidin exerted a similarly immunomodulatory effect on the intestine (Estruel-Amades et al., 2019). SIgA plays an essential role in protecting the intestinal barrier function by balancing lumen bacteria and immune cells homeostasis. This concept is supported by the work from Khounlotham et al. (2012), which showed the production of commensal bacteria-specific SIgA effectively compensated for the barrier dysfunction in mice with an architectural damage of TJs, and further reduced the risk for development of colitis. In a diarrheic murine model evoked by LPS from *Aeromonas hydrophila*, hesperidin significantly restored SIgA level and increased IgM (another immunoglobulin, a positive helper of SIgA) secretion, which reconfirmed the immunomodulatory potential of hesperidin (Abuelsaad, Mohamed, Allam, & Al-Solumani, 2013).

IBD is a group of intestinal disorders with inflammatory symptoms, the pathogenesis of which is extremely complicated and remains elusive. Most experts, however, agree on the significant involvement of the intestinal immune system. The potential immunomodulation of CFs on IBD have been extensively investigated not only *in vitro* but also in animal models. For example, tangeretin attenuated murine colitis by modulating the intestinal immunity, for example, induction of T cell differentiation, and suppressing the inflammatory responses (Eun, Woo, & Kim, 2017). Considering the high concentrations of these CFs reaching at the intestinal lumen, it is possible that their anti-inflammatory and immunomodulatory effects could be beneficial in IBD.

4.4 | Antioxidant property to benefit for intestinal barrier

A large body of experimental and clinical evidence suggests that a dysregulated intestinal immune system can lead to a sustained overproduction of reactive oxygen/nitrogen species, which can directly damage the intestinal epithelial tissue, and lead to the intestinal barrier injury and dysfunction (Conner, Brand, Davis, Kang, & Grisham, 1996; Pavlick et al., 2002). It has been well established that CFs possess prominent antioxidant property, particularly free radicals scavenging and metal-ion chelating actions (Jayaraman, Subramani, Sheik Abdullah, & Udaiyar, 2018; Wang et al., 2018).

Some CFs and their metabolites can exert the antioxidant effect at the intestinal lumen in a direct way. Although a direct antioxidant action of flavonoids in most tissues and compartments are impossible since an insufficient accumulation, the gastrointestinal tract (more specifically refers to the stomach and the upper part of the intestine) is considered as the only site of the body in which the biological relevance of a direct free radicals scavenging is realistic (Galleano, Verstraeten, Oteiza, & Fraga, 2010; Treml & Smejkal, 2016). Deprez, Mila, Huneau, Tome, and Scalbert (2001) estimated that dietary flavonoids can reach the gastrointestinal tract with a concentration up to 300 μM. After oral administration, about 24% tangeretin accumulated in the gastrointestinal tract of mice at 4 hr, which was dramatically higher than that in the peripheral organs including liver, kidney, brain, spleen, heart, and lung (Hung et al., 2018). A convincing evidence is from a very recent study showing that gavage feeding of luteolin (30 mg/kg b.w.) almost abolished irinotecan-induced oxidative damage to the duodenum of mice, associated with significant reduction of reactive oxygen species (ROS) levels, and complete restoration of reduced glutathione amount and catalase activity (Boeing et al., 2020). Such effects, however, was not observed in the colon. These results stress the direct antioxidative role of luteolin in the upper part of the intestine, which may contribute, at least in part, for their barrier protective function.

In addition to the direct action, the antioxidant activity of CFs can be mediated by nuclear factor erythroid 2-related factor 2/oxygenase-1 (Nrf2/HO-1) signaling pathway. In LPS-stimulated RAW 264.7 macrophages, xanthomicrol, a major colonic metabolite of 5-demethyltangeretin, was shown to decrease the produc-

tion of proinflammatory cytokine, cyclooxygenase-2, and inducible nitric oxide synthase to exert its anti-inflammatory effect, while increase the expression of antioxidative enzyme HO-1 to exert its antioxidant action (Guo et al., 2018). Also, 4′-demethylnobiletin and *Citrus sinensis* extract were discovered to scavenge ROS and increase HO-1 expression (Pepe et al., 2017; Wu, Song, Rakariyatham, Zheng, Guo, et al., 2015). Such effects may be of relevance to the activation of the transcriptional activity of Nrf2, which further induced the expression of Nrf2-dependant Phase II antioxidant enzymes, such as HO-1 and NADH quinone oxidoreductase 1 (NQO1) (Guo et al., 2012). In a mice model of colitis-associated colon carcinogenesis, oral administration of nobiletin promoted the nuclear translocation of Nrf2 and significantly up-regulated the protein levels of HO-1 and NQO1 in the colonic mucosa. Accumulated antioxidant enzymes could produce multiple antioxidants, such as bilirubin and carbon oxide, to improve the cellular antioxidative capacity and ultimately confer an anti-inflammatory effect (Wang, Smith, & Zucker, 2004; Wu et al., 2017). The evidence supports the Nrf2-dependent antioxidant action of PMFs in gut to benefit for the intestinal barrier.

4.5 | Regulatory effects of CFs on gut microbiota

In the past 20 years, a great deal of research has underscored the important role of gut microbiota in the digestion of dietary phytochemicals, including CFs (Almeida, Borge, Piskula, Tudose, & Santos, 2018; Hu et al., 2019). Indeed, the relationship between CFs and gut microbiota is bidirectional. That is to say, in addition to gut microbiome metabolizing CFs as previously discussed, CFs shape microorganism.

4.5.1 | CFs-based alteration of gut microbiota profiles

Gut microbiota plays a critical role in maintaining body health for human, and a disruption to this microecology will lead to microbial dysbiosis and then develop gut-related and even systematic diseases (Honda & Littman, 2016). In this scenario, the role of specific dietary phytochemicals has been robustly linked in recent years to changes in the gut microbial flora (Pei et al., 2020; Zhao, Hu, Zuo, & Wang, 2018).

CFs are known to positively shape gut microbiota composition (Table 2), for example, normalizing the relative abundance of *Firmicutes*-to-*Bacteroidetes* ratio (*F*/*B* ratio) under the pathological condition such as obesity. *Firmicutes* and *Bacteroidetes* are two major dominant phyla in human gut microbiota, and *F*/*B* ratio is considered as a rough indicator of bacterial shifts. A shift towards an increase in *F*/*B* ratio was identified in obese mice and humans, while weight loss intervention was accompanied by an improved abundance of *Bacteroidetes* (Ley et al., 2005; Ley, Turnbaugh, Klein, & Gordon, 2006; Turnbaugh, Bäckhed, Fulton, & Gordon, 2008). PMFs enriched extract has demonstrated the amelioration of high-fat diet-induced obesity and obesity-related metabolic syndrome including hepatic steatosis, dyslipidemia, and insulin resistance in mice (Zeng et al., 2020). The mechanism of the metabolically protective effect of citrus PMFs was associated with its modulatory action on gut dysbiosis, characterized by lowering *F*/*B* ratio and enriching *Bacteroidetes* (Zeng et al., 2020).

Akkermansia muciniphila has been emerging as a promising species of gut-protective bacteria since it was first proposed by Derrien, Vaughan, Plugge, and de Vos (2004). In healthy individuals, *A. muciniphila* is relatively abundant, accounting for up to 4% of the intestinal bacteria. However, its abundance decreases in certain disease states, for example, IBD, T2DM, and obesity (Everard et al., 2013; Png et al., 2010). Citrus PMFs such as nobiletin, tangeretin, and 5-demethylnobiletin are found to well shape *A. muciniphila* in gut. For example, Zhang, Zhu, et al. (2020) demonstrated that PMFs-rich citrus peels extract significantly prevented high-fat diet-induced obesity in mice by improving microflora dysbiosis, mainly manifested in enriching the relative abundance of *Akkermansia* and *Allobaculum*. Furthermore, this antiobesity effect of PMFs treatment presented in a dose- and time-dependent manner (Zhang, Zhu, et al., 2020). A comparative study between flavonoids respectively from citrus peels and oolong tea found that both these dietary flavonoids protected carnitine-feeding mice from vascular inflammation (Chen et al., 2019). Subsequent genus-level analysis of the gut microbiota in the cecum showed that oolong tea-derived flavonoids up-regulated *Lactobacillus* genus, whereas citrus PMFs increased *Akkermansia*. Recently, *A. muciniphila*, together with *Faecalibacterium prausnitzii* and *Ruminococcus bromii* was considered as the next-generation health-promoting gut bacteria (Anhê et al., 2016; Lordan, Thapa, Ross, & Cotter, 2020). Furthermore, the ability of dietary flavonoids consumption to shape *Akkermansia* genus has been identified in humans (Bekiares, Krueger, Meudt, Shanmuganayagam, & Reed, 2018). In this context, we postulate that citrus PMFs might represent a group of promising dietary phytochemicals that favor the presence of *A. muciniphila* in the gut microbiota to alleviate intestinal dysbiosis and even consequently bring metabolic benefits to the host.

TABLE 2 Representative studies on positive modification of gut microbiota profiles by flavonoid compound/component from citrus species, as well as resulting local and systemic benefits

Bioactive compound/component	Model	Treatment	Outcome vs. control[Proposal mechanism]	Reference
PMFs	High-fat diet-induced metabolic syndrome in male C57BL/6J mice	Citrus PMFs-rich extract, 120 mg/kg b.w., oral gavage daily for 8 weeks	Improvement of host metabolic features including alleviating glucose tolerance and insulin resistance, reducing the lipids levels and fat accumulation in liver tissues, and lowering body weight gain, and correction of gut dysbiosis and BCAAs levels; ↑*Bacteroides ovatus*; ↓ *F*/*B* ratio [Systemically metabolic protective effects via alleviation of gut dysbiosis and further regulation of BCAAs metabolism]	Zeng et al. (2020)
PMFs	High-fat diet-induced obesity in male C57BL/6J mice	PMFs-rich citrus peels extract, 0.25% and 0.5% mixed in diet for 11 weeks, respectively	↑ SCFAs levels in feces, *Akkermansia* and *Allobaculum* in a dose- and time-dependent manner; ↓ *F*/*B* ratio [Antiobesity effects by reshaping gut microbiota profiles]	Zhang et al. (2020)
PMFs	Carnitine-induced vascular inflammation in female C57BL/6J mice	1% mixed in diet for 6 weeks	↓ Trimethylamine-*N*-oxide level in plasma, vascular inflammatory markers; ↑ *Akkermansia*	Chen et al. (2019)
Hesperidin	Healthy Lewis rats	100 and 200 mg/kg b.w., respectively, three times a week for 4 weeks	↑ *Staphylococcus* proportion in cecal content (both dosages), *Lactobacillus* proportion (high dose group)	Estruel-Amades et al. (2019)
Flavanones	Healthy women ($n = 10$)	Orange juice, 300 mL/day/person, consumption for 2 months	↓ Glucose, triglycerides, total cholesterol, low-density lipoprotein-cholesterol in blood serum; ↑ insulin sensitivity, SCFAs levels, population of *Bifidobacterium* and *Lactobacillus* in faeces [Alteration of gut microbiota profiles and metabolites, and improvement of host blood biochemical parameters]	Lima et al. (2019)
Hesperetin	Male Wistar rats	1.0% mixed in diet for 3 weeks	↓ Weight of abdominal adipose tissues including the mass of mesenteric, perirenal, and epididymal adipose tissues, level of *Clostridium* subcluster XIVa; ↑ cecum content weight, levels of faecal *Clostridium* clusters IV and XVIII, SCFAs levels [Gaining a lower weight of abdominal adipose tissues by positively shaping gut microbiota to reduce starch digestion and stimulate SCFAs productions]	Unno et al. (2015)

(Continues)

TABLE 2 (Continued)

Bioactive compound/componen	Model	Treatment	Outcome vs. control[Proposal mechanism]	Reference
Flavonoids	Broad-spectrum antibiotics exposure-induced microbiota dysbiosis and BAs dysmetabolism	8.7 g/kg b.w., oral gavage once daily for 4 weeks	Correction of dysbiosis, recovery of intestinal permeability, reshaping BAs homeostasis [Maintenance of gut microbiota and BAs homeostasis via up-regulation of the liver-gut axis]	Liu et al. (2020)

Abbreviations: b.w., body weight; BAs, bile acids.; BCAAs, branched-chain amino acids; *F*/*B* ratio, *Firmicutes*-to-*Bacteroidetes* ratio; PMFs, polymethoxyflavones; SCFAs, short-chain fatty acids.
Mark caption: ↑ increased/active effect; ↓ decreased/inhibitory effect.

Additionally, some CFs can increase a variety of other beneficial bacteria and/or inhibit the growth of pathogens. In healthy rats, a dietary supplementation of hesperidin (100 and 200 mg/kg, three times a week for 4 weeks, respectively) greatly enriched the number of total bacteria in the caecum: both dosages resulted in an increased proportion of *Staphylococcus*, while an additional increase in the *Lactobacillus* level was observed in the high dose group (Estruel-Amades et al., 2019). Thus, we can see that not only the structure but the consumptive dose might affect the modulatory action of CFs on bacteria composition. A controlled clinical trial found a higher level of two dominated probiotics, that is, *Bifidobacterium* and *Lactobacillus*, in feces of 10 healthy women who continuously consumed orange juice for two months (Lima et al., 2019). Interestingly, after the daily treatment of orange juice, the subjects exhibited a significantly improved blood biochemical parameters including lipids, glucose, and insulin sensitivity.

The above discussion illustrates that CFs shaping microbiota composition is usually accompanied by a series of positive changes in host metabolic physiology such as lipoprotein, glucose, and insulin sensitivity. The next question would be to find how CFs exert metabolic protective effects and even prevent or ameliorate metabolic syndrome (MetS), what role dose gut microbiota play in this process, and how the participation of gut microbiota works in host.

4.5.2 | Modulatory action of CFs on gut microbiota: mechanism and outcome

Over the past two decades, gut microbiota has been well identified as a key role in the establishment and maintenance of host health, as well as in the pathogenesis of disease, particularly metabolic diseases including obesity, T2DM, hyperlipidemia, hepatic steatosis and IBD among others (Dabke, Hendrick, & Devkota, 2019; Lavelle & Sokol, 2020). The interaction modes between gut microbiota and its host is primarily by means of metabolites, a group of small molecules that are produced as intermediate or end products of microbial metabolism. These reported metabolites mostly focused on short-chain fatty acids (SCFAs), bile acids (BAs), and branched-chain amino acids (BCAAs) (Bloomgarden, 2018; Dalile, Van Oudenhove, Vervliet, & Verbeke, 2019; Wang & Zhao, 2018). Given above, we conceive of a cycle of circular causality to explain the complicated relationship between the intestinal microbial ecosystem and the host systemically metabolic homeostasis (Figure 8). Specifically, the altered composition of gut microbiota might cause changes in microbiome metabolome, which might further affect host whole metabolome. When microbiota dysbiosis occurs, the metabolic homeostasis of the microbiome in gut is broken, and followed by host metabolic disequilibrium, then MetS in host might appear. Exactly, CFs-based dietary intervention can end this aberrant cycle by rebuilding gut microecosystem and getting the metabolism back on track. Table 2 showed representative studies on modification of microbiome and metabolome by CFs to afford systemic metabolism-protective benefits.

The anaerobic microbiome fermentation degrades nondigestible carbohydrates to produce SCFAs, mainly acetate, propionate, and butyrate, in the cecum and colon (Cummings, Pomare, Branch, Naylor, & Macfarlane, 1987). SCFAs are cardinal examples of gut microbiota-derived beneficial metabolites and involved in epithelial barrier function maintenance, fatty acid oxidation, energy balance, and glucose homeostasis in humans (Cummings et al., 1987; Tan et al., 2014; Zheng et al., 2017). Microbiome-wide association studies have demonstrated a general reduction in levels of faecal SCFAs in patients with MetS, for example, obesity, T2DM, and IBD (Dugas et al., 2018; Sanna et al., 2019). Some CFs are capable of optimizing the microbiota profiles to increase SCFAs level and ultimately benefiting host for their metabolic homeostasis. For example, a diet feeding study with mice found that hesperetin administration at a dose of 16.4 mmol/kg for 3 weeks modified the composition of fecal microbiota by increasing the levels of *Clostridium* clusters IV and

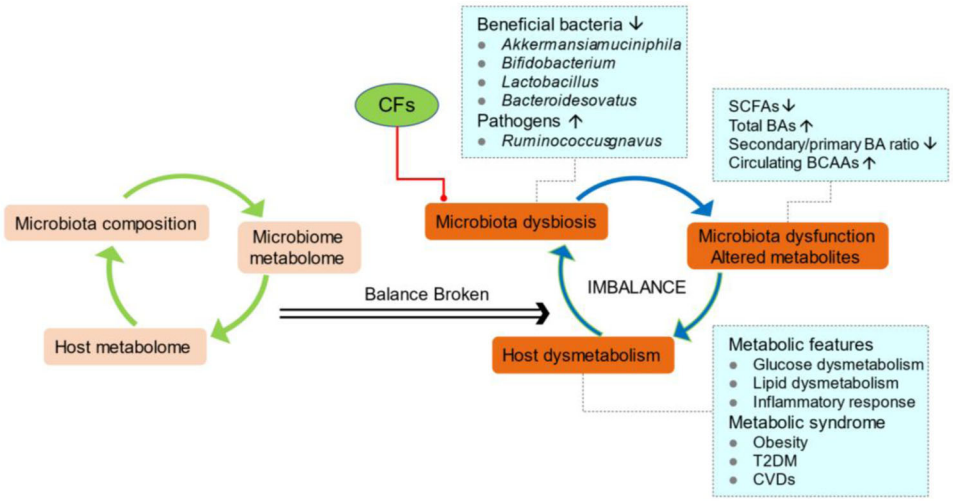

FIGURE 8 Schematic of the circular causality relationship between the intestinal microbial ecosystem and the host systemically metabolic homeostasis. Gut microbiota can modify the host systemic metabolism by means of their metabolites. In MetS, dysbiosis leads to alteration of the microbiome metabolome, causing the host dysmetabolism. CFs-based dietary intervention can end this aberrant cycle by reshaping microbiota profiles and ultimately getting the host metabolism back on track. SCFAs, short-chain fatty acids; BA, bile acids; BCAAs, branched-chain amino acids; T2DM, type 2 diabetes mellitus; CVDs, cardiovascular diseases

XVIII while reducing *Clostridium* subcluster XIVa level (Unno, Hisada, & Takahashi, 2015). Such changes in the microbial community led to a reduction in starch digestion, and parts of undigested starch were utilized to produce SCFAs in the cecal pools, and elevated SCFAs levels eventually contributed to the a lower weight gain of abdominal adipose tissues in mice (Unno et al., 2015).

BAs are small molecules that are synthesized from cholesterol by the liver, which can form micelles to aid in lipid digestion and absorption. There is a subtle interplay between the intestinal microbes and BAs. That is to say, gut microbiota is involved in BAs metabolism, for example, converting primary to secondary BAs in the colon, whereas BAs have a strong influence on microbiota composition and density (Lorenzo-Zúñiga et al., 2003; Ridlon, Kang, & Hylemon, 2006). In this regard, it is reasonable to assume that microbiota dysbiosis co-occurs with BAs dysmetabolism. This unbalanced condition, however, might be a precursor to MetS (Chávez-Talavera, Tailleux, Lefebvre, & Staels, 2017; Saad, Santos, & Prada, 2016). A very recent study established an antibiotics-induced mice model of dysbiosis, paralleled by BAs dysmetabolism and the intestinal dysfunction, to evaluate the metabolically modulatory effect of CFs *in vivo* (Liu et al., 2020). Results showed that the CFs-rich extract from *Citrus aurantium L.* counteracted the dysbiosis and recovered the intestinal permeability. This function of CFs was demonstrated to be associated with its capacity to reshape the homeostasis of BAs via up-regulation of the liver-gut axis related farnesoid X receptor (FXR)/fibroblast growth factor 15 (FGF15) pathway (Liu et al., 2020). FXR is a major BAs-sensing receptor, whose activation leads to alterations in energy metabolism-related pathways and therefore known as a key regulator of whole-body energy metabolism (Teodoro, Rolo, & Palmeira, 2011). Currently, FXR is under study as a promising target for the treatment of diseases of excess, such as T2DM. For example, silymarin, a natural flavonoid from the milk thistle seeds, was identified to directly interact with FXR to ameliorate diet-induced obesity by improving insulin resistance, dyslipidaemia, and inflammation, and shaping the BAs pool in liver (Gu et al., 2016). Since being potential in stimulating FXR signaling and rebuilding the BAs-related homeostasis, CFs, particularly PMFs, might deserve further research as FXR agonists to battle with BAs dysmetabolism-associated MetS.

BCAAs, namely, leucine, isoleucine, and valine, are a cluster of essential amino acids that can be taken up by skeletal muscle and used for energy, muscle repair, or building (Kimball & Jefferson, 2006). However, emerging evidence suggests that inappropriately high levels of circulating BCAAs is of relevance to increased risks for overweight, insulin resistance, and T2DM (Lu et al., 2020; Neis, Dejong, & Rensen, 2015; Wolak-Dinsmore et al., 2018). Therefore, prevention or mitigation of MetS by means of modulating BCAAs levels has become a research hotspot (Meslier et al., 2020). In this regard, citrus PMFs was found to potently ameliorate high-fat diet-induced MetS by shaping gut microbiota and further altering BCAAs levels (Zeng et al., 2020). Specifically, therapeutic interventions with PMFs-rich citrus extract greatly enriched the abundance of *Bacteroides ovatus*, a beneficial commensal bacterium responsible for decreased BCAAs

concentrations—identified by a gavage experiment with *Bacteroides ovatus*, and thereby reduced BCAAs levels in the host serum and feces, and ultimately improved host metabolic features, including alleviating glucose tolerance and insulin resistance, reducing the lipids levels and fat accumulation in liver tissues, and lowering body weight gain (Zeng et al., 2020). Interestingly, this action process of PMFs in protection of host metabolism is completely consistent with the circular causality mode we proposed in Figure 8, that is, modulation of host metabolic phenotype by means of modifying gut microbiome and metabolome.

In brief, selected CFs are capable of reshaping gut microbiota profiles and modulating their important metabolites and as an outcome, might be a systemically metabolic protection for the host. Therefore, some CFs might give not only local benefits within the intestine, but also systemic effects, such as prevention of obesity and T2DM as mentioned above. These evidences suggest a dietary phytochemical-based therapeutic strategy for a wide array of MetS associated with dysbiosis. Actually, the complex interplay of diet–microbiome–host has become clear and concrete gradually with the development of metabolomics, which provides an opportunity for the phytochemical-based dietary intervention. At the same time, however, much is left undetermined, for example, how to control the range and degree of the ultimate effects postintake of CFs since its extensive metabolism and influence *in vivo?* What differences of these actions are there between health and disease conditions? How to deal with the extremely complicated inter-individual variation in microbiota profiles, dietary habit, and host metabolic state to guarantee the dietary intervention effects? All these issues warrant approach to provide convincing evidence for future application of CFs—as well as other phytochemicals-based therapeutics.

4.5.3 | Inclusion of CFs in prebiotics?

As discussed above, the connection between gut microbiota and CFs is bidirectional and, more significantly, benefits each other. Some types of CFs or CFs extract were therefore referred to as the prebiotics. Whether CFs should be considered a prebiotic depends on the exact definition of prebiotics—a subject of some recent controversy (Bindels, Delzenne, Cani, & Walter, 2015; Hutkins et al., 2016).

The prebiotics concept was first defined by Gibson in 1995, describing as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon (Gibson & Roberfroid, 1995). According to this definition, prebiotics are usually limited to nondigestible and readily fermentable dietary oligosaccharides, such as fructo-oligosaccharides, galacto-oligosaccharides, mannan-oligosaccharide, xylo-oligosaccharide and inulin. In December 2016, the International Scientific Association for Prebiotics and Probiotics updated the definition of a prebiotic "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). On the one hand, this revision is more inclusive, which focused on "utilization," as opposed to "fermentation" in the older definition, to define the mode of selective microbial modification of prebiotic substrates, which provides an opportunity for noncarbohydrate substances, for example, CFs, to be incorporated in prebiotics. On the other hand, this updated definition retains the requirement for selective microbiota-mediated mechanisms for benefiting the target host to be a prebiotic. Emerging evidence from animal studies showing potential of CFs in shaping gut microbiota profiles and prevention or treatment of some types of MetS. These activities of CFs in these studies were referred as prebiotic potential or prebiotic-like effects. Thus, convincing weight of evidence is required to demonstrate the specific microbiota modification by CFs and causality linked to health outputs in the target host before calling CFs a real 'prebiotic'.

The cross-talk between CFs and microbes is complicated, combined with the potential to both local benefits within gut and systemic bioeffects, which highlights the need to study both CFs and the microbiome in concert. Understanding how CFs are metabolized by the microbiome and how the microbiome and metabolome in turn reshaped by CFs may further clarify the intestinal barrier protective effects of CFs as well as underlying mechanism.

5 | CONCLUSION

Knowledge on the actions of naturally occurring phytochemicals at the intestinal barrier is significant to design dietary, supplementary, or pharmacological patterns for the prevention and/or treatment of disease. This review highlights the potential candidates of dietary flavonoids derived from *Citrus* genus, as well as their metabolites, which perform well in improving multiple conditions of gut physiology to benefit for the intestinal barrier homeostasis. Still, such protective effects of CFs on gut barrier are supported by evidence from studies limited to *in vitro* or in animal level, whereas available data from epidemiologic and well-designed human trials are relatively scarce. Moreover, given the wide effects of natural phytochemicals on the body system, future research may dig into the underlying action mechanisms of CFs, including determination of involved molecular targets and signaling pathway, microbiota/CFs crosstalk, possibly discrepant influences on the small intestine and the large intestine, and ultimate health effects. It is hoped that this review will inspire further studies to provide more scientific evidence to promote

future application of citrus fruits and their flavonoid components in the intestinal barrier loss-related local and systemic diseases.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant No. 81803548), Tianjin Innovative Research Team Grant (TD13-5087), Hubei Province Technical Innovation Special Project (2019ABA100), and Natural Science Foundation of Tianjin (19JCQNJC12400).

AUTHOR CONTRIBUTIONS

Meiyan Wang wrote the first draft, prepared tables and figures, and took responsibility of submissions. Shiming Li revised, further enriched, and finalized the manuscript. Hui Zhao and Xiang Wen compiled the manuscript. Chi-Tang Ho designed the framework of the review and revised the draft.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Meiyan Wang https://orcid.org/0000-0001-7914-3060

REFERENCES

Abuelsaad, A. S., Mohamed, I., Allam, G., & Al-Solumani, A. A. (2013). Antimicrobial and immunomodulating activities of hesperidin and ellagic acid against diarrheic Aeromonas hydrophila in a murine model. *Life Sciences*, *93*(20), 714–722.

Almeida, A. F., Borge, G. I. A., Piskula, M., Tudose, A., Tudoreanu, L. Valentová, K., … Santos, C. N. (2018). Bioavailability of quercetin in humans with a focus on interindividual variation. *Comprehensive Reviews in Food Science & Food Safety*, *17*(3), 714–731.

Ameer, B., Weintraub, R. A., Johnson, J. V., Yost, R. A., & Rouseff, R. L. (1996). Flavanone absorption after naringin, hesperidin, and citrus administration. *Clinical Pharmacology and Therapeutics*, *60*(1), 34–40.

Anhê, F. F., Pilon, G., Roy, D., Desjardins, Y., Levy, E., & Marette, A. (2016). Triggering Akkermansia with dietary polyphenols: A new weapon to combat the metabolic syndrome? *Gut Microbes*, *7*(2), 146–153.

Aura, A. M., O'Leary, K. A., Williamson, G., Ojala, M., Bailey, M., Puupponen-Pimia, R., … Poutanen, K. (2002). Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora *in vitro*. *Journal of Agricultural and Food Chemistry*, *50*(6), 1725–1730.

Auricchio, S., Rubino, A., Landolt, M., Semenza, G., & Prader, A. (1963). Isolated intestinal lactase deficiency in the adult. *Lancet*, *2*(7303), 324–326.

Awad, W. A., Hess, C., & Hess, M. (2017). Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. *Toxins (Basel)*, *9*(2), 60.

Azuma, T., Shigeshiro, M., Kodama, M., Tanabe, S., & Suzuki, T. (2013). Supplemental naringenin prevents intestinal barrier defects and inflammation in colitic mice. *Journal of Nutrition*, *143*(6), 827–834.

Bang, S. H., Hyun, Y. J., Shim, J., Hong, S. W., & Kim, D. H. (2015). Metabolism of rutin and poncirin by human intestinal microbiota and cloning of their metabolizing α-L-rhamnosidase from *Bifidobacterium dentium*. *Journal of Microbiology and Biotechnology*, *25*(1), 18–25.

Bekiares, N., Krueger, C. G., Meudt, J. J., Shanmuganayagam, D., & Reed, J. D. (2018). Effect of Sweetened Dried Cranberry Consumption on Urinary Proteome and Fecal Microbiome in Healthy Human Subjects. *OMICS: A Journal of Integrative Biology*, *22*(2), 145–153.

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *12*(5), 303–310.

Bloomgarden, Z. (2018). Diabetes and branched-chain amino acids: What is the link? *Journal of Diabetes*, *10*(5), 350–352.

Boeing, T., de Souza, P., Speca, S., Somensi, L. B., Mariano, L. N. B., Cury, B. J., … de Andrade, S. F. (2020). Luteolin prevents irinotecan-induced intestinal mucositis in mice through antioxidant and anti-inflammatory properties. *British Journal of Pharmacology*, *177*(10), 2393–2408. doi: https://doi.org/10.1111/bph.14987

Borges, G., Lean, M. E., Roberts, S. A., & Crozier, A. (2013). Bioavailability of dietary (poly)phenols: A study with ileostomists to discriminate between absorption in small and large intestine. *Food & Function*, *4*(5), 754–762.

Borges, G., Mullen, W., Mullan, A., Lean, M. E., Roberts, S. A., & Crozier, A. (2010). Bioavailability of multiple components following acute ingestion of a polyphenol-rich juice drink. *Molecular Nutrition & Food Research*, *54*(Suppl 2), S268–277.

Brand, W., Oosterhuis, B., Krajcsi, P., Barron, D., Dionisi, F., van Bladeren, P. J., … Williamson, G. (2011). Interaction of hesperetin glucuronide conjugates with human BCRP, MRP2 and MRP3 as detected in membrane vesicles of overexpressing baculovirus-infected Sf9 cells. *Biopharmaceutics and Drug Disposition*, *32*(9), 530–535.

Braune, A., & Blaut, M. (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes*, *7*(3), 216–234.

Braune, A., Gütschow, M., Engst, W., & Blaut, M. (2001). Degradation of quercetin and luteolin by Eubacterium ramulus. *Applied and Environmental Microbiology*, *67*(12), 5558–5567.

Bredsdorff, L., Nielsen, I. L., Rasmussen, S. E., Cornett, C., Barron, D., Bouisset, F., … Williamson, Gary. (2010). Absorption, conjugation and excretion of the flavanones, naringenin and hesperetin from alpha-rhamnosidase-treated orange juice in human subjects. *British Journal of Nutrition*, *103*(11), 1602–1609.

Brett, G. M., Hollands, W., Needs, P. W., Teucher, B., Dainty, J. R., Davis, B. D., … Kroon, Paul A. (2009). Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. *British Journal of Nutrition*, *101*(5), 664–675.

Burapan, S., Kim, M., & Han, J. (2017). Demethylation of Polymethoxyflavones by human gut bacterium, *Blautia* sp. MRG-PMF1. *Journal of Agricultural and Food Chemistry*, *65*(8), 1620–1629.

Caderni, G., Femia, A. P., Giannini, A., Favuzza, A., Luceri, C., Salvadori, M., Dolara, P. (2003). Identification of mucin-depleted foci

in the unsectioned colon of azoxymethane-treated rats: Correlation with carcinogenesis. *Cancer Research*, *63*(10), 2388–2392.

Camilleri, M. (2019). Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut*, *68*(8), 1516–1526.

Camps-Bossacoma, M., Franch, A., Perez-Cano, F. J., & Castell, M. (2017). Influence of hesperidin on the systemic and intestinal rat immune response. *Nutrients*, *9*(6), 580.

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, *57*(6), 1470–1481.

Cao, H., Chen, X., Jassbi, A. R., & Xiao, J. (2015). Microbial biotransformation of bioactive flavonoids. *Biotechnology Advances*, *33*(1), 214–223.

Cao, H., Liu, J., Shen, P., Cai, J., Han, Y., Zhu, K., … Cao, Yongguo (2018). Protective effect of naringin on DSS-induced ulcerative colitis in mice. *Journal of Agricultural and Food Chemistry*, *66*(50), 13133–13140.

Cermak, R., Landgraf, S., & Wolffram, S. (2004). Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. *British Journal of Nutrition*, *91*(6), 849–855.

Chambers, C. S., Viktorova, J., Rehorova, K., Biedermann, D., Turkova, L., Macek, T., … Valentová, Kateřina. (2020). Defying multidrug resistance! modulation of related transporters by flavonoids and flavonolignans. *Journal of Agricultural and Food Chemistry*, *68*(7), 1763–1779.

Chang, J., Leong, R. W., Wasinger, V. C., Ip, M., Yang, M., & Phan, T. G. (2017). Impaired intestinal permeability contributes to ongoing bowel symptoms in patients with inflammatory bowel disease and mucosal healing. *Gastroenterology*, *153*(3), 723–731.e1.

Chávez-Talavera, O., Tailleux, A., Lefebvre, P., & Staels, B. (2017). Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology*, *152*(7), 1679–1694.e3.

Chen, P. Y., Li, S., Koh, Y. C., Wu, J. C., Yang, M. J., Ho, C. T., & Pan, Min-Hsiung. (2019). Oolong tea extract and citrus peel polymethoxyflavones reduce transformation of L-carnitine to trimethylamine-N-oxide and decrease vascular inflammation in l-carnitine feeding mice. *Journal of Agricultural and Food Chemistry*, *67*(28), 7869–7879.

Chiou, Y. S., Wu, J. C., Huang, Q., Shahidi, F., Wang, Y. J., Ho, C. T., & Pan, M.-H. (2014). Metabolic and colonic microbiota transformation may enhance the bioactivities of dietary polyphenols. *Journal of Functional Foods*, *7*, 3–25.

Choi, W., Yeruva, S., & Turner, J. R. (2017). Contributions of intestinal epithelial barriers to health and disease. *Experimental Cell Research*, *358*, 71–77.

Chun, O. K., Chung, S. J., & Song, W. O. (2007). Estimated dietary flavonoid intake and major food sources of U.S. adults. *Journal of Nutrition*, *137*(5), 1244–1252.

Cirmi, S., Ferlazzo, N., Lombardo, G. E., Maugeri, A., Calapai, G., Gangemi, S., & Navarra, Michele. (2016). Chemopreventive agents and inhibitors of cancer hallmarks: May citrus offer new perspectives? *Nutrients*, *8*(11), 698.

Clemente-Postigo, M., Oliva-Olivera, W., Coin-Aragüez, L., Ramos-Molina, B., Giraldez-Perez, R. M., Lhamyani, S., … Tinahones, F. J. (2019). Metabolic endotoxemia promotes adipose dysfunction and inflammation in human obesity. *American Journal of Physiology: Endocrinology and Metabolism*, *316*(2), E319–E332.

Conner, E. M., Brand, S. J., Davis, J. M., Kang, D. Y., & Grisham, M. B. (1996). Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease: Toxins, mediators, and modulators of gene expression. *Inflammatory Bowel Diseases*, *2*(2), 133–147.

Cornick, S., Kumar, M., Moreau, F., Gaisano, H., & Chadee, K. (2019). VAMP8-mediated MUC2 mucin exocytosis from colonic goblet cells maintains innate intestinal homeostasis. *Nature Communications*, *10*(1), 4306.

Cox, A. J., West, N. P., & Cripps, A. W. (2015). Obesity, inflammation, and the gut microbiota. *Lancet Diabetes & Endocrinology*, *3*(3), 207–215.

Cremonini, E., Daveri, E., Mastaloudis, A., Adamo, A. M., Mills, D., Kalanetra, K., … Oteiza, P. I. (2019). Anthocyanins protect the gastrointestinal tract from high fat diet-induced alterations in redox signaling, barrier integrity and dysbiosis. *Redox Biology*, *26*, 101269.

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, *28*(10), 1221–1227.

Cuyckens, F., & Claeys, M. (2005). Determination of the glycosylation site in flavonoid mono-O-glycosides by collision-induced dissociation of electrospray-generated deprotonated and sodiated molecules. *Journal of Mass Spectrometry*, *40*(3), 364–372.

Dabke, K., Hendrick, G., & Devkota, S. (2019). The gut microbiome and metabolic syndrome. *Journal of Clinical Investigation*, *129*(10), 4050–4057.

Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota-gut-brain communication. *Nature Reviews Gastroenterology & Hepatology*, *16*(8), 461–478.

Damiano, S., Sasso, A., De Felice, B., Di Gregorio, I., La Rosa, G., Lupoli, G. A., … Santillo, M. (2018). Quercetin increases MUC2 and MUC5AC gene expression and secretion in intestinal goblet cell-like LS174T via PLC/PKCα/ERK1-2 pathway. *Frontiers in Physiology*, *9*, 357.

Daniels, L. B., Coyle, P. J., Chiao, Y. B., Glew, R. H., & Labow, R. S. (1981). Purification and characterization of a cytosolic broad specificity beta-glucosidase from human liver. *Journal of Biological Chemistry*, *256*(24), 13004–13013.

Daveri, E., Cremonini, E., Mastaloudis, A., Hester, S. N., Wood, S. M., Waterhouse, A. L., … Oteiza, Patricia I. (2018). Cyanidin and delphinidin modulate inflammation and altered redox signaling improving insulin resistance in high fat-fed mice. *Redox Biology*, *18*, 16–24.

Davis, C., Bryan, J., Hodgson, J., & Murphy, K. (2015). Definition of the Mediterranean diet: A literature review. *Nutrients*, *7*(11), 9139–9153.

Day, A. J., DuPont, M. S., Ridley, S., Rhodes, M., Rhodes, M. J., Morgan, M. R., & Williamson, Gary. (1998). Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Letters*, *436*(1), 71–75.

Day, A. J., Gee, J. M., DuPont, M. S., Johnson, I. T., & Williamson, G. (2003). Absorption of quercetin-3-glucoside and quercetin-4'-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology*, *65*(7), 1199–1206.

De Lisle, R. C., Roach, E., & Jansson, K. (2007). Effects of laxative and *N*-acetylcysteine on mucus accumulation, bacterial load, transit, and inflammation in the cystic fibrosis mouse small intestine. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *293*(3), 577–584.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, *18*(14), 1818–1892.

Deprez, S., Mila, I., Huneau, J. F., Tome, D., & Scalbert, A. (2001). Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxidants and Redox Signaling*, *3*(6), 957–967.

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *54*(Pt 5), 1469–1476.

Dugas, L. R., Lie, L., Plange-Rhule, J., Bedu-Addo, K., Bovet, P., Lambert, E. V., … Layden, Brian T. (2018). Gut microbiota, short chain fatty acids, and obesity across the epidemiologic transition: The METS-Microbiome study protocol. *BMC Public Health*, *18*(1), 978.

Estruel-Amades, S., Massot-Cladera, M., Perez-Cano, F. J., Franch, A., Castell, M., & Camps-Bossacoma, M. (2019). Hesperidin effects on gut microbiota and gut-associated lymphoid tissue in healthy rats. *Nutrients*, *11*(2), 324.

Eun, S. H., Woo, J. T., & Kim, D. H. (2017). Tangeretin inhibits IL-12 expression and NF-kappaB activation in dendritic cells and attenuates colitis in mice. *Planta Medica*, *83*(6), 527–533.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., … Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(22), 9066–9071.

Felgines, C., Texier, O., Morand, C., Manach, C., Scalbert, A., Regerat, F., Rémésy, C. (2000). Bioavailability of the flavanone naringenin and its glycosides in rats. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *279*(6), G1148–1154.

Fridge, J. L., Conrad, C., Gerson, L., Castillo, R. O., & Cox, K. (2007). Risk factors for small bowel bacterial overgrowth in cystic fibrosis. *Journal of Pediatric Gastroenterology and Nutrition*, *44*(2), 212–218.

Galleano, M., Verstraeten, S. V., Oteiza, P. I., & Fraga, C. G. (2010). Antioxidant actions of flavonoids: Thermodynamic and kinetic analysis. *Archives of Biochemistry and Biophysics*, *501*(1), 23–30.

Gattuso, G., Caristi, C., Gargiulli, C., Bellocco, E., Toscano, G., & Leuzzi, U. (2006). Flavonoid glycosides in bergamot juice (*Citrus bergamia* Risso). *Journal of Agricultural and Food Chemistry*, *54*(11), 3929–3935.

Gervais, L., & Bardin, A. J. (2017). Tissue homeostasis and aging: New insight from the fly intestine. *Current Opinion in Cell Biology*, *48*, 97–105.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., … Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, *14*(8), 491–502.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*(6), 1401–1412.

Gil-Cardoso, K., Gines, I., Pinent, M., Ardevol, A., Blay, M., & Terra, X. (2016). Effects of flavonoids on intestinal inflammation, barrier integrity and changes in gut microbiota during diet-induced obesity. *Nutrition Research Reviews*, *29*(2), 234–248.

Groschwitz, K. R., & Hogan, S. P. (2009). Intestinal barrier function: Molecular regulation and disease pathogenesis. *Journal of Allergy and Clinical Immunology*, *124*(1), 3–22.

Gu, M., Zhao, P., Huang, J., Zhao, Y., Wang, Y., Li, Y., … Huang, C. (2016). Silymarin ameliorates metabolic dysfunction associated with diet-induced obesity via activation of farnesyl X receptor. *Frontiers in Pharmacology*, *7*, 345.

Guirro, M., Gual-Grau, A., Gibert-Ramos, A., Alcaide-Hidalgo, J. M., Canela, N., Arola, L., Mayneris-Perxachs, J. (2020). Metabolomics elucidates dose-dependent molecular beneficial effects of hesperidin supplementation in rats fed an obesogenic diet. *Antioxidants (Basel)*, *9*(1), 79.

Guo, S., Qiu, P., Xu, G., Wu, X., Dong, P., Yang, G., … Xiao, H. (2012). Synergistic anti-inflammatory effects of nobiletin and sulforaphane in lipopolysaccharide-stimulated RAW 264.7 cells. *Journal of Agricultural and Food Chemistry*, *60*(9), 2157–2164.

Guo, S., Wu, X., Zheng, J., Charoensinphon, N., Dong, P., Qiu, P., … Xiao, H. (2018). Anti-inflammatory effect of xanthomicrol, a major colonic metabolite of 5-demethyltangeretin. *Food & Function*, *9*(6), 3104–3113.

He, B., Nohara, K., Park, N., Park, Y. S., Guillory, B., Zhao, Z., … Chen, Z. (2016). The small molecule nobiletin targets the molecular oscillator to enhance circadian rhythms and protect against metabolic syndrome. *Cell Metabolism*, *23*(4), 610–621.

Heazlewood, C. K., Cook, M. C., Eri, R., Price, G. R., Tauro, S. B., Taupin, D., … Mcguckin, M. A. (2008). Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Medicine*, *5*(3), e54.

Hollman, P. C., de Vries, J. H., van Leeuwen, S. D., Mengelers, M. J., & Katan, M. B. (1995). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *American Journal of Clinical Nutrition*, *62*(6), 1276–1282.

Honda, K., & Littman, D. R. (2016). The microbiota in adaptive immune homeostasis and disease. *Nature*, *535*(7610), 75–84.

Hu, H., Zhang, S., Liu, F., Zhang, P., Muhammad, Z., & Pan, S. (2019). Role of the gut microbiota and their metabolites in modulating the cholesterol-lowering effects of citrus pectin oligosaccharides in C57BL/6 mice. *Journal of Agricultural and Food Chemistry*, *67*(43), 11922–11930.

Hung, W. L., Chang, W. S., Lu, W. C., Wei, G. J., Wang, Y., Ho, C. T., & Hwang, L. S. (2018). Pharmacokinetics, bioavailability, tissue distribution and excretion of tangeretin in rat. *Journal of Food and Drug Analysis*, *26*(2), 849–857.

Hutkins, R. W., Krumbeck, J. A., Bindels, L. B., Cani, P. D., Fahey, G. Jr., Goh, Y. J., … Sanders, M. E. (2016). Prebiotics: Why definitions matter. *Current Opinion in Biotechnology*, *37*, 1–7.

Ijssennagger, N., van der Meer, R., & van Mil, S. W. C. (2016). Sulfide as a mucus barrier-breaker in inflammatory bowel disease? *Trends in Molecular Medicine*, *22*(3), 190–199.

Jayaraman, R., Subramani, S., Sheik Abdullah, S. H., & Udaiyar, M. (2018). Antihyperglycemic effect of hesperetin, a citrus flavonoid, extenuates hyperglycemia and exploring the potential role in antioxidant and antihyperlipidemic in streptozotocin-induced diabetic rats. *Biomedicine and Pharmacotherapy*, *97*, 98–106.

Jin, M. J., Kim, U., Kim, I. S., Kim, Y., Kim, D. H., Han, S. B., … Yoo, H. H. (2010). Effects of gut microflora on pharmacokinetics of hesperidin: A study on non-antibiotic and pseudo-germ-free rats. *Journal of Toxicology and Environmental Health. Part A*, *73*(21-22), 1441–1450.

Johansson, M. E., Sjovall, H., & Hansson, G. C. (2013). The gastrointestinal mucus system in health and disease. *Nature Reviews Gastroenterology & Hepatology*, *10*(6), 352–361.

Junyuan, Z., Hui, X., Chunlan, H., Junjie, F., Qixiang, M., Yingying, L., … Yue, Z. (2018). Quercetin protects against intestinal barrier disruption and inflammation in acute necrotizing pancreatitis through TLR4/MyD88/p38 MAPK and ERS inhibition. *Pancreatology*, *18*(7), 742–752.

Kanaze, F. I., Bounartzi, M. I., Georgarakis, M., & Niopas, I. (2007). Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *European Journal of Clinical Nutrition*, *61*(4), 472–477.

Khounlotham, M., Kim, W., Peatman, E., Nava, P., Medina-Contreras, O., Addis, C., … Parkos, C. A. (2012). Compromised intestinal epithelial barrier induces adaptive immune compensation that protects from colitis. *Immunity*, *37*(3), 563–573.

Kim, M., Kim, N., & Han, J. (2014). Metabolism of *Kaempferia parviflora* polymethoxyflavones by human intestinal bacterium *Bautia* sp. MRG-PMF1. *Journal of Agricultural and Food Chemistry*, *62*(51), 12377–12383.

Kimball, S. R., & Jefferson, L. S. (2006). Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. *Journal of Nutrition*, *136*(Suppl 1), 227S–231S.

Klepsch, V., Gerner, R. R., Klepsch, S., Olson, W. J., Tilg, H., Moschen, A. R., … Hermann-Kleiter, N. (2018). Nuclear orphan receptor NR2F6 as a safeguard against experimental murine colitis. *Gut*, *67*(8), 1434–1444.

Koga, N., Ohta, C., Kato, Y., Haraguchi, K., Endo, T., Ogawa, K., … Yano, M. (2011). *In vitro* metabolism of nobiletin, a polymethoxyflavonoid, by human liver microsomes and cytochrome P450. *Xenobiotica*, *41*(11), 927–933.

Lai, C. S., Tsai, M. L., Cheng, A. C., Li, S., Lo, C. Y., Wang, Y., … Pan, M.-H. (2011). Chemoprevention of colonic tumorigenesis by dietary hydroxylated polymethoxyflavones in azoxymethane-treated mice. *Molecular Nutrition & Food Research*, *55*(2), 278–290.

Lavelle, A., & Sokol, H. (2020). Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nature Reviews Gastroenterology & Hepatology*, *17*(4), 223–237.

Lavoie, S., Chun, E., Bae, S., Brennan, C. A., Gallini Comeau, C. A., Lang, J. K., … Garrett, W. S. (2020). Expression of FFAR2 by dendritic cells prevents their expression of IL27 and is required for maintenance of mucosal barrier and immune response against colorectal tumors in mice. *Gastroenterology*, *158*(5), 1359–1372.

Lee, J. Y., Hall, J. A., Kroehling, L., Wu, L., Najar, T., Nguyen, H. H., … Littman, Dan R. (2020). Serum amyloid A proteins induce pathogenic Th17 cells and promote inflammatory disease. *Cell*, *180*(1), 79–91.

Leese, H. J., & Semenza, G. (1973). On the identity between the small intestinal enzymes phlorizin hydrolase and glycosylceramidase. *Journal of Biological Chemistry*, *248*(23), 8170–8173.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11070–11075.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023.

Li, J., Lin, S., Vanhoutte, P. M., Woo, C. W., & Xu, A. (2016). *Akkermansia muciniphila* protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe-/- Mice. *Circulation*, *133*(24), 2434–2446.

Li, S., Wang, Z., Sang, S., Huang, M. T., & Ho, C. T. (2006). Identification of nobiletin metabolites in mouse urine. *Molecular Nutrition & Food Research*, *50*(3), 291–299.

Lima, A. C. D., Cecatti, C., Fidelix, M. P., Adorno, M. A. T., Sakamoto, I. K., Cesar, T. B., & Sivieri, K. (2019). Effect of daily consumption of orange juice on the levels of blood glucose, lipids, and gut microbiota metabolites: Controlled clinical trials. *Journal of Medicinal Food*, *22*(2), 202–210.

Lin, N., Sato, T., Takayama, Y., Mimaki, Y., Sashida, Y., Yano, M., & Ito, A. (2003). Novel anti-inflammatory actions of nobiletin, a citrus polymethoxy flavonoid, on human synovial fibroblasts and mouse macrophages. *Biochemical Pharmacology*, *65*(12), 2065–2071.

Lissner, D., Schumann, M., Batra, A., Kredel, L.-I., Kühl, A. A., Erben, U., … Siegmund, B. (2015). Monocyte and M1 macrophage-induced barrier defect contributes to chronic intestinal inflammation in IBD. *Inflammatory Bowel Diseases*, *21*(6), 1297–1305.

Liu, L., Liu, Z., Li, H., Cao, Z., Li, W., Song, Z., … Liu, Y. (2020). Naturally occurring TPE-CA maintains gut microbiota and bile acids homeostasis via FXR signaling modulation of the liver-gut axis. *Frontiers in Pharmacology*, *11*, 12.

Liu, X., Lin, C., Ma, X., Tan, Y., Wang, J., & Zeng, M. (2018). Functional characterization of a flavonoid glycosyltransferase in sweet orange (*Citrus sinensis*). *Frontiers in Plant Science*, *9*, 166.

Liu, Y., Heying, E., & Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety*, *11*(6), 530–545.

Liu, Z., & Hu, M. (2007). Natural polyphenol disposition via coupled metabolic pathways. *Expert Opinion on Drug Metabolism & Toxicology*, *3*(3), 389–406.

Lordan, C., Thapa, D., Ross, R. P., & Cotter, P. D. (2020). Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes*, *11*(1), 1–20.

Lorenzo-Zúñiga, V., Bartolí, R., Planas, R., Hofmann, A. F., Viñado, B., Hagey, L. R. … Gassull, M. A. (2003). Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. *Hepatology*, *37*(3), 551–557.

Lu, J., Gu, Y., Liu, H., Wang, L., Li, W., Li, W., … Hu, G. (2020). Daily branched-chain amino acid intake and risks of obesity and insulin resistance in children: A cross-sectional study. *Obesity (Silver Spring)*, *28*(7), 1310–1316.

Luca, S. V., Macovei, I., Bujor, A., Miron, A., Skalicka-Wozniak, K., Aprotosoaie, A. C., & Trifan, A. (2020). Bioactivity of dietary polyphenols: The role of metabolites. *Critical Reviews in Food Science and Nutrition*, *60*(4), 626–659.

Manach, C., Morand, C., Gil-Izquierdo, A., Bouteloup-Demange, C., & Remesy, C. (2003). Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *European Journal of Clinical Nutrition*, *57*(2), 235–242.

Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., … Evans, R. M. (1995). The nuclear receptor superfamily: The second decade. *Cell*, *83*(6), 835–839.

Martens, E. C., Neumann, M., & Desai, M. S. (2018). Interactions of commensal and pathogenic microorganisms with the intestinal mucosal barrier. *Nature Reviews: Microbiology*, *16*(8), 457–470.

Matsumoto, H., Ikoma, Y., Sugiura, M., Yano, M., & Hasegawa, Y. (2004). Identification and quantification of the conjugated metabolites derived from orally administered hesperidin in rat plasma. *Journal of Agricultural and Food Chemistry*, *52*(21), 6653–6659.

Mellor, J. D., & Layne, D. S. (1971). Steroid-D-glucosidase activity in rabbit tissues. *Journal of Biological Chemistry*, *246*(14), 4377–4380.

Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high-performance liquid chromatography: A review. *Journal of Agricultural and Food Chemistry*, *48*(3), 577–599.

Meslier, V., Laiola, M., Roager, H. M., De Filippis, F., Roume, H., Quinquis, B., … Ercolini, D. (2020). Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut*, *69*(7), 1258–1268.

Miyake, Y., Shimoi, K., Kumazawa, S., Yamamoto, K., Kinae, N., & Osawa, T. (2000). Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats. *Journal of Agricultural and Food Chemistry*, *48*(8), 3217–3224.

Miyake, Y., Yamamoto, K., & Osawa, T. (1997). Metabolism of antioxidant in lemon fruit (*Citrus limon* $B_{URM.\,f.}$) by human intestinal bacteria. *Journal of Agricultural and Food Chemistry*, *45*, 3738–3742.

Murakami, A., Koshimizu, K., Ohigashi, H., Kuwahara, S., Kuki, W., Takahashi, Y., … Matsuoka, Y. (2002). Characteristic rat tissue accumulation of nobiletin, a chemopreventive polymethoxyflavonoid, in comparison with luteolin. *Biofactors*, *16*(3–4), 73–82.

Nagata, N., Xu, L., Kohno, S., Ushida, Y., Aoki, Y., Umeda, R., … Ota, T. (2017). Glucoraphanin ameliorates obesity and insulin resistance through adipose tissue browning and reduction of metabolic endotoxemia in mice. *Diabetes*, *66*(5), 1222–1236.

Navarra, M., Femia, A. P., Romagnoli, A., Tortora, K., Luceri, C., Cirmi, S., … Caderni, G. (2020). A flavonoid-rich extract from bergamot juice prevents carcinogenesis in a genetic model of colorectal cancer, the Pirc rat (F344/NTac-Apc(am1137)). *European Journal of Nutrition*, *59*(3), 885–894.

Nectoux, A. M., Abe, C., Huang, S. W., Ohno, N., Tabata, J., Miyata, Y., … Matsui, T. (2019). Absorption and metabolic behavior of hesperidin (rutinosylated hesperetin) after single oral administration to Sprague–Dawley rats. *Journal of Agricultural and Food Chemistry*, *67*(35), 9812–9819.

Neis, E. P., Dejong, C. H., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients*, *7*(4), 2930–2946.

Nielsen, I. L., Chee, W. S., Poulsen, L., Offord-Cavin, E., Rasmussen, S. E., Frederiksen, H., … Williamson, G. (2006). Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: A randomized, double-blind, crossover trial. *Journal of Nutrition*, *136*(2), 404–408.

Nielsen, S. E., Breinholt, V., Cornett, C., & Dragsted, L. O. (2000). Biotransformation of the citrus flavone tangeretin in rats. Identification of metabolites with intact flavane nucleus. *Food and Chemical Toxicology*, *38*(9), 739–746.

Nielsen, S. E., Breinholt, V., Justesen, U., Cornett, C., & Dragsted, L. O. (1998). *In vitro* biotransformation of flavonoids by rat liver microsomes. *Xenobiotica*, *28*(4), 389–401.

Odenwald, M. A., & Turner, J. R. (2017). The intestinal epithelial barrier: A therapeutic target? *Nature Reviews Gastroenterology & Hepatology*, *14*(1), 9–21.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, *7*, 688–693.

Orrego-Lagarón, N., Vallverdú-Queralt, A., Martínez-Huélamo, M., Lamuela-Raventos, R. M., & Escribano-Ferrer, E. (2016). Metabolic profile of naringenin in the stomach and colon using liquid chromatography/electrospray ionization linear ion trap quadrupole-Orbitrap-mass spectrometry (LC-ESI-LTQ-Orbitrap-MS) and LC-ESI-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis*, *120*, 38–45.

Pavlick, K. P., Laroux, F. S., Fuseler, J., Wolf, R. E., Gray, L., Hoffman, J., & Grisham, M. B. (2002). Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radical Biology and Medicine*, *33*(3), 311–322.

Pearce, S. C., Al-Jawadi, A., Kishida, K., Yu, S., Hu, M., Fritzky, L. F., … Ferraris, R. P. (2018). Marked differences in tight junction composition and macromolecular permeability among different intestinal cell types. *BMC Biology*, *16*(1), 19.

Pei, R., Liu, X., & Bolling, B. (2020). Flavonoids and gut health. *Current Opinion in Biotechnology*, *61*, 153–159.

Pepe, G., Pagano, F., Adesso, S., Sommella, E., Ostacolo, C., Manfra, M., … Campiglia, P. (2017). Bioavailable *Citrus sinensis* extract: Polyphenolic composition and biological activity. *Molecules*, *22*(4), 623.

Pereira-Caro, G., Borges, G., van der Hooft, J., Clifford, M. N., Del Rio, D., Lean, M. E., … Crozier, Alan. (2014). Orange juice (poly)phenols are highly bioavailable in humans. *American Journal of Clinical Nutrition*, *100*(5), 1378–1384.

Pereira-Caro, G., Fernandez-Quiros, B., Ludwig, I. A., Pradas, I., Crozier, A., & Moreno-Rojas, J. M. (2018). Catabolism of citrus flavanones by the probiotics *Bifidobacterium longum* and *Lactobacillus rhamnosus*. *European Journal of Nutrition*, *57*(1), 231–242.

Pereira-Caro, G., Ludwig, I. A., Polyviou, T., Malkova, D., Garcia, A., Moreno-Rojas, J. M., & Crozier, A. (2016). Identification of plasma and urinary metabolites and catabolites derived from orange juice (poly)phenols: Analysis by high-performance liquid chromatography-high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry*, *64*(28), 5724–5735.

Pereira-Caro, G., Polyviou, T., Ludwig, I. A., Nastase, A. M., Moreno-Rojas, J. M., Garcia, A. L., … Crozier, A. (2017). Bioavailability of orange juice (poly)phenols: The impact of short-term cessation of training by male endurance athletes. *American Journal of Clinical Nutrition*, *106*(3), 791–800.

Pero, R. W. (2010). Health consequences of catabolic synthesis of hippuric acid in humans. *Current Clinical Pharmacology*, *5*(1), 67–73.

Png, C. W., Lindén, S. K., Gilshenan, K. S., Zoetendal, E. G., McSweeney, C. S., Sly, L. I., … Florin, T. H. J. (2010). Mucolytic bacteria with increased prevalence in IBD mucosa augment *in vitro* utilization of mucin by other bacteria. *American Journal of Gastroenterology*, *105*(11), 2420–2428.

Porras, D., Nistal, E., Martinez-Florez, S., Olcoz, J. L., Jover, R., Jorquera, F., … Sánchez-Campos, S. (2019). Functional interactions between gut microbiota transplantation, quercetin, and high-fat diet determine non-alcoholic fatty liver disease

development in germ-free mice. *Molecular Nutrition & Food Research*, *63*(8), e1800930.

Rehman, M. U., Rahman Mir, M. U., Farooq, A., Rashid, S. M., Ahmad, B., Bilal Ahmad, S., … Ahmad Ganaie, M. (2018). Naringenin (4,5,7-trihydroxyflavanone) suppresses the development of precancerous lesions via controlling hyperproliferation and inflammation in the colon of Wistar rats. *Environmental Toxicology*, *33*(4), 422–435.

Rera, M., Clark, R. I., & Walker, D. W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(52), 21528–21533.

Ridlon, J. M., Kang, D. J., & Hylemon, P. B. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research*, *47*(2), 241–259.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, Kieran. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. *European Journal of Nutrition*, *57*(1), 1–24.

Saad, M. J., Santos, A., & Prada, P. O. (2016). Linking gut microbiota and inflammation to obesity and insulin resistance. *Physiology (Bethesda)*, *31*(4), 283–293.

Sanna, S., van Zuydam, N. R., Mahajan, A., Kurilshikov, A., Vich Vila, A., Võsa, U., … Mccarthy, M. I. (2019). Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nature Genetics*, *51*(4), 600–605.

Schneider, H., & Blaut, M. (2000). Anaerobic degradation of flavonoids by Eubacterium ramulus. *Archives of Microbiology*, *173*(1), 71–75.

Schoefer, L., Mohan, R., Schwiertz, A., Braune, A., & Blaut, M. (2003). Anaerobic degradation of flavonoids by *Clostridium orbiscindens*. *Applied and Environmental Microbiology*, *69*(10), 5849–5854.

Sesink, A. L., Arts, I. C., Faassen-Peters, M., & Hollman, P. C. (2003). Intestinal uptake of quercetin-3-glucoside in rats involves hydrolysis by lactase phlorizin hydrolase. *Journal of Nutrition*, *133*(3), 773–776.

Shakour, Z. T. A., Fayek, N. M., & Farag, M. A. (2020). How do biocatalysis and biotransformation affect Citrus dietary flavonoids chemistry and bioactivity? A review. *Critical Reviews in Biotechnology*, *40*(5), 1–26. doi: https://doi.org/10.1080/07388551.2020.1753648, .

Shen, C. Y., Wan, L., Wang, T. X., & Jiang, J. G. (2019). *Citrus aurantium* L. var. amara Engl. inhibited lipid accumulation in 3T3-L1 cells and *Caenorhabditis elegans* and prevented obesity in high-fat diet-fed mice. *Pharmacological Research*, *147*, 104347.

Silberberg, M., Morand, C., Mathevon, T., Besson, C., Manach, C., Scalbert, A., & Remesy, C. (2006). The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. *European Journal of Nutrition*, *45*(2), 88–96.

Stevens, Y., Rymenant, E. V., Grootaert, C., Camp, J. V., Possemiers, S., Masclee, A., & Jonkers, Daisy. (2019). The intestinal fate of citrus flavanones and their effects on gastrointestinal health. *Nutrients*, *11*(7), 1464.

Sukkar, S. G., Schenone, E., Foppiani, L., & Nobile, M. T. (2004). Experimental assessment of chemotherapy-induced early intestinal damage in colon cancer the lactulose-mannitol permeability test. *Tumori*, *90*(5), 461–463.

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The role of short-chain fatty acids in health and disease. *Advances in Immunology*, *121*, 91–119.

Teodoro, J. S., Rolo, A. P., & Palmeira, C. M. (2011). Hepatic FXR: Key regulator of whole-body energy metabolism. *Trends in Endocrinology and Metabolism*, *22*(11), 458–466.

Thaiss, C. A., Levy, M., Grosheva, I., Zheng, D., Soffer, E., Blacher, E., … Elinav, E. (2018). Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science*, *359*(6382), 1376–1383.

Thevaranjan, N., Puchta, A., Schulz, C., Naidoo, A., Szamosi, J. C., Verschoor, C. P., … Bowdish, D. M. E. (2017). Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host Microbe*, *21*(4), 455–466.e4.

Thilakarathna, S. H., & Rupasinghe, H. P. (2013). Flavonoid bioavailability and attempts for bioavailability enhancement. *Nutrients*, *5*(9), 3367–3387.

Treml, J., & Smejkal, K. (2016). Flavonoids as potent scavengers of hydroxyl radicals. *Comprehensive Reviews in Food Science & Food Safety*, *15*(4), 720–738.

Tresserra-Rimbau, A., Rimm, E. B., Medina-Remon, A., Martinez-Gonzalez, M. A., Lopez-Sabater, M. C., Covas, M. I., … Lamuela-Raventós, R. M. (2014). Polyphenol intake and mortality risk: A re-analysis of the PREDIMED trial. *BMC Medicine*, *12*, 77.

Tung, Y. C., Chang, W. T., Li, S., Wu, J. C., Badmeav, V., Ho, C. T., Pan, M.-H. (2018). Citrus peel extracts attenuated obesity and modulated gut microbiota in mice with high-fat diet-induced obesity. *Food & Function*, *9*(6), 3363–3373.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*, *3*(4), 213–223.

Unno, T., Hisada, T., & Takahashi, S. (2015). Hesperetin modifies the composition of fecal microbiota and increases cecal levels of short-chain fatty acids in rats. *Journal of Agricultural and Food Chemistry*, *63*(36), 7952–7957.

Walgren, R. A., Karnaky, K. J. Jr., Lindenmayer, G. E., & Walle, T. (2000). Efflux of dietary flavonoid quercetin 4'-beta-glucoside across human intestinal Caco-2 cell monolayers by apical multidrug resistance-associated protein-2. *Journal of Pharmacology and Experimental Therapeutics*, *294*(3), 830–836.

Walgren, R. A., Lin, J. T., Kinne, R. K., & Walle, T. (2000). Cellular uptake of dietary flavonoid quercetin 4'-beta-glucoside by sodium-dependent glucose transporter SGLT1. *Journal of Pharmacology and Experimental Therapeutics*, *294*(3), 837–843.

Walker, E. H., Pacold, M. E., Perisic, O., Stephens, L., Hawkins, P. T., Wymann, M. P., & Williams, R. L. (2000). Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Molecular Cell*, *6*(4), 909–919.

Wang, M., Meng, D., Zhang, P., Wang, X., Du, G., Brennan, C., … Zhao, H. (2018). Antioxidant protection of nobiletin, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin from citrus peel in *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry*, *66*(12), 3155–3160.

Wang, W. W., Smith, D. L., & Zucker, S. D. (2004). Bilirubin inhibits iNOS expression and NO production in response to endotoxin in rats. *Hepatology*, *40*(2), 424–433.

Wang, Z., & Zhao, Y. (2018). Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell*, *9*(5), 416–431.

Wei, G. J., Sheen, J. F., Lu, W. C., Hwang, L. S., Ho, C. T., & Lin, C. I. (2013). Identification of sinensetin metabolites in rat urine by an isotope-labeling method and ultrahigh-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*, *61*(21), 5016–5021.

Wen, X., & Walle, T. (2006). Methylated flavonoids have greatly improved intestinal absorption and metabolic stability. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *34*(10), 1786–1792.

Wen, X., Zhao, H., Wang, L., Wang, L., Du, G., Guan, W., … Li, S. (2020). Nobiletin attenuates DSS-induced intestinal barrier damage through the HNF4alpha-claudin-7 signaling pathway. *Journal of Agricultural and Food Chemistry*, *68*(16), 4641–4649.

Williamson, G., Kay, C. D., & Crozier, A. (2018). The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. *Comprehensive Reviews in Food Science & Food Safety*, *17*, 1054–1112.

Wolak-Dinsmore, J., Gruppen, E. G., Shalaurova, I., Matyus, S. P., Grant, R. P., Gegen, R., … Dullaart, R. P. F. (2018). A novel NMR-based assay to measure circulating concentrations of branched-chain amino acids: Elevation in subjects with type 2 diabetes mellitus and association with carotid intima media thickness. *Clinical Biochemistry*, *54*, 92–99.

Wolffram, S., Block, M., & Ader, P. (2002). Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. *Journal of Nutrition*, *132*(4), 630–635.

Wong, Y. C., Zhang, L., Lin, G., & Zuo, Z. (2009). Structure-activity relationships of the glucuronidation of flavonoids by human glucuronosyltransferases. *Expert Opinion on Drug Metabolism & Toxicology*, *5*(11), 1399–1419.

Wu, G. A., Terol, J., Ibanez, V., Lopez-Garcia, A., Perez-Roman, E., Borreda, C., … Talon, M. (2018). Genomics of the origin and evolution of Citrus. *Nature*, *554*(7692), 311–316.

Wu, X., Song, M., Gao, Z., Sun, Y., Wang, M., Li, F., … Xiao, H. (2017). Nobiletin and its colonic metabolites suppress colitis-associated colon carcinogenesis by down-regulating iNOS, inducing antioxidative enzymes and arresting cell cycle progression. *Journal of Nutritional Biochemistry*, *42*, 17–25.

Wu, X., Song, M., Rakariyatham, K., Zheng, J., Guo, S., Tang, Z., … Xiao, H. (2015). Anti-inflammatory effects of 4'-demethylnobiletin, a major metabolite of nobiletin. *Journal of Functional Foods*, *19*(Pt A), 278–287.

Wu, X., Song, M., Rakariyatham, K., Zheng, J., Wang, M., Xu, F., … Xiao, H. (2015). Inhibitory effects of 4'-demethylnobiletin, a metabolite of nobiletin, on 12-o-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mouse ears. *Journal of Agricultural and Food Chemistry*, *63*(51), 10921–10927.

Wu, X., Song, M., Wang, M., Zheng, J., Gao, Z., Xu, F., … Xiao, H. (2015b). Chemopreventive effects of nobiletin and its colonic metabolites on colon carcinogenesis. *Molecular Nutrition & Food Research*, *59*(12), 2383–2394.

Yang, G., Bibi, S., Du, M., Suzuki, T., & Zhu, M. J. (2017). Regulation of the intestinal tight junction by natural polyphenols: A mechanistic perspective. *Critical Reviews in Food Science and Nutrition*, *57*(18), 3830–3839.

Zeng, S. L., Li, S. Z., Xiao, P. T., Cai, Y. Y., Chu, C., Chen, B. Z., … Liu, E.-H. (2020). Citrus polymethoxyflavones attenuate metabolic syndrome by regulating gut microbiome and amino acid metabolism. *Science Advances*, *6*(1), eaax6208.

Zeng, X., Su, W., Bai, Y., Chen, T., Yan, Z., Wang, J., … Yao, H. (2017). Urinary metabolite profiling of flavonoids in Chinese volunteers after consumption of orange juice by UFLC-Q-TOF-MS/MS. *Journal of Chromatography. B: Analytical Technologies in the Biomedical and Life Sciences*, *1061–1062*, 79–88.

Zeng, X., Su, W., Zheng, Y., He, Y., He, Y., Rao, H., … Yao, H. (2019). Pharmacokinetics, tissue distribution, metabolism, and excretion of naringin in aged rats. *Frontiers in Pharmacology*, *10*, 34.

Zhang, J., Lei, H., Hu, X., & Dong, W. (2020). Hesperetin ameliorates DSS-induced colitis by maintaining the epithelial barrier via blocking RIPK3/MLKL necroptosis signaling. *European Journal of Pharmacology*, *873*, 172992.

Zhang, M., Zhu, J., Zhang, X., Zhao, D. G., Ma, Y. Y., Li, D., … Huang, Q. (2020). Aged citrus peel (chenpi) extract causes dynamic alteration of colonic microbiota in high-fat diet induced obese mice. *Food & Function*, *11*(3), 2667–2678.

Zhao, Y., Hu, X., Zuo, X., & Wang, M. (2018). Chemopreventive effects of some popular phytochemicals on human colon cancer: A review. *Food & Function*, *9*(9), 4548–4568.

Zheng, J., Bi, J., Johnson, D., Sun, Y., Song, M., Qiu, P., … Xiao, H. (2015). Analysis of 10 metabolites of polymethoxyflavones with high sensitivity by electrochemical detection in high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *63*(2), 509–516.

Zheng, L., Kelly, C. J., Battista, K. D., Schaefer, R., Lanis, J. M., Alexeev, E. E., … Colgan, S. P. (2017). Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. *Journal of Immunology*, *199*(8), 2976–2984.

Zheng, Y. Y., Zeng, X., Peng, W., Wu, Z., & Su, W. W. (2019). Characterisation and classification of Citri Reticulatae Pericarpium varieties based on UHPLC-Q-TOF-MS/MS combined with multivariate statistical analyses. *Phytochemical Analysis*, *30*(3), 278–291.

How to cite this article: Wang M, Zhao H, Wen X, Ho C-T, Li S. Citrus flavonoids and the intestinal barrier: Interactions and effects. *Compr Rev Food Sci Food Saf*. 2021;20:225–251.
https://doi.org/10.1111/1541-4337.12652