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Review

The path toward using microbial metabolites as therapies

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ABSTRACT

Metabolites have emerged as the quintessential effectors mediating the impact of the commensal microbiome on human physiology, both locally at the sites of microbial colonization and systemically. The endocrine activity of the microbiome and its involvement in a multitude of complex diseases has made microbiome-modulated metabolites an attractive target for the development of new therapies. Several properties make metabolites uniquely suited for interventional strategies: natural occurrence in a broad range of concentrations, functional pleiotropy, ease of administration, and tissue bioavailability. Here, we provide an overview of recently discovered physiological effects of microbiome-associated small molecules that may serve as the first examples of metabolite-based therapies. We also highlight challenges and obstacles that the field needs to overcome on the path toward successful clinical trials of microbial metabolites for human disease.

Contents

1. Introduction

Large-scale genome-wide association studies (GWAS) have revealed numerous genetic contributions to modern human diseases, including metabolic, inflammatory, and neurodegenerative disorders. At the same time, the cases in which GWAS have come close to reaching saturation with respect to the number of genetic associations to a particular disease suggest that these diseases do not solely arise from human genetic determinants. Rather, environmental and lifestyle factors play a major role in disease pathogenesis. A significant component of our environmental exposure is represented by diet and by symbiotic microbes that reside in the gastrointestinal tract. The microbiota has been recognized to play a role in human disease, and the mechanisms by which these microorganisms contribute to host health have been extensively investigated over the past decade. Commensal bacteria can directly engage host responses through physical interactions or through

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secreted molecules that gain access to the systemic circulation and thereby affect human physiology and disease. Recent technical progress in the fields of untargeted metabolomics – applied to well-characterized clinical cohorts and coupled with mechanistic studies in animal models – have enabled the characterization of human disease-associated gut microorganisms and their metabolites. Given the pervasiveness and pleiotropy of metabolite effects on human physiology, the translational potential of these molecules for "post-biotic" therapies is enormous [1,2]. Herein, we broadly summarize recent advances in the identification and characterization of microbiome-associated metabolites that are of potential therapeutic value for clinical use (Fig. 1). These metabolites are either solely derived from bacterial metabolism or dietary nutrients that are modulated by intestinal bacteria.

1.1. Trimethylamine-N-oxide (TMAO)

TMAO may serve as a prototypical example for a microbiome-derived metabolite with strong translational potential. TMAO is linked to a broad range of diseases. In addition to cardiovascular and metabolic diseases, including atherosclerosis, type 2 diabetes, myocardial infarction, and stroke [3–7], studies identified TMAO as a potential predictive biomarker of chronic kidney disease and end-stage renal disease [8], although conflicting results were recently published [9]. Recent evidence also showed that TMAO is elevated in the cerebrospinal fluid of patients with mild cognitive impairment and Alzheimer's disease [10]. Initially, untargeted metabolomic analysis of human plasma samples from independent cohorts of patients who subsequently experienced myocardial infarction or stroke versus subjects who did not, led to the identification of TMAO as a metabolite strongly correlating with increased cardiovascular risk [11]. TMAO is formed by a diet-derived meta-organismal pathway. Eggs, milk, red meat and some seafood are rich in the lipid phosphatidylcholine, which is a source of the essential nutrient choline in omnivores. Choline and other trimethylamine (TMA)-containing species (L-carnitine; betaine) are catabolized by intestinal microbial TMA lyases and form the gas TMA [12]. TMA is rapidly absorbed by the host, enters the portal circulation, and is metabolized by the hepatic flavin monooxygenase FMO3 to TMAO [13]. An intact gut microbiome is required for the formation of TMAO from dietary choline, as germ-free mice failed to form TMAO after administration of deuterium-labelled PC, while the d9 isotopomer of TMAO was successfully produced in control mice [11]. TMAO supplementation in apolipoprotein E (ApoE)-deficient mice was observed to foster enhanced macrophage foam cell formation and to promote aortic root atherosclerotic plaque development [11]. TMAO induces alterations in cholesterol (reductions in reverse cholesterol transport), sterol metabolism, as well as in bile acid pool size and composition [14,15]. In experimental atherosclerosis, induced by 2% choline diet for 6 weeks in rodent, TMAO levels were reduced by oral supplementation with guggulsterone, a farnesoid X receptor antagonist, resulting in ameliorated experimental disease [16]. Although the receptor for TMAO is not yet known, this metabolite was shown to enhance platelet hyperreactivity and thrombosis risk [17].

Importantly, a natural inhibitor targeting the microbial TMA-generating enzyme gave promising results in a preclinal study of atherosclerosis [18] and protected against thrombosis risk. Indeed, a single oral dose of a structural analog to choline, 3,3-dimethyl-1-butanol (DMB), or iodomethylcholine (IMC), an inhibitor of the microbial CutC/D TMA-generating enzyme pair, significantly reduced plasma TMAO for 3 days in mice, rescuing the diet-induced hyperreactivity of platelets [19]. This effort represents a proof of concept that a microbial enzyme can be targeted to create new drugs for human diseases. Given that TMAO can be established as a cause rather than consequence of disease-promoting activity [20], the next step toward clinical application will be to establish the safety, efficacy and pharmacodynamics for this inhibitor in humans.

1.2. Short-chain fatty acids (SCFAs)

SCFAs are among the most concentrated metabolites in the gastrointestinal tract. Acetate, butyrate, and propionate are produced by members of the intestinal microbial community through fermentation of dietary fibers and starches, which are unable to be broken down by host-metabolism [21]. In turn, these metabolites are sensed by host cells through various G-protein coupled receptors (GPRs), known as free fatty acid receptors (FFARS), and the intracellular receptor PPARγ. Furthermore, SCFAs can also regulate cellular responses through inhibition of histone deacetylases (HDACs). Examples of SCFA effects on the host include regulatory T (Treg) cell and macrophage differentiation and downregulation of pro-inflammatory mediators. These effects underline the fine balance that SCFAs help maintain between intestinal immunity and inflammation [21–24]. Furthermore, SCFAs have been

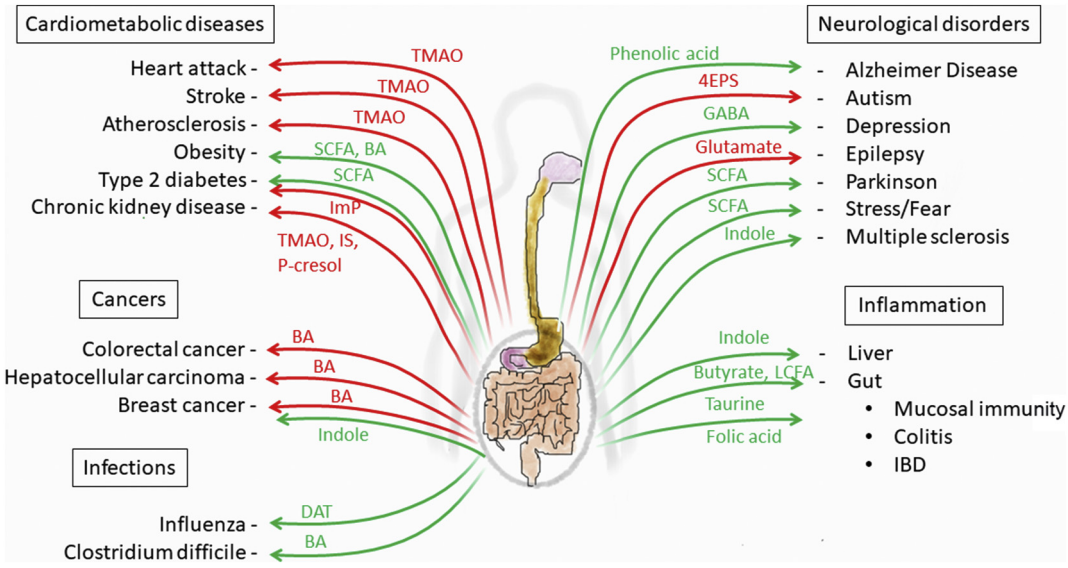

Fig. 1. The impact of gut microbiome-derived metabolites in disease. Arrows represent a beneficial (green) or detrimental (red) role for selected gut microbiome-associated metabolites in different diseases. 4EPS: 4-ethylphenylsulfate. BA: Bile acid. DAT: desaminotyrosine. GABA: gamma-aminobutyric acid. IBD: inflammatory bowel disease. ImP: Imidazole propionate. IS: Indoxyl sulfate. LCFA: Long-chain fatty acids. TMAO: Trimethylamine-*N*-oxide. SCFA: short-chain fatty acids.

seen to specifically alter hematopoiesis, promote IgA responses, alter T cell homeostasis, modulate fat accumulation and insulin sensitivity, and promote neurogenesis [25–30]. Due to their clear role at the host-microbiota interface in shaping the immune system and metabolism, there has been increased interest to explore their potential roles in disease and uses as therapy. SCFAs are being used in clinical trials to treat a range of diseases affecting neurological functions, metabolism, and gastrointestinal health. Alterations in the microbiome have been correlated to the emergence of diabetes, obesity, and inflammatory diseases such as inflammatory bowel disease (IBD) [31]. Blood glucose regulation and lipid metabolism is highly implicated in metabolic diseases and some SCFAs, in particular butyrate, have been shown to affect these processes [32]. Due to butyrate's potential role, it is currently in Phase 2 and 3 trials to study its effect on insulin resistance and liver damage in children with obesity (NCT02721953). Furthermore, another clinical study administered butyrate enemas in patients in remission from ulcerative colitis or irritable bowel syndrome; however, relatively minor effects were observed [33]. Two studies aimed to look at the effect of either a SCFA mixture or acetate as interventional treatment in overweight male individuals with the potential of controlling obesity and type 2 diabetes mellitus. The results demonstrated whole body increase of fasting fat oxidation and plasma PYY, attenuation of fasting free glycerol concentrations, and decreased lipolysis, indicating the potential of acetate or SCFA colonic infusions as therapies for these metabolic conditions [34–36]. The potential therapeutic role of SCFAs is also being examined with respect to the gut-brain axis. Butyrate as an HDAC inhibitor, GPCR activator, and cellular energy source has been proposed to ameliorate symptoms in Alzheimer's, Parkinson's, autism, Huntington's, and schizophrenia [37–42]. Trials are underway to assess butyrate's effect of cognitive function in patients with schizophrenia, in particular in regard to its epigenetic modulation of inflammation (NCT03010865). Currently, a SCFA mixture is being examined for its role in affective processing such as in stress, fear, and emotions (NCT03688854). Though these clinical studies are using SCFA directly as a therapy, there is also a growing interest in the use of pre-biotics such as resistant starches, which can be used by the microbiota to generate SCFA [40,43].

1.3. Long-chain fatty acids (LCFAs)

LCFAs have long been used in dietary supplementation, either as linseed or perilla oil containing linoleic acid and fish oils containing omega-3 and omega-6 fatty acids (FA). Considered to be essential, as they cannot be produced by mammals, LCFAs are obtained through diet and eventually metabolized to generate bioactive lipid mediators. These have been seen to reduce blood pressure (arachidonic acid) or modulate inflammation through attenuation of inflammatory cytokines [44,45]. Recently, LCFAs have been identified as microbial metabolites. Various conjugated linoleic acids (CLAs), oxy FA, and hydroxy FA are generated by commensal bacteria in the intestine: *Lactobacillus plantarum, Enterococcus faecalis, and Bacteroides thetaiotaomicron* [46]. Indeed, it has been shown that these microbial-derived linoleic acids can target PPARγ receptors in macrophages [47], while also fortifying epithelial barrier integrity through upregulation of tight junctions. Specifically, 10-hydroxy-cis-12 octadecenoic acid is one CLA which has been explored as a potential therapeutic for its role in epithelial barrier integrity [48,49]. Omega acids have long been shown to be beneficiary to the heart and brain, and now with accumulating evidence of microbially generated LCFAs, recent studies explore using CLAs in therapy as post-biotics. Indeed, studies have shown the potential of CLA to limit body fat percentage in healthy, overweight and obese adults possibly through modulation of fatty acid metabolism [50,51]. Additionally, one Phase 3 clinical study was performed to look for a role in attenuating atherosclerosis with a CLA, yet no effect was seen [52]. Another study posited that CLAs used as a supplement with vitamin and fiber could be effective as an adjuvant to ulcerative colitis therapy [53].

1.4. 4-ethylphenylsulfate (4-EPS)

4-EPS is a dietary fermentation product, suspected to be a uremic toxin. Specific pathogen-free and germ-free mice naturally have very low concentrations of 4-EPS. Interest in this metabolite stems from a report which showed an increase in 4-EPS in the maternal immune activation (MIA) mouse model of autism spectrum disorder (ASD). This elevated 4-EPS level correlated with behavioral abnormalities. These effects were then ameliorated through treatment with *B. fragilis, indicating the possible use of pro-biotic and post-biotic therapies for ASD* [54].

1.5. Indole

Amino-acid metabolism from the intestinal microbiota represents a major source of bioactive metabolites to the host [55]. The essential amino acid tryptophan is catalyzed into indole by tryptophanase, which is solely encoded in microbial genomes [56]. Tryptophan derivatives such as 3-indolepropionic acid (IPA) converted by *Clostridium sporogenes* or indole-3-aldehyde by *Lactobacillus*-encoded tryptophanase are recognized by the aryl hydrocarbon receptor (AhR). AhR is a ligand-activated transcription factor that integrates environmental, dietary, microbial and metabolic cues to control complex transcriptional programs in a ligand-specific, cell-type-specific, and context-specific manner [57]. Intestinal epithelial cell-specific deletion of Ahr results in failure to control *C. rodentium* infection [58]. AhR also mediates the effect of the microbiome on intra-epithelial lymphocytes [59]. Indole-3-aldehyde signaling through AhR mediates IL-22 production in type 3 innate lymphoid cells under pathogenic infection by *Candida albicans* [60]. Additional commensal microbiota metabolites are also endowed with AhR agonistic activity, such as indole-3-acetic acid, indole-3-acetylaldehyde, indole-3-aldehyde, 3-methylindole, and 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE). These metabolites are thought to limit intestinal inflammation via AhR-dependent mechanisms and may be dysfunctional in IBD [61]. Indole metabolites can also counteract detrimental inflammation in LPS-mediated liver inflammation through the modulation of the NLRP3 pathway [62]. In experimental autoimmune encephalomyelitis, a preclinal model of multiple sclerosis, indole metabolites modulated central nervous system inflammation through the reduction of pathogenic activity of astrocytes [63–65]. In neurodegenerative disorders, cerebellar syndrome is thought to be related to a neurochemical deficit of 5-hydroxytryptamine (5-HT). Current clinical trials will evaluate indole-3-propionic acid supplementation, a 5-HT precursor, as a therapeutic strategy for Friedreich's ataxia and multiple sclerosis. Indole can be further converted into indoxyl and indoxyl sulfate by host hepatic oxidases (CYP2E1 and SULT1A1). Indoxyl sulfate is suspected to be a central “uremic toxin”, whose removal from the host via renal excretion is impaired in kidney disease [66]. Indoxyl sulfate is also a potential vascular toxin that can induce oxidative stress in endothelial cells, increase vascular smooth muscle cell proliferation, and potentially contribute to the pathophysiology of atherosclerosis and sarcopenia in subjects with renal disease [66,67]. Over 90% of dietary tryptophan, however, is metabolized by the kynurenine pathway, which generates AhR ligands identified as trace-extended aromatic condensation products. Administration of a pharmacologically optimized kynureninase that degrades kynurenine into an immunologically inert, nontoxic metabolite inhibits tumor growth in a mouse model [68].

Several attempts to modulate indole metabolites for therapeutic purposes are under investigation. *Arabinoxylan oligosaccharides* (AXOS) are pre-biotic carbohydrates with promising health-promoting properties that stimulate the activity of specific colon bacteria, in particular *Bifidobacteria*. AXOS are tested in chronic kidney disease for lowering indole derivatives (indoxyl sulfate). An alternative to AXOS is AST-120 (also called Kremezin), which binds to indole and is currently tested in clinical trials as a therapeutic for chronic kidney disease (reduction of uremic toxin). An alternative therapeutic strategy would be to directly

inhibit AhR by delivering antagonists alone or in nanoparticles or design enzyme optimized to metabolize AHR agonists [57].

p-cresol (or 4-methylphenol), and its derivative *p*-cresyl sulfate, is another bacterial metabolite from protein fermentation, not produced by human enzymes [69]. It is suspected to alter endothelial function [70], to promote vascular calcification [71] and has been associated with increased mortality in hemodialysis patients [72], pathogenesis of ulcerative colitis, and *Clostridium difficile* infection [73]. While increased dietary fat consumption in healthy subject results in an increase of p-cresol and indole [74], a dietary strategy with oligofructose-enriched inulin contributes to a lower generation of protein fermentation metabolites and constitutes a significant improvement for chronic kidney disease patients [75,76]. AXOS intake increases fecal bifidobacteria and reduces urinary p-cresol excretion [77].

1.6. Other protein-derived metabolites

Taurine is an amino sulfonic acid whose levels are modulated by commensal bacteria deconjugation of primary bile acids [78,79], causing an increase in luminal taurine levels. Taurine was observed to induce NLRP6 inflammasome signaling and was thus proposed to modulate inflammatory bowel diseases [80]. Taurine is also being studied as a protective metabolite in colorectal cancer and diabetes. One study looked at the abundance of sulfidogenic bacteria in African Americans with diets rich or low in taurine to observe for risk elements for colorectal cancer, such as oxidative stress, primary and secondary bile acid pools, and inflammation markers [81]. Meanwhile, two clinical studies administer taurine to treat vascular complications in diabetes that may arise through oxidative stress (NCT03410537, NCT01226537).

The protective effect of ketogenic diet (reduced carbohydrates and high-fat diet) has been shown to be mediated by the gut microbiome through the reduction of amino acid y-glutamylation, ultimately leading to increased GABA/glutamate content in the brain [82].

Desaminotyrosine (DAT) is a degradation product of tyrosine and naturally present in mammalian urine. A recent study showed that an intact gut microbiome, and in particular the presence of *Clostridium orbiscendens*, is required for detectable DAT levels in the feces and serum [83]. Reduction of DAT production was associated with increased susceptibility to viral infection, due to suboptimal amplification of the interferon type I signaling loop. Yet, no registered clinical study is assessing the translation of this finding to the clinic.

Imidazole propionate is a recently identified histidine-derived metabolite that plays an important role in the pathophysiology of type 2 diabetes. Higher concentrations of this metabolite tend to be found in type 2 diabetes subjects versus healthy subjects, and result in the impairment of the insulin signaling by activating p38γ, p62, and mTORC1 [84]. Therefore, clinical trials will evaluate diets (low-protein versus high-protein) that can inhibit imidazole propionate production (NCT03732690).

1.7. Polyamines

Polyamines have been implicated in the regulation of inflammation pathways and induction of inflammatory bowel diseases, such as colitis [80]. Metabolites such as spermidine are either generated by the microbiota, by the host, or consumed through the diet, and have pleiotropic effects on multiple different tissues [85]. Spermine can act on the NLRP6 inflammasome pathway, which subsequently impacts microbial composition. Indeed, several members of the *Lactobacillus* genus mediate polyamine synthesis through biosynthetic and transport pathways of spermine [80]. Spermine, which is produced through decarboxylation of amino acids, was observed to reduce colonic IL-18 levels and suppress NLRP6 inflammasome assembly. In patients with inflammatory bowel disease and in mice with chronic DSS-induced colitis, increased levels of the polyamine precursor spermidine were observed [86]. Insights into a possible role for polyamine inhibition as a viable strategy against inflammatory bowel disease in humans await clinical studies [87].

Microbiota-associated polyamines have also been implicated in the regulation of epithelial circadian rhythms [88]. The microbiome undergoes diurnal oscillations, and so do intestinal polyamine levels [88–91]. Alteration of polyamine rhythms is associated with clock dysfunction [92], and thus control of intestinal polyamine levels might present a rational approach to controlling transcriptional oscillations in intestinal epithelial cells.

1.8. Retinoic acid

Retinoic acid, derived from dietary vitamin A, has been implicated largely in the development and regulation of the immune system. Retinoic acid concentration in the gut is in turn maintained by *Clostridia* commensal bacteria by suppression of retinol dehydrogenase 7 in intestinal epithelial cells [93]. This lipid metabolite has been shown to contribute to the modulation of immune responses through IgA production [27]. Moreover, retinoic acid maintains T-cell immunity through regulatory T (Treg) cell differentiation and T helper 17 cell control [94]. Indeed, deficiencies in retinoic acid or its receptors have been associated with disease states such as colitis-associated colon cancer and IBD [95]. Due to several studies implicating its role in T cell development, all trans-retinoic acid (ATRA) is being explored as a potential therapy in diseases of deregulated immune states such as thrombocytopenia, rheumatoid arthritis, cancer, and auto-immune disorders [96–99]. To date there has been one completed Phase 2 study demonstrating that patients with primary immune thrombocytopenia treated with ATRA showed enhanced response of sustained platelet levels (NCT01667263). ATRA is also currently used to treat leukemia and acne under the name tretinoin, though its mechanism of action is still currently unknown. Further studies are required to explore the use of retinoic acid in ATRA form or 13-cis retinoic form in modulation of inflammation in the gut.

1.9. Bile acids (BAs)

Primary BAs, chenodeoxycholic acid (CDCA) and cholic acid (CA), are synthesized in the liver from cholesterol by hepatocytes and represent more than 75% of the body's total BA content. In the small and large intestine, the bacterial deconjugation, dehydrogenation, 7α-dehydroxylation, and epimerization of the primary BAs produces the "secondary" BAs. CA is converted to dihydroxy deoxycholic acid (DCA) and CDCA to the monohydroxy lithocholic acid (LCA). Colonic bacteria can further metabolize 7a-oxo-LCA to a "tertiary" BA, dihydroxy ursodeoxycholic acid (UDCA) [100]. BAs play a key role in maintaining cholesterol homeostasis through the absorption and emulsification of lipids. Bacteria with bile salt hydrolase activity, such as *Lactobacillus and Clostridium,* facilitate various aspects of BA metabolism including deconjugation, dehydrogenation, and dihydroxylation [101,102]. Germ-free mice present higher overall BA levels, mostly ursodeoxycholic acid (UDCA), as well as bigger gallbladders due to activation of the transmembrane G protein-coupled receptor 5 (TGR5) pathway [103]. Exposure to cold temperatures in mice triggers the transformation of cholesterol to BAs in the liver, alters gut microbiome composition, stimulates lipoprotein processing in brown adipose tissue, and generates heat [104].

Recent studies have indicated that UDCA improves insulin sensitivity and promotes weight loss in patients with type 2 diabetes (NCT02033876). It also plays a key role in fighting infection. UDCA inhibited the growth of *Clostridium difficile* in fecal samples from patients with recurring *C. difficile* infections and was effective in treating a patient with *C. difficile*-associated pouchitis [105]. A clinical trial will aim to validate this result by administering ursidol/UDCA to patients with recurring *C. difficile* infections (NCT02748616). Additionally, higher concentrations of deoxycholic acid (DCA) are associated with

colonic crypt regeneration after wounding [106]. DCA utilized FXR to hinder prostaglandin E2 synthesis (a substance that inhibits crypt repair), thereby promoting wound repair [106].

Moreover, studies have focused on inhibiting secondary BAs as a form of treatment. Many studies have linked high concentrations of secondary BAs to increased risk for various types of cancer (colorectal, pancreatic, and liver cancer) and recognized their ability to promote DNA mutations and cell death. For example, one paper reported that in a murine model of liver cancer, the administration of secondary BAs (e.g. ω-muricholic acid) reduced natural killer T (NKT) cell accumulation and decreased CXCL16 expression, thereby reversing the antitumor effect of primary BAs [107]. Other studies have reported significantly higher DCA concentrations in colorectal cancer patients, and increased hepatocellular carcinoma (HCC) risk with rising DCA levels [108,109]. However, lowering DCA levels (using either difructose anhydride III or ursodeoxycholic acid) decreased HCC development in obese mice [109].

1.10. Flavonoids

Flavonoids are polyphenolic secondary plant metabolites that are known to possess a vast range of biological abilities, including antioxidant function, protective effects on vascular endothelial cells, and anti-carcinogenic activity. These metabolites are a specific subgroup within the larger category of polyphenols, and the microbiome plays a significant role in flavonoid metabolism [110]. Members of the colonic microbiota are responsible for mediating both the hydrolysis and fermentation of ingested flavonoids [111], and germ-free or antibiotics-treated mice consequently have high levels of flavonoids in their intestines [112]. For example, *Bacteroides distasonis* and *Bacteroides uniformis* are two of several microbial species that are known to be involved in the hydrolysis of polyphenols. Therefore, the gut microbiota is key to facilitating these processes, but the polyphenols and flavonoids themselves may also alter gut microbiota composition. For example, a study revealed that the flavonoids rutin and quercetin can inhibit *E. coli* and *Serratia marscens* growth [113].

Furthermore, flavonoids seem to have a disease-ameliorating effect in obese mice. A study reported a decrease in hyperlipidemia, hepatic steatosis, and insulin resistance in high-fat diet mice after oral gavage with a flavonoid-rich *Paulownia fortunei* extract [114]. Clinical trials aim at evaluating the post-biotic effect of polyphenols derived from dietary plant Extra Virgin Olive Oil and/or Red Wine (NCT03101436) in obese and diabetic patients. In addition, the flavonoid-metabolizing activity of the gut microbiome becomes physiologically relevant in the post-obesity situation [115]. In a mouse model of post-dieting weight regain, persistent post-obesity microbiome alterations maintained a paucity of the two flavonoids apigenin and naringenin in the intestine [112]. Post-biotic supplementation of these metabolites increased host energy expenditure and ameliorated relapsing obesity.

Moreover, studies have reported that flavonoids like luteolin are powerful antioxidants that can inhibit mast cells, which are known to be involved in inflammatory responses. A study reported that tetramethoxyluteolin, a luteolin analog, inhibited the release of inflammatory mediators such as beta-hexosaminidase, histamine secretion, and tumor necrosis factor (TNF) from mast cells [116]. Additionally, luteolin was also shown to inhibit the release of IL-6 [106]. Treatment with luteolin and diosmin, another luteolin analog, inhibits the IL-6 induced JAK2/STAT3 pathway in MIA mice and improves social skills [117]. This result has also been replicated in human studies. A study reported decreased levels of IL-6 and TNF, as well as improvements in social skills, in ASD children after luteolin formulation treatment [118]. Indeed, flavonoids seem to have beneficial affects across various types of disorders and diseases of the brain. For example, flavonoids have shown promising results in Alzheimer's disease (AD). Grape seed polyphenolic extract (GSPE), a potent antioxidant, has been reported to inhibit amyloid-β (Aβ) oligomerization in Tg2576 mice and tau peptide aggregations *in vitro* [119].

Together, these studies indicate that modulating the microbiome to increase intestinal flavonoid levels might be beneficial for a wide range of conditions.

1.11. N-acyl amides

N-acyl amides are bioactive lipids that play multiple roles in mammalian physiology. Commendamide (or *N*-acyl-3-hydroxypalmitoylglycine) is a metabolite from the human gut microbiome that was identified by functional metagenomic screening for NF-kB activators. It resembles long chain *N*-acyl-amides and activates a G-protein coupled receptor G2A/GPR132, implicated in autoimmunity and atherosclerosis [120]. Genes predicted to encode similar lipids were further identified, synthesized, and inserted into *E. coli*. These lipids were then tested against G-protein coupled receptors (GPCR). The microbial lipid *N*-acyl serinol is similar to a human ligand for GPR119, which once activated stimulates glucagon-like peptide-1 (GLP-1) release from the intestinal L-cells and regulated metabolic hormone activity and glucose homeostasis. Similar results were found when *N*-acyl serinol is fed to mice [121]. No registered clinical trial is yet evaluating its potential translation to human pathologies.

2. Conclusion

The examples discussed here illustrate the functional versatility of metabolites that are produced or modulated by the intestinal microbiome. While the concept of adjusting the levels of these small molecules for therapeutic purposes is intuitive and congruent with decades of experience in the field of pharmacology, we believe that numerous challenges need to be overcome on the path toward using microbial metabolites for therapies.

First, as discussed here, the function of these metabolites is pleiotropic, but highly context-dependent. SCFAs can serve as energetic fuel, modulate gene expression via modification of histone residues, and function as signaling molecules by binding to receptors and triggering a downstream signaling cascade. The precise therapeutic effect is thus highly dependent on cell type, concentration, tissue context, and receptor expression patterns.

Second, depending on the effect of a particular metabolite on the molecular etiology of a disease, different strategies can be envisioned for pharmacological intervention (Table 1). In case the metabolite levels sink below physiological levels, supplementation strategies need to consider the route and frequency of administration, side effects of exceeding physiological concentrations, and inter-individual differences in pharmacokinetics. In case the metabolite contributes to disease pathophysiology, the inhibition of enzymatic steps in the production of the metabolite is promising; however, tools to study pharmacological inhibition of prokaryotic proteins are currently limited.

Finally, given the dynamic nature of the microbiome and its disease relevance [122,123], optimal concentrations of intestinal metabolites might be highly context-dependent. As such, time-resolved studies are necessary to determine the optimal timing for metabolite interventions of the course of disease development.

These difficulties notwithstanding, targeting the meta-organismal pathways involved in the generation and signaling of microbiome-associated metabolites, presents an enormous and mostly untapped opportunity to modulate disease susceptibility.

2.1. Outstanding Questions

- How can we interfere with disease-associated microbiome-derived metabolites and enhance the effect of beneficial microbial metabolites in a durable and context-dependent manner?
- How can we identify and validate microbial metabolite synthesis pathways using metagenomic sequencing information, and how

Table 1
Gut-derived metabolites, from bench to bedside.

Metabolite	Major physiological effect	Reference for basic science study	Clinical trial (condition, intervention, stage)	Reference for clinical trial
TMAO	Alterations of cholesterol, sterol metabolism and bile acid pool size and composition	[11,14]	Chronic kidney disease, Rifaximin antibiotic, phase 4	NCT03718988
	Platelet hyperreactivity and thrombosis risk	[13,18]	Heart failure, Rifaximin vs S. boulardii, phase 2	NCT02637167
			Chronic kidney disease, Sevelamer Carbonate, phase 3	NCT03596749
			Chronic Kidney Disease, AXOS vs Maltodextrine, phase 2	NCT02141815
SCFAs	Hematopoietic alterations through HDACs, gut-brain axis	[24,30,41,42]	Stress and attention, SCFA oral supplementation, N/A	NCT03688854
Butyrate	Epigenetic modulation of inflammation		Schizophrenia	NCT03010865
	Increased fatty acid oxidation	[124]	Childhood Obesity: Liver damage and insulin resistance, dietary supplement, Phase 2 and 3	NCT02721953
	Inflammation and oxidative stress	[124,125]	Minor effects on oxidative stress, enemas sodium butyrate, N/A	NCT00696098
Conjugated linoleic acid (CLA)	Fortification of epithelial barrier integrity	[48,49]	obesity, oral supplementation, N/A	PMID: 11592727
N-3 long chain fatty acid	Attenuation of inflammation	[126]	Ulcerative Colitis, oral supplement, Phase 3	PMID: 15822041
Indole-3-carbinol	Ahr mediated control of intestinal epithelial cells proliferation and differentiation	[58]	Squamous Cell Head and Neck Cancer, SCB01A, phase 2	NCT03020823
			Prostate cancer, phase 2/3	NCT00579332
			Breast Cancer, DIM supplementation, phase 3	NCT02525159
Indole	Ahr mediated mucosal immunity	[59,61]	Healthy, *L. reuteri* recolonization, N/A	NCT03501082
Indole-3-carbinol	Ahr mediated immunoregulatory effects	[127]	Systemic Lupus Erythematosus, DIM supplementation, phase 1	NCT02483624
			Obesity, Indole 3 carbinol supplementation, phase 2	NCT00988845
Indole-3-propionic acid			Friedreich's Ataxia, VP 20629, phase1	NCT01898884
Indoxyl sulfate	Uremic and vascular toxin	[67]	Chronic kidney disease, AST-120 (Kremezin®), phase 4	NCT01157260
p-cresol	Uremic toxin altering of endothelial cells function and promoting vascular calcification	[70,71]	Chronic Kidney Disease, BENEO synergy1 (inulin/oligofructose), Phase1/2	NCT00695513
			Chronic Renal Failure, Synbiotic Probinul-Neutro®, phase 4	NCT02008331
Taurine	Modulation of gut bile acid metabolism, anti-inflammatory, oxidative stress	[79,80]	Diabetes, oral supplement, N/A	NCT03410537
				NCT01226537
Imidazole propionate	Impairment of the insulin signaling	[84]	type 2 diabetes, high vs low protein diets, N/A	NCT03732690
Retinoic Acid	T-Cell development	[93]	Primary immune thrombocytopenia, ATRA supplement, phase 2	NCT01667263
UDCA	Ameliorate insulin insensitivity		Type 2 diabetes, Ursodiol, phase 2	NCT02033876
	Inhibition of *Clostridium difficile* spore germination and vegetative growth	[105]	Diarrhea, Ursodiol, phase 4	NCT02748616
DCA	Colonic crypt regeneration	[106]	Esophageal Carcinoma, Ursodiol, phase 2	NCT01097304
Flavonoid	Antioxidant and anti-inflammatory properties	[116,117]	Autism Spectrum Disorders, Luteolin, Quercetin and Rutin dietary supplement, phase 2	NCT01847521
			Metabolic Syndrome, chlorogenic acid and luteolin, N/A	NCT03444558
			Alzheimer's diseases or T2DM, polyphenolic extract, phase 1	NCT02502253
N-acyl amides	Ligand of G-protein coupled receptor	[120,121]	Type 2 Diabetes Mellitus, dietary supplementation, phase 1	NCT01453842

can the microbial versus dietary or host-derived sources for the metabolites be distinguished?
- Can we identify host sensors for theses metabolites and generate drugs targeting either microbial enzymes for metabolite production or their receptors on host tissues?
- How many clinically relevant metabolites are microbiome derived and how many of them can be therapeutically targeted?

2.2. Search strategy and selection criteria

Data for this Review were identified by searches of PubMed, Google Scholar, and clinical trial database (clinicaltrials.gov). References were used from relevant articles using the search terms “metabolite”, and “gut microbiota”/”microbiome”. Only articles published in English between 1980 and February 2019 were included. Particular emphasis was given on literature published in peer-reviewed journals in the past 5 years, reporting a mechanism of action or clinical data.

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Conflict of interest

Authors declare that they have no competing interests.

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