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Microbial Flavonoid Metabolism: A Cardiometabolic Disease Perspective

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Abstract

Cardiometabolic disease (CMD) is a leading cause of death worldwide and encompasses the inflammatory metabolic disorders of obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, and cardiovascular disease. Flavonoids are polyphenolic plant metabolites that are abundantly present in fruits and vegetables and have biologically relevant protective effects in a number of cardiometabolic disorders. Several epidemiological studies underscored a negative association between dietary flavonoid consumption and the propensity to develop CMD. Recent studies elucidated the contribution of the gut microbiota in metabolizing dietary intake as it relates to CMD. Importantly, the biological efficacy of flavonoids in humans and animal models alike is linked to the gut microbial community. Herein, we discuss the opportunities and challenges of leveraging flavonoid intake as a potential strategy to prevent and treat CMD in a gut microbe–dependent manner, with special emphasis on flavonoid-derived microbial metabolites.

Contents

1. INTRODUCTION

1.1. Cardiometabolic Disease and the Gut Microbial Contribution

Cardiometabolic disease (CMD) encompasses the metabolic disorders type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease (42, 50, 66, 136). Strikingly, one in three adults worldwide are afflicted with CMD, a condition that represents a fundamental metabolic imbalance (43). Furthermore, CMD increases one's propensity to develop coronary heart disease, which is the leading cause of death worldwide (47). Current CMD treatment strategies include aggressive management of traditional risk factors via weight reduction, physical activity, control of hypertension, and lowering of blood glucose and lipid indices in combination with pharmaceutical approaches (24). Pharmaceutical interventions directed at a number of human targets including 3-hydroxy-3-methylglutaryl–coenzyme A reductase (HMGCR), glucagon-like receptor 1, sodium-glucose cotransporter 2, dipeptidyl dipeptidase 4, adenosine monophosphate–activated protein kinase (AMPK), and peroxisome proliferator-activated receptors have some efficacy in abrogating the CMD burden (8, 19, 20, 88, 99, 127). However, as with many drugs targeting host enzymatic pathways, these treatment strategies have the potential to fall short in practice due to undesirable side effects, thus forcing both the patient and clinician to make risk-benefit decisions (3, 41, 48, 86, 102). Alternative CMD treatment strategies grounded in the nutritional value of one's diet have gained traction in recent years, in part due to the benefits of a lack of prescription costs and no need for health insurance, a reduced risk of negative side effects, and appreciable efficacy (83). Consequently, the emerging field of personalized nutrition and nutraceutical intervention represents a unique opportunity to complement traditional CMD treatment strategies (121).

In recent years, the contribution of gut microbiota to human health and disease has been of intense focus and rigorous investigation in a variety of disease contexts. It is now appreciated that the gut microbial community collectively serves as a metaendocrine organ, altering the human physiologic condition via direct microbe–host interactions and indirectly through the production of gut microbial metabolites (17, 18, 46, 94, 103). An ever-expanding list of pathognomonic microbial metabolites has been implicated in the development and progression of CMD (67, 68, 85, 123, 133, 143), and importantly, these metabolites are dependent on dietary input (9, 119, 121,

144). The role of the gut microbiome as it relates to the processing of dietary input and ultimately the impact on host physiology cannot be understated, and for this reason it has been extensively reviewed as of late (69, 144). Unfortunately, with the exception of short-chain fatty acids (SCFAs), the field of beneficial microbes (probiotics) and microbial metabolites (postbiotics) remains largely understudied, particularly in the context of CMD. Subsequently, there exists an unmet need to balance the scale of knowledge with regard to the gut microbiome and its diet-dependent metabolome by further investigating microbial symbionts and the products thereof.

1.2. Flavonoids

Flavonoids are secondary plant metabolites that play a central role in plant physiology and are an integral component of the human diet, as they are the most common and diverse group of polyphenolic compounds in fruits and vegetables (71). The basic flavan structure consists of a 15-carbon skeleton that includes two phenyl rings, the A-ring and the B-ring, and a heterocyclic C-ring (**Figure 1*a***). There are six classes of flavonoids: flavanones, flavones, isoflavones, flavan-3-ols, flavonols, and anthocyanidins. Each class is further subdivided according to their oxidation

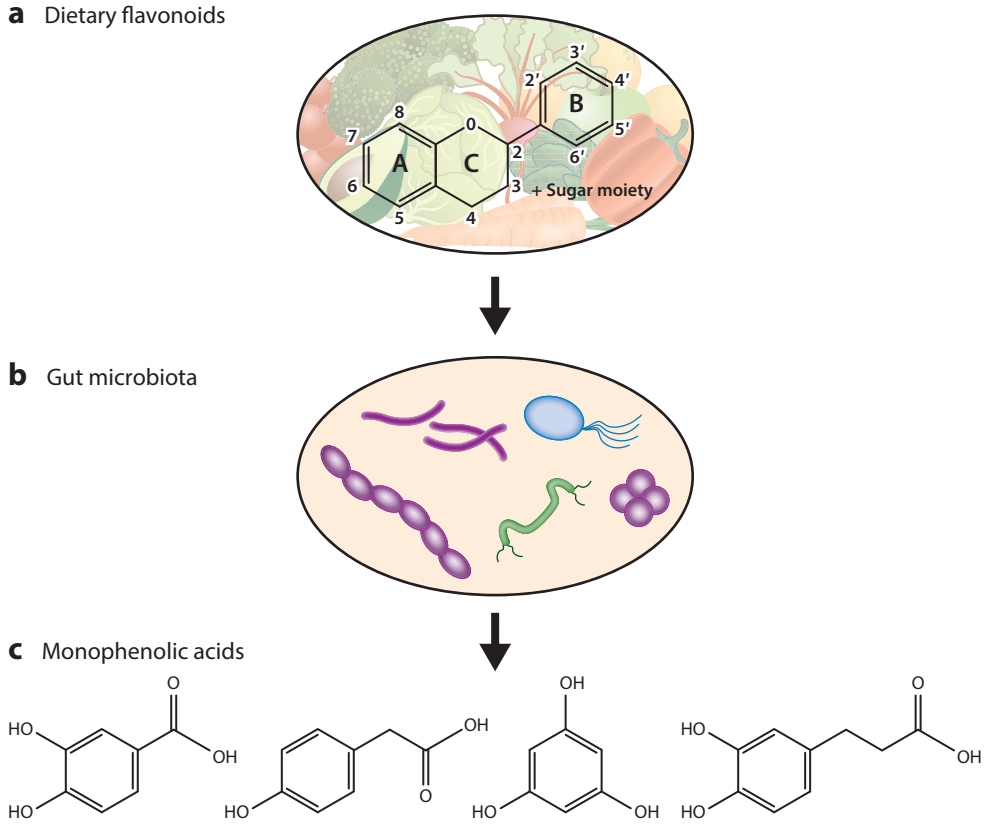

Figure 1

Gut microbial catabolism of dietary flavonoids into monophenolic acids. (*a*) Dietary flavonoids are an abundant group of polyphenols present in pigmented fruits and vegetables. Flavonoids exist in planta as either glycosylated or aglycone heterocyclic molecules. (*b*) Specific commensal members of the human gut microbiota catabolize both glycosylated and aglycone plant-derived flavonoids into smaller monophenolic acids (*c*).

status (71). Flavonoids are most commonly linked to a sugar moiety in planta as water-soluble flavonoid glycosides; however, smaller amounts of aglycone flavonoids are also present (57). Glycosylation may occur on any of the core flavan rings and ultimately impacts substrate specificity for host and microbial deglycosylase enzymes in the gastrointestinal tract (137).

The biological activity of flavonoids is dependent on the degree and position of glycosylation, hydroxylation, oxidation, and saturation (49, 71, 100). Flavonoids were initially appreciated as dietary antioxidants with the ability to scavenge free radicals via hydrogen donation (100). Consequently, flavonoids gained traction as a potential therapeutic for inflammatory metabolic disorders to prevent the formation of radical oxygen species and downstream lipid peroxidation—a characteristic feature of CMD (44, 70). To date, numerous epidemiological studies have investigated the relationship between dietary flavonoid consumption and cardiovascular disease. In their systemic review of 14 human prospective cohort studies with meta-analysis, Wang and colleagues (132) affirmed the therapeutic potential of flavonoids in human CMD by highlighting the inverse relationship between dietary consumption of all six flavonoid classes and cardiovascular disease. Moreover, in a prospective cohort study of more than 56,000 Danish adults, moderate consumption of flavonoids (~500 mg/day) was associated with a decreased risk of cardiovascular-related and all-cause mortality (11). Although many factors contribute to the development of CMD, the purpose of this review is to highlight the contribution of microbial flavonoid metabolism to the prevention and regression of CMD.

2. MICROBIAL FLAVONOID METABOLISM

2.1. Microbial Contribution to Flavonoid Bioavailability

The generally poor bioavailability of flavonoids has been extensively reviewed (57, 77, 125, 137) and is understood to be a consequence of limited absorption, extensive systemic metabolism, and rapid excretion. Moreover, flavonoids that are not absorbed in the small intestine are subject to gut microbial metabolism in the colon, where the parent flavan can be catabolized into monophenolic acids (**Figure 1*b*,*c***). These monophenolic acids have a bioavailability that far exceeds that of the heterocyclic-glycosylated parent (33, 92, 104, 130).

In vitro flavonoid catabolism studies using cultures of human colonic microflora revealed a marked interindividual variation in metabolic capacity. For example, the extent to which flavonoids naringin and rutin—and their aglycones naringenin and quercetin—are degraded is dependent on both the structural differences between the compounds and the composition of the donor gut microbiota that were used to seed the fermentation system (98). Similar variation in monophenolic acid catabolites was observed in a study using flavan-3-ol substrates for in vitro incubation with different human fecal microbiota cultures (107). This interindividual variation has been further corroborated in numerous studies of the human plasma metabolome following consumption of a flavonoid-rich substrate such as apple juice (126), wild blueberries (40), or flavonoid-enriched chocolate (23, 30) and was recently reviewed by Manach and colleagues (76) and Eker and colleagues (37). The marked interindividual variation in flavonoid catabolism in vitro and in the postprandial human plasma metabolome suggests a defined contribution of the gut microbiome and highlights the need for mechanistic studies on the molecular cause of these differential catabolic pathways.

2.2. Molecular Mechanisms of Microbial Flavonoid Metabolism

Although an appreciable number of bacterial species have been reported to metabolize flavonoids [comprehensively reviewed by Braune & Blaut (13)] (**Table 1**), the molecular mechanism underlying microbial-flavonoid bioconversion remains an understudied topic. Interest in the

Table 1 Microbial flavonoid metabolites

Flavonoid class	Microbial flavonoid metabolites[a]	Metabolite-producing bacteria	References
Flavanones	Phenylacetic acid 2-Hydroxyphenylacetic acid 4-Hydroxyphenylacetic acid 3-Phenylpropanoic acid 3-(3′-Hydroxyphenyl)propanoic acid 3-(4′-Hydroxyphenyl)propanoic acid 3-(3′,4′-Dihydroxyphenyl)propanoic acid 3-(3′-Hydroxy-4′-methoxyphenyl)propanoic acid 3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acid Benzene-1,3,5-triol	*Lachnospiraceae* strain CG19-1; *Flavonifractor plautii*; *Eubacterium ramulus* DSM 16296; *Clostridium butyricum* IFO139149; *Bifidobacterium longum* R0175; *Lactobacillus rhamnosus* ssp. *Rhamnosus* NCTC 10302; polymicrobial rat fecal culture	12, 16, 45, 80, 96, 108, 111, 114
Flavones	3-(4′-Hydroxyphenyl)propanoic acid 3-(2′,4′-Dihydroxyphenyl)propanoic acid 3-(3′,4′-Dihydroxyphenyl)propanoic acid	Polymicrobial rat fecal culture; *Eubacterium ramulus*; *Lachnospiraceae* strain CG19-1; *Flavonifractor plautii*	12, 16, 108, 111, 114
Isoflavones	(*S*)-Equol 5-Hydroxy-equol 6′-Hydroxy-*O*-desmethylangolensin *O*-Desmethylangolensin 2-(4-Hydroxyphenyl)propanoic acid Benzene-1,3-diol Benzene-1,3,5-triol	*Slackia equolifaciens* JCM 16059; *Eggerthella* sp. YY7918; *Adlercreutzia equolifaciens* JCM 14793; *Slackia isoflavoniconvertens* DSM 22006; *Lactococcus garvieae* 20-92; *Lachnospiraceae* strain CG19-1; *Eubacterium ramulus* DSM 16296; *Eubacterium ramulus* Julong 601; *Slackia* sp. NATTS; *Clostridium* sp. HGH 136	14, 58
Flavan-3-ols[b,c]	Phenylacetic acid 3-Hydroxyphenylacetic acid 4-Hydroxyphenylacetic acid 3,4-Dihydroxyphenylacetic acid Phenylpropanoic acid 3-Phenylpropanoic acid 3-(3′-Hydroxyphenyl)propanoic acid 3-(4′-Hydroxyphenyl)propanoic acid 3-(3′,4′-Dihydroxyphenyl)propanoic acid 3-Hydroxy-3-(3-hydroxyphenyl)propanoic acid 3,4,5-Trihydroxybenzoic acid Benzene-1,2,3-triol	Polymicrobial human fecal culture	6, 29, 79, 137
Flavonols	Phenylacetic acid 2-Hydroxyphenylacetic acid 3-Hydroxyphenylacetic acid 4-Hydroxyphenylacetic acid 3,4-Dihydroxyphenylacetic acid 3-(3′-Hydroxyphenyl)propanoic acid 3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acid 3′,4′-Dihydroxycinnamic acid 4-Hydroxybenzoic acid 3,4-Dihydroxybenzoic acid Benzene-1,3,5-triol	*Flavonifractor plautii*; *Eubacterium ramulus* DSM 16296; *Enterococcus casseliflavus*; *Lachnospiraceae* strain CG19-1; *Clostridium perfringens*; *Bacteroides fragilis* polymicrobial human fecal culture; polymicrobial rat fecal culture	16, 45, 60, 95, 98, 108, 109, 111, 114, 138

(*Continued*)

Table 1 (*Continued*)

Flavonoid class	Microbial flavonoid metabolites[a]	Metabolite-producing bacteria	References
Anthocyanidins	(2,5-Dihydroxyphenyl)acetic acid 3,4-Dihydroxybenzoic acid 3,4,5-Trihydroxybenzoic acid 3,5-Dimethoxy-4-hydroxybenzoic acid 4-Hydroxy-3-methoxybenzoic acid 2,4,6-Trihydroxybenzaldehyde 3′,4′-Dihydroxycinnamic acid 4′-Hydroxy-3′-methoxycinnamic acid Benzene-1,3,5-triol	*Bifidobacterium animalis* ssp. *lactis* BB-12; *Lactobacillus plantarum* IFPL722; *Lactobacillus casei* LC-01; *Eubacterium ramulus* DSM 16296; *Clostridium saccharogumia* DSM 17460; *Streptococcus thermophiles* GIM 1.321; *Lactobacillus plantarum* GIM 1.35; human fecal culture	5, 7, 28, 53, 55, 65

[a]The nomenclature ascribed herein is consistent with the recommendations set forth by Kay et al. (64).
[b]Only end-product monophenolic acid metabolites of flavan-3-ols are summarized herein. Intermediate propan-2-ols, phenyl valeric acids, and valerolactone metabolites have been elegantly characterized using radiolabeled ^{14}C(−)-epicatechin (92) and extensively reviewed by Aura (4), Márquez Campos et al. (79), Monagas et al. (82), and Williamson et al. (137). The bacterial communities and species involved in these intermediate steps are summarized by Aura (4) and Braune & Blaut (13), respectively.
[c]Although several other flavan-3-ol metabolites were reported by Ottaviani et al. (92), the host contribution to flavonoid metabolism cannot be ruled out in such in vivo studies, and consequently these metabolites are not included in the table.

flavonoid catabolizing capacity of human-derived bacterial species can be traced back to 1987, when Bokkenheuser and colleagues (10) reported the hydrolysis of flavonoid glycosides by *Bacteroides* spp. In subsequent years, the same group reported the ability of the human gut commensal *Clostridium oribscindens* (now reclassified as *Flavonifractor plautii*) to cleave the C-ring of quercetin (139). Since then, Braune, Blaut, and colleagues have greatly contributed to the field—first reporting the degradation of the flavone luteolin, and the flavonol quercetin by the human gut commensal *Eubacterium ramulus* (16), followed by the functional characterization of a chalcone isomerase and phloretin hydrolase, the first identified bacterial enzymes involved in the catabolism of flavonoids (54, 110). In an elegant biochemical study, Schröder and colleagues (112) described an eight-gene cluster in *Slackia isoflavoniconvertens* responsible for the conversion of the dietary isoflavone daidzein to equol, a metabolite exclusively produced by bacteria that has been negatively correlated with CMD (113, 116). The enzymatic deglycosylation of *O*- and *C*-coupled flavonoid glucosides was characterized by Braune and colleagues (14) and determined to be carried out by the *dfg* gene cluster in *Eubacterium cellulosolvens* and *Lachnospiraceae* strain CG19-1. This finding is particularly noteworthy considering that host enzymes cannot cleave *C*-coupled flavonoid glucosides (12). Most recently, a flavanone- and flavanonol-cleaving reductase was identified in *Eubacterium ramulus* and heterologously expressed in *Escherichia coli*, where its specificity and NADH dependency were demonstrated (15).

Despite the seminal works described above, there remains an unmet need to identify and characterize the molecular mechanisms responsible for flavonoid catabolism in more bacterial species, with special emphasis on polymicrobial catabolism. The current challenges include the limitations of in vitro growth medium to fully recapitulate in vivo colonic conditions, the lack of systems to study complex polymicrobial cross-catabolism reactions, the marked discrepancy between the microbiomes of humans and animal models, and the requirement for labor- and cost-intensive quantitation methods used to measure flavonoids and their microbial metabolites, such as liquid chromatography with tandem mass spectrometry. Addressing these pitfalls will lead to future studies providing a more complete picture of the three-way diet–gut microbiome–host interface as it relates to CMD prevention, progression, and treatment.

2.3. Bacterial Flavonoid Metabolites

The bioactivity of flavonoids has been shown to be dependent on the gut microbial community in a number of disease contexts and may suggest that microbial flavonoid metabolites are actually the bioactive molecules as opposed to (or in addition to) the parent flavonoid. Of note, monophenolic acids stemming from microbial flavonoid metabolism are more readily absorbed than their parent flavonoids and, as a consequence, are more abundant in human plasma, reaching up to micromolar concentrations (25, 33, 92, 104). Although monophenolic acids themselves may be consumed in a plant-based diet, the capacity for microbial production of monophenolic acid production far exceeds what is realistically achievable through dietary intake (87, 105).

Given the vast heterogeneity of the human microbiome, further compounded by the roughly 6,000 reported flavonoids, it is not surprising that microbial flavonoid metabolism can yield a plethora of phenolic acid metabolites (93). Unfortunately, only a handful of bacterial species with catabolic activity toward flavonoids have been reported to date. Compounded by a lack of high-throughput screening approaches, the complexity of polymicrobial fecal cultures, and limitations in detection methods, little progress has been made in recent years to identify bacterial species capable of catabolizing any particular flavonoid into its respective phenolic acid product(s). **Table 1** summarizes the phenolic acid products of each flavonoid class and the corresponding microbes responsible for flavonoid catabolism reported to date. Since the purpose of this review is to highlight microbial flavonoid metabolites exclusively, human or animal studies identifying plasma phenolic acids resulting from flavonoid consumption were excluded, as the role of host metabolism cannot be ruled out. Furthermore, differentially abundant bacterial species identified using 16S ribosomal RNA (rRNA) sequencing of fecal material following flavonoid consumption were also excluded, due to a lack of causative evidence for flavonoid catabolism by such species.

3. MICROBIAL FLAVONOID METABOLITES IN CARDIOMETABOLIC DISEASE

3.1. Microbial Flavonoid Metabolites: Obesity

Although many studies have demonstrated the role of flavonoids in abrogating obesity in humans or mice (1, 21, 22, 56, 82), few of these investigated the contribution of the gut microbiome to the antiobesogenic properties of flavonoids. Esposito and colleagues (39) reported that an anthocyanin-rich extract attenuated high-fat diet (HFD)-induced obesity in male C57BL/6 mice with an intact gut microbiome but not in animals that had their gut microbial community ablated with the commonly used antibiotic cocktail VNAM (0.5 g/L vancomycin, 1 g/L neomycin sulfate, 1 g/L ampicillin, 1 g/L metronidazole). Furthermore, VNAM-treated mice had a 16- to 25-fold increase in fecal anthocyanin content, suggesting that gut microbes contribute to the catabolism and antiobesogenic properties of flavonoids. In a separate study on the effects of dieting in obese mice, gut microbial metabolism of apigenin and naringenin was implicated in the extent of secondary weight regain (124).

Currently, animal models of HFD-induced obesity are being leveraged to study the role of single microbial flavonoid catabolites in the prevention or regression of obesity. The flavonoid metabolites 3-(3′-hydroxyphenyl)propanoic acid and 4-hydroxyphenylacetic acid significantly decreased the messenger RNA (mRNA) expression of the lipogenesis genes fatty acid synthase (*Fas*), stearoyl-CoA-desaturase 1 (*Scd1*), and acetyl-CoA carboxylase alpha (*Acaca*) while concomitantly increasing the fatty acid oxidation genes peroxisome proliferator-activated receptor alpha (*Ppara*), acetyl-CoA carboxylase beta (*Acacb*), and hormone-sensitive lipase (*Hsl*) in white adipocytes derived from the inguinal fat pad of male C57BL/6 mice (131). Additionally, the

flavonol and anthocyanidin catabolite 3′,4′-dihydroxycinnamic acid has been demonstrated to decrease HFD-induced obesity by reducing adiposity in mice while improving the serum lipid profile (74, 140). Another anthocyanidin metabolite, 3,5-dimethoxy-4-hydroxybenzoic acid, reduced body mass and adiposity in a high-fat/high-cholesterol (HFHC) murine model of obesity (51). Similar results were observed by administering the promiscuous flavonoid catabolite 3-(4′-hydroxyphenyl)propanoic acid in the drinking water of HFD-fed mice for 11 weeks (135). In two separate HFD-feeding studies, 3′,4′-dihydroxycinnamic acid was shown to decrease body mass and adiposity in male C57BL/6 mice (74, 140). Using male C57BL/6J mice fed a high-fat/high-fructose diet (HFFD), 4-hydroxy-3-methoxybenzoic acid ameliorated body mass gain and adiposity while promoting thermogenesis in brown adipose tissue and the beiging of inguinal white adipose tissue (52). Moreover, 4-hydroxy-3-methoxybenzoic acid decreased body mass and adiposity and improved serum lipids in HFD-fed rats (26). These findings are further supported by the amelioration of obesity and adiposity in leptin receptor–deficient mice (*db/db*) following daily oral gavage of 4-hydroxy-3-methoxybenzoic acid via the upregulation of thermogenic factors in adipose tissue (62). Additionally, daily intraperitoneal injection of the microbial anthocyanidin catabolite 3,4,5-trihydroxybenzoic acid was demonstrated to be antiobesogenic in an HFD-induced model of obesity in C57BL/6 mice (27) through the activation of the AMPK/Sirt1/Pgc1α pathway (35). The antiobesogenic properties of 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid were reported by Ohue-Kitano and colleagues (89) in an HFD murine model. In vivo and ex vivo studies examining the contribution of microbial flavonoid catabolites in the context of obesity are summarized in **Table 2** and depicted in **Figure 2**.

3.2. Microbial Flavonoid Metabolites: Diabetes

In a 1-year, double-blind, randomized, placebo-controlled trial, postmenopausal women with T2DM consuming 27 g/day of flavonoid-enriched chocolate benefited from improved insulin sensitivity and reduced insulin resistance when compared with placebo controls (30). While these data suggest the potential efficacy of flavonoids to be used clinically in the treatment of T2DM, the gut microbial contribution to the observed improvement in glucose homeostasis remains unclear and warrants further investigation. Furthermore, recent cross-sectional epidemiological evidence has revealed an inverse association between dietary hydroxycinnamic acid intake and insulin resistance (106). Taken together, these findings solidify the necessity for further investigation into the missing microbial link between the antidiabetic associations of flavonoids and phenolic acids.

Demonstrating the bioactivity of microbial flavonoid catabolites in the context of T2DM, Ormazabal and colleagues (91) treated explanted human visceral adipose tissue (VAT) from obese subjects with 3,4-dihydroxybenzoic acid (3,4-DHBA). Overall insulin responsiveness was improved through the phosphorylation of insulin receptor substrate-1 and protein kinase B (Akt) in 3,4-DHBA-treated VAT. Furthermore, 3,4-dihydroxybenzoic acid treatment decreased the activity of protein tyrosine phosphatase 1B (PTP1B), which has been implicated in the blunting of insulin responsiveness (38). After inducing hyperglycemia in male C57BL/6 mice with chronic HFD feeding, 3′,4′-dihydroxycinnamic acid was shown to decrease serum insulin and blood glucose levels (74, 140). The microbial anthocyanidin catabolite 4-hydroxy-3-methoxybenzoic acid exerted antihyperglycemic effects in HFD-fed rats and reduced serum triglycerides (TG), free fatty acids (FFAs), hepatic monocyte chemoattractant protein 1 (MCP-1), and hepatic cyclooxygenase-2 (COX-2), while increasing the phosphorylated acetyl-CoA carboxylase/acetyl-CoA carboxylase ratio (pACC/ACC) (26). Following intraperitoneal injection of 4-hydroxy-3-methoxybenzoic acid, obese male C57BL/6 mice benefited from improved glucose tolerance and insulin resistance in sequential glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) (52). Similar GTT

Table 2 Microbial flavonoid metabolite effects on obesity

Microbial flavonoid metabolite (concentration/dose)	Duration of study	Disease model	Key outcome	Reference
3-(4′-Hydroxyphenyl)propanoic acid (100 mM in drinking water)	11 weeks	HFD-induced obesity using male C57BL/6 mice	Decreased body mass, adiposity	135
4-Hydroxyphenylacetic acid (0.5–2 μM)	48 h	Primary adipocytes from inguinal white adipose tissue; male C57BL/6J mice	Decreased *Fasn*, *Scd1*, and *Acaca* mRNA expression; increased *Ppara*, *Acacb*, and *Hsl* mRNA; decreased lipid accumulation	131
3-(3′-Hydroxyphenyl)propanoic acid (0.5–2 μM)	48 h	Primary adipocytes from inguinal white adipose tissue; male C57BL/6J mice	Decreased *Fasn*, *Scd1*, and *Acaca* mRNA expression; increased *Ppara*, *Acacb*, and *Hsl* mRNA; decreased lipid accumulation	131
3′,4′-Dihydroxycinnamic acid (50 mg/kg BW daily gavage)	12 weeks	HFD-induced obesity; male C57BL/6 mice	Decreased body mass, adiposity	140
3′,4′-Dihydroxycinnamic acid (0.2% and 0.8% w/w in diet)	6 weeks	HFHC-induced obesity; male C57BL/6 mice	Decreased body mass, adiposity; improved serum lipids	74
4-Hydroxy-3-methoxybenzoic acid (0.5% w/w in diet)	16 weeks	HFFD-induced obesity; male C57BL/6J mice	Decreased body mass, adiposity; induction of thermogenesis in BAT and beiging of iWAT	52
4-Hydroxy-3-methoxybenzoic acid (30 mg/kg BW daily gavage)	16 weeks	HFD-induced obesity; male SD rats	Decreased body mass, adiposity; improved serum lipids	26
4-Hydroxy-3-methoxybenzoic acid (100 mg/kg BW daily gavage)	6 weeks (WT) 4 weeks (*db/db*)	HFD-induced obesity in WT male mice; *Lepr*$^{-/-}$ (*db/db*) male mice	Decreased body mass, adiposity; improved serum lipids in both WT and *db/db* mice	62
3,4,5-Trihydroxybenzoic acid (10 mg/kg BW daily IP)	9 weeks	HFD-induced obesity; male C57BL/6 mice	Decreased body mass; activation of AMPK, SIRT1, and PGC1α	35
3,4,5-Trihydroxybenzoic acid (50 and 100 mg/kg daily gavage)	16 weeks	HFD-induced obesity; male C57BL/6 mice	Decreased body mass	27
3,5-Dimethoxy-4-hydroxybenzoic acid (0.05% w/w in diet)	16 weeks	HFHC-induced obesity; male C57BL/6J mice	Decreased body mass, adiposity	51
3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acid (1% w/w in diet)	12 weeks	HFD-induced obesity; male C57BL/6J mice	Decreased body mass	91

Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; BAT, brown adipose tissue; BW, body weight; HFD, high-fat diet; HFFD, high-fat/high-fructose diet; HFHC, high-fat/high-cholesterol diet; IP, intraperitoneal injection; iWAT, inguinal white adipose tissue; mRNA, messenger RNA; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; SD, Sprague-Dawley rats; SIRT1, sirtuin 1; WT, wild-type; w/w, weight by weight.

and ITT results were obtained in obese mice treated daily with 3,4,5-trihydroxybenzoic acid (at 10 mg/kg of body weight) (35). Strikingly, incorporation of 3,5-dimethoxy-4-hydroxybenzoic acid into an HFHC diet for 9 weeks reduced serum glucose and insulin to levels indistinguishable from those of mice fed a normal chow diet (51). More recently, the ability of 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid to lower fasting blood glucose levels in mice with HFD-induced hyperglycemia was reported by Ohue-Kitano and colleagues (89). In vivo and ex vivo studies

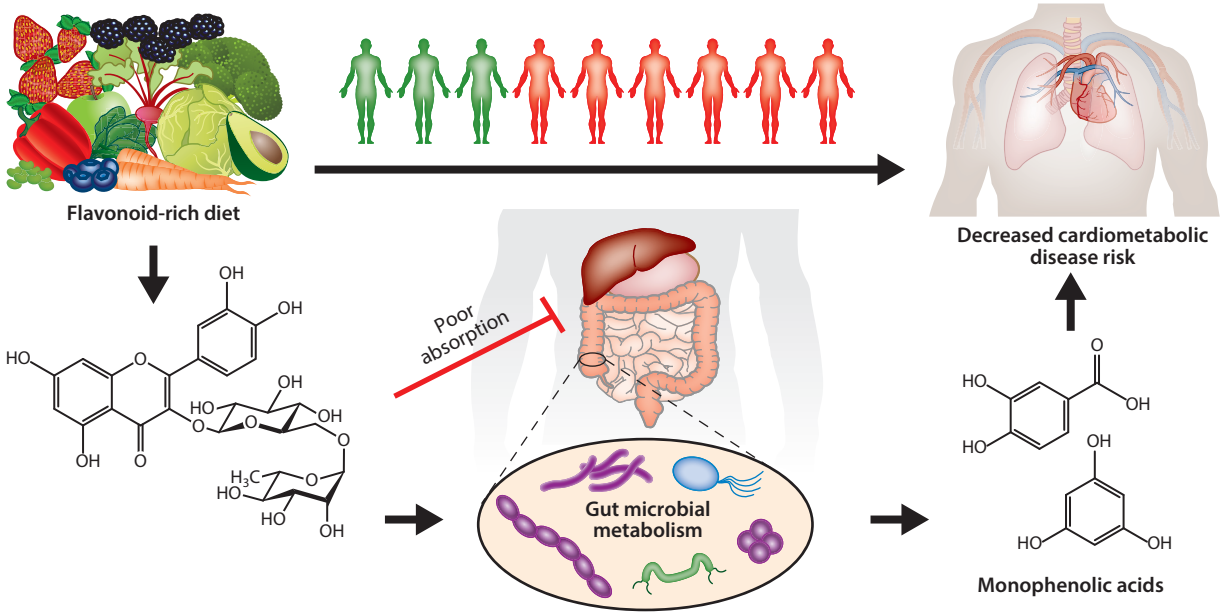

Figure 2

The cardiometabolic abrogating effects of flavonoids are gut microbe dependent. A diet rich in colorful fruits and vegetables is typically considered beneficial for cardiometabolic health. However, the successful outcomes of human dietary intervention trials are often dampened by a high interpersonal variability. Dietary flavonoids generally have poor bioavailability and are subject to extensive metabolism by colonic microflora, resulting in the production of monophenolic acids. These microbial flavonoid catabolites reach plasma concentrations far exceeding those of their parent flavonoid molecules. Various animal models have demonstrated that monophenolic acids mediate cardiometabolic disease development and progression. In addition, the postprandial monophenolic acid profile is highly dependent on the gut microbial community.

examining the contribution of microbial flavonoid catabolites in the context of T2DM are summarized in **Table 3** and depicted in **Figure 2**.

3.3. Microbial Flavonoid Metabolites: Cardiovascular Disease

Recent human epidemiological studies have associated flavonoid consumption with decreased risk for cardiovascular disease and all-cause mortality (11, 132). These associations have been further corroborated by animal models demonstrating the antiatherosclerotic properties of flavonoids (21). However, with the exception of the soy-derived, exclusively microbial isoflavone metabolite (*S*)-equol, the gut microbial contribution to the cardioprotective benefits of flavonoid consumption remains understudied in humans. The stratification of subjects by (*S*)-equol production status repeatedly reveals that consumption of dietary isoflavones is associated with cardioprotection in (*S*)-equol producers but not in those who lack a gut microbial community capable of producing (*S*)-equol, a concept that is comprehensively reviewed by Sekikawa and colleagues (113). Given the marked discrepancy in (*S*)-equol production between populations (only 30% of the Western population versus 60% of the Asian population) (115–117), compounded by the discrepant risk for cardiovascular disease based on (*S*)-equol production status, (*S*)-equol serves as a prime example of why the gut microbial contribution to dietary flavonoid metabolism should be rigorously investigated.

Table 3 Microbial flavonoid metabolite effects on diabetes

Microbial flavonoid metabolite (concentration/dose)	Duration of study	Disease model	Key outcome	Reference
3,4-Dihydroxybenzoic acid (100 μmol/L)	24 h	VAT explant; NW and OB humans	Increased insulin sensitivity via IRS-1 and Akt phosphorylation in OB-VAT; decreased protein expression of phospho-p65 NF-κB and IL-6 in OB-VAT; decreased PTP1B activity in OB-VAT; decreased protein expression of IL-6 in NW-VAT	91
3′,4′-Dihydroxycinnamic acid (50 mg/kg BW daily gavage)	12 weeks	HFD-induced T2DM; male C57BL/6 mice	Decreased fasting serum insulin	140
3′,4′-Dihydroxycinnamic acid (0.2% and 0.8% w/w in diet)	6 weeks	HFHC-induced T2DM; male C57BL/6 mice	Decreased fasting blood glucose	74
4-Hydroxy-3-methoxybenzoic acid (0.5% w/w in diet)	16 weeks	HFFD-induced T2DM; male C57BL/6J mice	Decreased insulin resistance and increased glucose tolerance	52
4-Hydroxy-3-methoxybenzoic acid (30 mg/kg BW daily gavage)	16 weeks	HFD-induced T2DM; male SD rats	Decreased serum insulin, glucose; decreased AUC following GTT; decreased HOMA-IR	26
3,4,5-Trihydroxybenzoic acid (10 mg/kg body weight daily IP)	9 weeks	HFD-induced T2DM; male C57BL/6 mice	Decreased insulin resistance and increased glucose tolerance	35
3,5-Dimethoxy-4-hydroxybenzoic acid (0.05% w/w in diet)	16 weeks	HFHC-induced T2DM; male C57BL/6J mice	Decreased serum glucose and insulin; decreased HOMA-IR; decreased AUC following GTT	51
3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acid (1% w/w in diet)	12 weeks	HFD-induced T2DM; male C57BL/6J mice	Decreased fasting blood glucose	89

Abbreviations: Akt, protein kinase B; AUC, area under the curve; BW, body weight; GTT, glucose tolerance test; HFD, high-fat diet; HFFD, high-fat/high-fructose diet; HFHC, high-fat/high-cholesterol diet; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin 6; IP, intraperitoneal; IRS-1, insulin receptor substrate 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NW, normal weight; OB, obese; PTP1B, protein-tyrosine phosphatase 1B; SD, Sprague-Dawley rats; T2DM, type 2 diabetes mellitus; VAT, visceral adipose tissue; w/w, weight by weight.

As perhaps the most well-studied monophenolic acid microbial flavonoid catabolite, 3,4-DHBA is consistently and repeatedly reported to be a potent cardioprotective molecule. In 2010, Wang and colleagues (129) reported that 3,4-DHBA reduces the atherosclerotic burden of apolipoprotein E (ApoE)-deficient mice. In an elaborate follow-up study, the same group established that 3,4-DHBA is a gut microbial metabolite derived from cyanidin-3-*O*-β-glucoside (C3G). As 3,4-DHBA is more bioavailable than C3G, the antiatherosclerotic effects of C3G consumption are attributed to 3,4-DHBA rather than C3G, in part by mediating cholesterol efflux through the repression of the microRNA miRNA-10b (130). Most recently, it was reported that 3,4-DHBA inhibits vulnerable lesion progression in aged ApoE-deficient mice by activation of MER proto-oncogene tyrosine kinase (MERTK) and suppression of mitogen-activated protein kinase 3/1 (MAPK3/1) (142). Moreover, 3,4-DHBA reduced plasma-soluble vascular cell adhesion molecule 1 (sVCAM-1) expression in human umbilical vein endothelial cells (HUVECs) following tumor necrosis factor alpha (TNF-α) stimulation at physiologically attainable concentrations while reducing *VCAM1* mRNA expression at supraphysiologic

concentrations (31, 32, 128, 134). Similarly, 3,4-DHBA reduced both protein and mRNA expression of interleukin 6 (IL-6) and VCAM-1 in oxidized low-density lipoprotein (oxLDL)- and CD40L-stimulated HUVECs (2). In the same study, 4-hydroxy-3-methoxybenzoic acid phenocopied the effects of 3,4-DHBA, while 2,4,6-trihydroxybenzaldehyde reduced VCAM-1 protein and mRNA expression in CD40L but not in oxLDL-stimulated HUVECs. A combination of 3,4-DHBA and 4-hydroxybenzoic acid, in the presence or absence of 4-hydroxy-3-methoxybenzoic acid, decreased the secretion of TNF-α in lipopolysaccharide (LPS)-stimulated THP-1 monocytes (34). It was also reported that 4-hydroxybenzoic acid alone decreased the protein expression of IL-1β in the same LPS-stimulated THP-1 monocyte model of CMD inflammation (34). In vitro experiments with aortic rings isolated from Wistar rats revealed 3-(3′-hydroxyphenyl)propanoic acid as a potent vasodilator. These vasodilatory properties were further confirmed in vivo using normotensive and spontaneously hypertensive rats and 3-(3′-hydroxyphenyl)propanoic acid was found to act through a nitric oxide–dependent mechanism (84). 3,4,5-Trihydroxybenzoic acid significantly attenuated elevated systolic blood pressure, aortic wall thickness, and left ventricular hypertrophy in spontaneously hypertensive rats (61).

Investigations by the Kay group (2, 34, 134) were among the first to comprehensively include phase II conjugated monophenolic acids as part of a preliminary in vitro screen—a necessary inclusion for prior in vitro studies lacking broader biological context. The inclusion of phase II conjugates is necessary, as it appreciates the canonical route of absorption for molecules originating from the gastrointestinal tract and draining into the liver, where they are subject to hepatic first-pass metabolism prior to entering the peripheral circulation. These liver-conjugated catabolites are often among the predominant forms seen in the peripheral circulation (97).

Studies examining the contribution of microbial flavonoid catabolites to cardiovascular disease prevention and regression are summarized in **Table 4** and depicted in **Figure 2**. **Table 4** also includes select in vitro studies investigating the role of microbial flavonoid metabolites in a cardiovascular disease context (2, 34, 134). These particular in vitro studies are characterized by their judicious use of physiologically attainable concentrations and inclusion of phase II phenolic acid conjugates—which are deemed rigorous features and are required for more accurate in vitro modeling (63, 134, 137).

3.4. Microbial Flavonoid Metabolites: Nonalcoholic Fatty Liver Disease

A sedentary lifestyle, dietary caloric surplus, and excess adiposity all contribute to the deposition of free fatty acids in the liver and dysregulation of de novo hepatic lipogenesis, leading to NAFLD (36) and closely related nonalcoholic steatohepatitis (NASH). NAFLD progression to NASH has recently been appreciated to be subject to regulation by the gut microbial community (72), resulting in a flurry of research aiming to connect the interface between diet, the gut microbiome, and NAFLD. Recent epidemiological evidence revealed an inverse association with dietary phenolic acid intake and NAFLD, even when adjusting for confounding factors such as smoking status, body mass index, and dietary consumption of SCFAs and sweetened beverages (106). Further corroborating these correlative data, and expanding upon the bioefficacy of 3,4-DHBA, Liu and colleagues (75) reported the antisteatotic properties of this monophenolic acid through reduced hepatic inflammation and downregulation of hepatic lipogenesis after feeding mice a trans fatty acid–rich diet. Additional mechanistic insight was provided by Sun and colleagues (122), who reported that 3,4-DHBA abrogates hepatic steatosis in a *Sirt3*-dependent manner, partially through the deacetylation of acyl-CoA-synthetase family member 3 (ACSF3), a key regulator of de novo fatty acid synthesis and oxidation in the liver (118). After 16 weeks of an HFD, male C57BL/6 mice treated daily with up to 100 mg/kg of 3,4,5-trihydroxybenzoic acid had decreased hepatic

Table 4 Microbial flavonoid metabolite effects on cardiovascular disease

Microbial flavonoid metabolite (concentration/dose)[a]	Duration of study	Disease model	Key outcome	Reference
3,4-Dihydroxybenzoic acid (0.003% w/w in diet)	20 weeks	Male *ApoE*$^{-/-}$ mice	Decreased aortic lesion size and aortic cholesterol; decreased protein expression of sVCAM-1 and sICAM-1; decreased NF-κB binding activity	129
3,4-Dihydroxybenzoic acid (5 mg/kg BW daily gavage)	4 weeks	Aged (30-week-old) male *ApoE*$^{-/-}$ mice	Decreased aortic lesion size and aortic cholesterol; decreased miRNA-10b expression; increased *Abca1*/*Abcg1*-mediated RCT	130
3,4-Dihydroxybenzoic acid (0.003% w/w in diet)	20 weeks	Aged (30-week-old) male *ApoE*$^{-/-}$ mice	Decreased vulnerable lesion progression through activation of MERTK and inhibition of MAPK3/1	142
3,4-Dihydroxybenzoic acid (1–100 μM)	18 h	oxLDL-stimulated HUVECs	Decreased sVCAM-1 protein expression; decreased *VCAM1* mRNA expression at 100 μM	134[b]
3,4-Dihydroxybenzoic acid (1–10 μM)	24 h	oxLDL and CD40L-stimulated HUVECs	Decreased IL-6 protein and mRNA expression; decreased VCAM1 protein and mRNA expression	2[b]
4-Hydroxybenzoic acid (1 μM)	24 h	LPS-stimulated THP-1 monocytes	Decreased IL-1β protein expression	34[b]
4-Hydroxybenzoic acid + 3,4-dihydroxybenzoic acid combination (0.1–10 μM)	3 h	LPS-stimulated THP-1 monocytes	Decreased TNF-α protein expression	34[b]
4-Hydroxybenzoic acid + 3,4-dihydroxybenzoic acid + 4-hydroxy-3-methoxybenzoic acid combination (1–10 μM)	3 h	LPS-stimulated THP-1 monocytes	Decreased TNF-α protein expression	34[b]
3-(3′-Hydroxyphenyl)propanoic acid (2.5 mg/kg BW to 25 mg/kg BW)	5 min	Normotensive male Wistar rats and SHR	NO-dependent decreased systolic and diastolic BP	84
3,4,5-Trihydroxybenzoic acid (1% w/v in drinking water)	16 weeks	Male SHR	Decreased systolic BP in SHR; decreased aortic wall thickness and left ventricular hypertrophy	61
4-Hydroxy-3-methoxybenzoic acid (0.1–10 μM)	24 h	oxLDL and CD40L-stimulated HUVECs	Decreased IL-6 protein and mRNA expression; decreased VCAM1 protein and mRNA expression	2[b]
2,4,6-Trihydroxybenzaldehyde (10 μM)	24 h	CD40L-stimulated HUVECs	Decreased VCAM1 protein and mRNA expression	2[b]

[a](*S*)-Equol was excluded from the table as its cardioprotective role in humans has been recently and comprehensively reviewed by Sekikawa et al. (113).
[b]Phase II monophenolic acid conjugates were excluded from the table as they are the result of host modification, a topic outside of the aims and scope of this review.
Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; ApoE, apolipoprotein E; BP, blood pressure; BW, body weight; HUVEC, human umbilical vein endothelial cell; IL-6, interleukin 6; LPS, lipopolysaccharide; MAPK3/1, mitogen-activated protein kinase 3/1; MERTK, MER proto-oncogene tyrosine kinase; mRNA, messenger RNA; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; RCT, reverse cholesterol transport; SHR, spontaneously hypertensive rats; sICAM-1, plasma-soluble intercellular adhesion molecule 1; sVCAM-1, plasma-soluble vascular cell adhesion molecule 1; TNF-α, tumor necrosis factor alpha; w/v, weight by volume; w/w, weight by weight.

steatosis, as quantified by reduced liver TG, FFAs, and cholesterol when compared with control animals (27). Similar results were recently revealed in male Swiss mice, where a marked reduction in the hepatic steatosis was observed in mice receiving a daily oral gavage of 3,4,5-trihydroxybenzoic acid (120).

Table 5 Microbial flavonoid metabolite effects on nonalcoholic fatty liver disease

Microbial flavonoid metabolite (concentration/dose)	Duration of study	Disease model	Key outcome	Reference
3,4-Dihydroxybenzoic acid (1%, 2%, 4% w/w in diet)	10 weeks	TFAD-induced hepatic steatosis; male C57BL/6 mice	Decreased hepatic TG and cholesterol; reduced hepatic protein expression of IL-1β, IL-6, TNF-α, and MCP-1	75
3,4-Dihydroxybenzoic acid (30 mg/kg BW daily gavage)	8 weeks	HFD-induced hepatic steatosis; WT male C57BL/6 and *Sirt3* $^{-/-}$ mice	Decreased hepatic steatosis and liver injury in a *Sirt3*-dependent manner	122
3,4,5-Trihydroxybenzoic acid (50 and 100 mg/kg BW daily gavage)	16 weeks	HFD-induced hepatic steatosis; male C57BL/6 mice	Decreased hepatic TG, FFAs, and cholesterol	27
3,4,5-Trihydroxybenzoic acid (100 mg/kg BW daily gavage)	8 weeks	HFD-induced hepatic steatosis; male Swiss mice	Decreased percent steatotic area in liver	120
3,5-Dimethoxy-4-hydroxybenzoic acid (0.05% w/w in diet)	16 weeks	HFHC-induced steatosis; male C57BL/6J mice	Decreased hepatic TG, FFAs, and cholesterol; decreased FAS and PAP activity; increased CPT activity and β-oxidation	51
3′,4′-Dihydroxycinnamic acid (0.8% w/w in diet)	6 weeks	HFHC-induced steatosis; male C57BL/6 mice	Decreased hepatic TG and cholesterol; decreased hepatic SREBP1c/2, FAS, and HMGCR protein expression; increased hepatic pAMPK	74
3-(4′-Hydroxy-3′-methoxyphenyl)propanoic acid (1% w/w in diet)	12 weeks	HFD-induced steatosis; male C57BL/6J mice	Decreased hepatic TG; decreased hepatic mRNA expression of *Cd36*, *Acc1*, *Fas*; increased hepatic mRNA expression of *Cpt1a/2*, *Ppargc1a*, *Gck*, *Pkm2*	89
4-Hydroxy-3-methoxybenzoic acid (100 mg/kg BW daily gavage)	6 weeks (WT) 4 weeks (*db/db*)	HFD-induced obesity in WT male mice; *Lepr*$^{-/-}$ (*db/db*) male mice	Decreased hepatic TG, cholesterol, and NF-κB protein expression in both WT and *db/db* mice	62

Abbreviations: BW, body weight; CPT, carnitine palmitoyltransferase; FAS, fatty acid synthase; FFA, free fatty acid; HFD, high-fat diet; HFHC, high-fat/high-cholesterol diet; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IL-1β, interleukin 1 beta; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; mRNA, messenger RNA; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; pAMPK, phosphorylated 5′ adenosine monophosphate-activated protein kinase; PAP, phosphatidate phosphatase; SREBP1, sterol regulatory element-binding transcription protein 1; TFAD, trans fatty acid–rich diet; TG, triglycerides; TNF-α, tumor necrosis factor alpha; WT, wild-type; w/w, weight by weight.

The incorporation of 3,5-dimethoxy-4-hydroxybenzoic acid into an HFHC diet decreased hepatic steatosis in mice while decreasing hepatic mRNA expression of *Cidea*, *Pparg*, *Fasn*, *Srebp-1c/2*, and *Hmgcr* and increasing hepatic mRNA expression of *Ppara*, *Acsl*, and *Cpt1/2* (51). Similarly, 3′,4′-dihydroxycinnamic acid reduced hepatic TG and cholesterol in mice fed an HFHC diet through the increased hepatic expression of SREBP1c/2, FAS, and HMGCR proteins and a concomitant increase in phosphorylated AMPK (74). 4-Hydroxy-3-methoxybenzoic acid was shown to reduce hepatic steatosis and NF-κB protein expression both in an HFD-induced model of steatosis and in mice lacking the leptin receptor (62). In vivo studies examining the role of microbial flavonoid catabolites in NAFLD are summarized in **Table 5** and depicted in **Figure 2**.

4. FUTURE ISSUES AND UNANSWERED QUESTIONS

An important concern for past, current, and future animal studies is that monophenolic acids administered via diet, drinking water, or oral gavage render the molecule of interest subject to further

metabolism in the upper gastrointestinal tract before reaching the colon. Moreover, these routes of administration may lead to prehepatic absorption of monophenolic acids. Prehepatic absorption is not consistent with the physiologic route of absorption, distribution, and metabolism, which involves venous drainage from the intestine into the liver via the portal vein. Alternative delivery methods such as intraperitoneal or intravenous injection have similar disadvantages with regard to nonphysiologic distribution. To circumvent this, we recently developed a surgical method for continuous intraportal delivery of the monophenolic acid metabolite 4-hydroxyphenylacetic acid (90). However, due to the complex nature of this surgery and associated animal housing requirements, this technique is not readily adaptable to investigations with a large number of metabolites, and physiologically relevant delivery is a topical challenge for the field.

Furthermore, the administration of complex communities of microbial flavonoid catabolites as they exist postprandially in vivo is necessary to understand the synergistic or antagonistic relationships between phenolic acids in the context of CMD. Despite the well-characterized plethora of phenolic acids resulting from microbial flavan-3-ol catabolism, the bacterial species and enzymatic mechanisms involved remain poorly investigated. Unfortunately, sex is not considered as a biological variable in the vast majority of microbial flavonoid metabolism animal models. This hinders progress made toward a comprehensive understanding of the three-way diet–gut microbiome–host interface and must be addressed in future studies (73). The gut microbiome predictively responds to flavonoid consumption, thus positioning fecal 16S rRNA sequencing as a candidate clinical tool to identify flavonoid responders and nonresponders (59). Furthermore, flavonoids and their microbial catabolites have recently been used as biomarkers in human epidemiological studies (92, 101). Innovative approaches utilizing flavonoid catabolites to mediate transcriptional control switches represent exciting research progress in the field (141). Collectively, the use of microbial sequencing data and plasma or urine phenolic acids concentrations may one day inform clinical dietary decision-making in an evidence-based manner. These types of analyses should enhance the precision and efficacy of personalized nutrition and nutraceutical intervention to abrogate the disease burden of CMD.

SUMMARY POINTS

1. An inverse association between flavonoid consumption and cardiometabolic disease (CMD) incidence has been established, yet the microbial contribution to these findings remains unclear.
2. The bioavailability of flavonoids as they exist in planta is limited, rendering dietary flavonoids subject to extensive catabolism by colonic microflora, where they are converted into more readily absorbed monophenolic acids.
3. Animal models of obesity indicate that microbial flavonoid catabolites are capable of abrogating high fat diet–induced obesity and adiposity through the suppression of de novo hepatic lipogenesis and induction of thermogenic factors in adipose tissue.
4. Studies in animal models of type 2 diabetes mellitus demonstrate improved glucose homeostasis and insulin resistance following the administration of microbial flavonoid catabolites, though the molecular mechanisms remain underdeveloped.
5. The treatment of animal models of cardiovascular disease with microbial flavonoid catabolites reduced systolic blood pressure, aortic wall thickness, and left ventricular hypertrophy. These animals also benefited from decreased expression of the

proatherosclerotic adhesion molecules VCAM-1 and ICAM-1 and reduced levels of proinflammatory factors.

6. Recent epidemiological studies have revealed a negative correlation between phenolic acid intake and hepatic steatosis. These findings were recapitulated in animal models of hepatic steatosis and have been demonstrated to be the consequence of altered hepatic lipid metabolism.
7. Future studies in humans and mice are required to more fully appreciate the role of the gut microbiome as a central mediator of dietary flavonoid consumption in CMD.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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