Gut microbiota as a target for prevention and treatment of type 2 diabetes: Mechanisms and dietary natural products

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Abstract

Type 2 diabetes mellitus (T2DM) is among the most remarkable public health concerns globally. Accumulating research evidence documents that alteration of gut microbiota has an indispensable role in the onset and progression of obesity and T2DM. A reduced microbial diversity is linked to insulin resistance and energy metabolism, especially for the rise of the Firmicutes/Bacteroidetes ratio. Changes in metabolites followed by the gut dysbacteriosis are linked to the presence of T2DM. Moreover, endotoxin leakage and gut permeability caused by gut dysbacteriosis is more of a trigger for the onset and progression of T2DM. Research documents that natural products are remarkable arsenals of bioactive agents for the discovery of anti-T2DM drugs. Many studies have elucidated that the possible mechanisms of the anti-T2DM effects of natural products are remarkably linked to its regulation on the composition of gut microflora and the successive changes in metabolites directly or indirectly. This review presents a brief overview of the gut microbiota in T2DM and several relevant mechanisms, including short-chain fatty acids, biosynthesis and metabolism of branched-chain fatty acids, trimethylamine N-oxide, bile acid signaling, endotoxin leakage, and gut permeability, and describes how dietary natural products can improve T2DM via the gut microbiota.

Key Words: Diabetes; Obesity; Gut microbiota; Mechanisms; Dietary natural products; Metabolites
INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia and insufficient insulin secretion and/or dysfunction. Epidemiological studies have implied that the number of DM patients will rise from 422 million in 2018 to 592 million in 2035[1]. The dominant risk factor for DM is becoming more prevalent over time in both developed and developing regions[2,3].

Guidelines show that type 2 DM (T2DM) accounts for nearly 95% of DM types, which include T1DM, gestational DM, and so on[3,4]. T2DM has always been the focus and key point of research on DM. The incidence of T2DM is related with diminished secretion of insulin secretion along with insulin resistance (IR) caused by individual genetics and acquired environmental factors, for instance, air pollution, unhealthy lifestyle, and poor mental state, causing multiple organ injury and several complications[5,6]. Current studies are investigating disorders of energy metabolism, endoplasmic reticulum (ER) stress, oxidative stress, inflammatory response, mitochondrial dysfunction, as well as gut microbiota[7-9].

The Human Microbiome Project has been leveraged to explore how gut microbiota influences the development of the human diseases that have started to emerge[10]. The human gut harbors trillions of microorganisms, including > 10^13 bacteria, which are mainly composed of six main phyla, i.e., Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria and Actinobacteria[11]. Numerous studies have illustrated that the gut microflora modulates diverse cellular processes, e.g., micronutrient synthesis, bowel motility, and minerals and electrolytes absorption[12], and provides signals to activate the immune response, inflammation, and oxidative stress in many metabolic diseases, for instance, nonalcoholic fatty liver disease and T2DM[13,14]. However, the type of microbes that contribute to DM and mechanisms associated are still not fully understood. Therefore, a systematic search of various electronic databases, including Google Scholar, PubMed, Sciedirect, and so on were performed with several keywords alone or in combination [diabetes, obesity, gut microbiota, short-chain fatty acids (SCFAs), branched-chain amino acids (BCAAs), inflammation, gut barrier, etc.]. Herein, we review the present studies on changes in the gut microflora and summarize the possible mechanism of the gut microbiota dysbiosis in T2DM as well as some relevant dietary natural products.

ALTERNATION OF GUT MICROBIOTA IN T2DM

It is estimated that > 80% of T2DM patients are overweight, which is recognized as the greatest risk factor for T2DM[15]. Apart from genetic and lifestyle factors, energy homeostasis disorder induced by gut microbial dysbiosis has an indispensable role in the onset and progression of T2DM. Many metagenome-wide association reports have shown remarkable correlations between variation of specific gut microbes, bacterial genes, and metabolic pathways in T2DM[16,17]. It has been verified that gut microflora is among the independent contributing factors for fat accumulation and IR, whereas germ-free (GF) mice, having no microbiota, had little weight gain and increase in body fat, and mild resistance to the diet relative to wild-type mice[18].

Core Tip: Numerous natural products possessing prebiotic effects like fruits, vegetables, and medicinal plants, have been found to ameliorate type 2 diabetes mellitus by modulating gut microbiota composition and abundance, reducing the gut permeability, and subsequently increasing the production of short-chain fatty acids and biosynthesis and metabolism of branched-chain fatty acids, decreasing the level of lipopolysaccharide, and inhibiting the inflammation.
Obese mice-derived microbiota (FMT) increased weight gain in GF mice when transplanted unlike FMT derived from thin mice[19]. Moreover, FMT was also carried out in numerous studies in humans, including obesity, ulcerative colitis, and so on [20]. Several studies have shown that there is a high Firmicutes/Bacteroidetes ratio in obese mice or mice fed Western diets[21]. Larsen et al[22] found a low number of Firmicutes and increased Betaproteobacteria in DM, relative to nondiabetic patients, which were positively related with the plasma glucose contents, especially for Betaproteobacteria. Besides Bacteroidetes and Firmicutes, Prevotella spp., Clostridium coccoides, and Eubacterium rectale were more prevalent in individuals with diabetes and positively and remarkably linked to plasma glucose, rather than body mass index, indicating that these bacteria can directly influence the level of glucose tolerance[23]. The number of Clostridiales, Streptococcus mutans, and Lactobacillus gasseri is increased whereas that of Aecalibacterium prausnitzii (both butyrate-producing bacteria) and Roseburia intestinalis is decreased in various groups of T2DM patients. These findings suggest that gut microbiota dysbiosis is strongly linked to the onset and progression of T2DM.

METABOLITES OF GUT MICROBIOТА AFFECT ENERGY METABOLISM

SCFAs
The gut microbiota acts as a real organ, which can generate monosaccharides and SCFAs by hydrolyzing and fermenting dietary polysaccharides from the host, including acetate, propionate, and butyrate. The mechanism involving the production of adipose-tissue-derived satiety hormone leptin under the action of SCFAs is the most studied[24]. Nevertheless, recent studies have illustrated that SCFAs act against obesity by preventing fat accumulation. The production of SCFAs is different in obese and insulin-resistant subjects via regulation of metabolism in several organs and tissues, for instance, adipose tissue, the pancreas, and the brain[25,26]. The increased level of butyrate is linked to improved insulin sensitivity[27,28], highlighting the significance of butyrate-secreting bacteria in modulation of glycemia. By further analyzing stool samples from patients, Psichas et al[29] illustrated that high rates of colonic fermentation were closely related with high SCFA products in obese individuals. Therefore, the effect of SCFAs on energy metabolism is multidimensional, including involving lipid oxidation, appetite modulation, as well as glucose metabolism (Figure 1)[24].

Several studies have shown that SCFAs trigger the secretion of Peptide YY (PYY) along with glucagon-like peptide 1 (GLP-1), by recognizing and stimulating G-protein-coupled receptor (GPR)41/43 in rodent and human cell lines[30-32]. Similarly, the same effect and mechanisms have been documented and confirmed in vivo[33]. Moreover, numerous studies have demonstrated that exogenous GLP-1 and PYY acutely reduce food intake in humans, which has been widely used in the treatment of T2DM[34-36]. Therefore, SCFAs not only contribute 5%-10% of energy to the host, but are also recognized by endogenous ligands of GPR41/43, and act as signaling molecules to participate in adjustment of energy[39-41]. Kimura documented that GPR43-modulated adipose-insulin cascades and sympathetic activity were controlled by GPR41, which detected SCFAs released from gut microbiota[42]. Nevertheless, in GPR41/FFAR3−/− and GPR43/FFAR2−/− knockout cells, GLP-1 secretion activated by SCFAs was remarkably attenuated. However, unlike GPR41/ FFAR3−/− mice, GPR43/FFAR2−/− showed markedly downregulated GLP-1 in circulation, indicating that GPR43/FFAR2 plays a more important role in these effects. Confusingly, the level of satiety hormones at 24 wk following supplementation of inulin propionate ester at 10 g/d was not different from those in groups treated with 10 g/d inulin alone in overweight adults[43], emphasizing mechanisms of crosstalk between the gut microflora and host. Therefore, SCFAs, as metabolites of the gut microflora, are important signaling molecules in the regulation of host energy metabolism. Relevant research on the metabolic influences of SCFA delivery or production on the host is urgently needed, especially the influences on satiety-inducing hormones.

Besides, in rodent receiving acute and chronic oral supplementation of SCFAs, besides affecting the production of satiety hormones, SCFAs could also advantageously impact body weight via impacting energy expenditure[44]. Another study documented that a single oral administration of 1.5% AcOH with a stomach tube, in comparison to distilled water, elevated energy expenditure along with lipid oxidation [45]. Similar to the above results, injection with acetate (5.2 mg/kg) elevated whole-body oxygen consumption and decreased the body weight at 6 mo post-treatment in
Figure 1  Mechanisms of action of short-chain fatty acids in type 2 diabetes mellitus. The effect of short-chain fatty acids on energy metabolism is multidimensional, including appetite regulation, energy intake/expenditure, and lipid oxidation, as well as glucose metabolism. SCFA: Short-chain fatty acid; GLP-1: Glucagon-like peptide 1; PYY: Peptide YY; ER: Endoplasmic reticulum; UCP-1: Uncoupling protein-1; mTOR: Mammalian target of rapamycin.

rats[46]. Moreover, butyrate (5% w/w) prevented high-fat diet (HFD)-induced obesity via enhanced lipid oxidation[47]. Studies on the mechanism showed that these effects were linked to the UCP-1 (uncoupling protein-1) and elevation of peroxisome proliferator-activated receptor-(PPAR)-γ co-activator 1α (PPARGC1A, coding for PGC1α) in brown adipose tissue[47]. The animal experimental data proved that SCFAs upregulate genes that modulate lipid oxidation and thermogenesis, thereby eliminating adiposity and weight gain[24]. Human studies have indicated that colonic infusion of SCFA mixtures, including acetate, butyrate, and propionate, reduces lipolysis and elevates energy expenditure and PYY, as well as fat oxidation in overweight/obese individuals[48]. In addition, acute oral sodium propionate ingestion elevated resting expenditure of energy along with lipid oxidation in 18 healthy volunteers in contrast with a sodium chloride control, and these effects were independent of insulin and glucose contents and sympathetic nervous system activity[49]. The result from a randomized double-blind crossover trial proved that sodium acetate infusion into the distal colon (180 mmol/L) enhanced lipid oxidation relative to sodium chloride placebo, and the resting energy expenditure between overnight-fasted overweight and obese individuals was similar[44]. Jocken et al[49] showed that SCFAs reduced lipolysis and promoted lipid oxidation in white adipose tissue (WAT), and that antilipolytic effect was orchestrated by FFAR3 and/or FFAR2 levels in WAT[50]. Therefore, these results strongly suggest that SCFAs are beneficial for weight control and influence energy expenditure. Research on the mechanism of action of SCFAs in DM has suggested that SCFAs induce GLP-1 and amylin secretion via FFA2 receptor, hence modulating glucose metabolism and insulin levels[51]. The Akt/mTOR pathway, ER stress, and release of intracellular Ca²⁺ play important roles in this process[51]. There is need to understand the pathways and regulators involved in a variety of cell models, for instance, human-derived adipocytes, hepatocytes, or skeletal muscle.

BCAAs

BCAAs have increasingly been studied as playing a role in diabetes[52]. BCAAs constitute nearly approximately 20% of the amino acids used to form proteins[53]. More studies have documented that plasma content elevations of BCAAs have been linked to obesity, as well as diabetes[54]. Previously, this phenomenon was thought as a consequence and not a cause of IR[55]. Nevertheless, recently, growing research
evidence opines that BCAAs elevations contribute to IR: (1) Exogenous BCAAs remarkably diminish the sensitivity to insulin, as illustrated via hyperinsulinemic euglycemic clamps measurements[36,57]; and (2) coadministration of BCAAs with HFD generally worsens the ensuing IR in rodents[58]. The presence of HFD or lipids in all these rodents played a critical role in this effect, and BCAAs alone elicited insignificant or no impact, illustrating that BCAAs crosstalk with fatty acids (FAs) to promote IR[59]. Further studies have proved that FA oxidation disorders elevated BCAAs contents in plasma, and aggregation of the intermediate metabolites of BCAAs, for instance, C3 and C5 acylcarnitines along with acetyl-CoA can inhibit complete FA oxidation[60]. The crosstalk of BCAAs with FAs induces energy metabolism disorder, including ATP production, TCA (tricarboxylic acid cycle) cycle, as well as oxidative phosphorylation, and leads to mitochondrial dysfunction and inflammation, which are critical for the progression of DM[61-63]. Inflammatory factor signaling pathways, including the nuclear factor (NF)-κB pathway and mammalian target of rapamycin complex 1 (mTORC1), might be candidate therapeutic targets in this process[34,64]. Moreover, BCAAs supplementation could repress stimulation of Akt2 via the mTORC1- and mTORC2-dependent cascades and enhanced degradation that is dependent on Akt2 ubiquitin-proteasomes via mTORC2 signaling, indicating that mTORC2 might also play an important role in this process[65]. More importantly, by using metagenomics and metabolomics methods, increased concentrations of BCAAs were linked to a gut microbiome that has an abundant biosynthetic ability for BCAAs. Prevotella copri coupled with Bacteroides vulgatus are recognized as the primary species modulating the relationship of biosynthesis of BCAAs with IR[66], indicating that gut microbes affect host serum metabolome along with insulin sensitivity via regulating the level of BCAAs (Figure 2).

**Trimethylamine N-oxide**

Terrestrial mammal trimethylamine N-oxide (TMAO) is derived from exogenous arsenals, with the TMA serving as the precursor, which is a metabolite of diverse other precursors, primarily choline, as well as carnitine originating from ingested foods[67]. Bacteria metabolize choline and L-carnitine into TMA, and flavin-containing monooxygenase (FMO)-3, a hepatic enzyme, oxidizes TMA into TMAO. There are two key steps for the generation of TMAO, illustrating that the gut microflora is an independent risk factor for DM[68,69]. Further analysis of the microbiota has shown that the primary bacterial phylum that degrades carnitine to TMA is Proteobacteria along with bacteria of the family Prevotellaceae, phylum Bacteroidetes. In contrast, the S24-7 family of Bacteroidetes (the family majorly involved in plant polysaccharides) is related with diminished TMAO contents[70-72].

The initial findings suggested a positive relationship of high plasma TMAO content with an elevated risk for major severe cardiovascular diseases including myocardial infarction, stroke, and atherosclerosis. More studies have documented that TMAO is opined to serve as a biomarker, as well as an independent predisposing factor for many diseases, for example, kidney failure, DM, and cancer[69]. Some research evidence opines that TMAO influences glucose metabolism, and remarkably higher median TMAO contents in plasma occur in individuals with diabetes in contrast with persons without DM[73,74]. A prospective mechanism connecting TMAO with IR is TMAO-dependent elevated concentrations of N-nitroso compounds and upregulated activity of FMO-3[75,76]. Research documents reduced TMAO and choline contents in the hepatic tissues of diabetic mice[77]. Metformin has been shown to decrease glucose and increase plasma TMAO[77]. TMAO measurements have low diagnostic significance in diabetic individuals who are obese due to the high variability of TMAO contents in plasma[78]. More importantly, treatment with TMAO promotes normal protein folding, counteracting ER stress in diabetic rats, illustrating a potentially beneficial impact of TMAO in DM[79]. Therefore, from the results so far, the characteristics of TMAO in DM are still controversial.

**GUT PERMEABILITY GIVES A NOVEL INSIGHT INTO T2DM**

Although chronic low inflammation induced by metabolic endotoxemia in serum is a risk factor for T2DM, gut permeability is more of a trigger for the onset and progression of T2DM. The intestinal mucosal lining functions as a barrier, preventing viruses, toxins, and pathogenic bacteria invading from the gut epithelium into the circulation[80]. Recent reports have chronicled that altered bowel function of the gut barrier is involved in DM pathogenesis[61]. Disruption of the gut barrier is docu-
Figure 2  Increased plasma levels of branched-chain amino acids induced by dysbiosis are closely associated with obesity and diabetes. The accumulation of branched-chain amino acids inhibits complete oxidation of fatty acids, induces energy metabolism disorder, including ATP production, tricarboxylic acid cycle, and oxidative phosphorylation, thereby causing energy metabolism disorder, and induces inflammation by targeting nuclear factor-κB and mammalian target of rapamycin complex 1. TCA: Tricarboxylic acid; NF-κB: Nuclear factor-κB; mTORC1: Mammalian target of rapamycin complex 1; BCAAs: Branched-chain amino acids.
dramatically reduced, and then the fasting glycemia, inflammation, and body weight are also improved[84]. Growing in vitro along with in vitro research evidence suggests that Toll-like receptors (TLRs) are responsible for LPS-induced inflammatory responses[90]. TLR4 recognizes bacterial LPS, thereby triggering the expression of proinflammatory cytokines along with chemokines, including tumor necrosis factor (TNF)-α[91], TNF-α is strongly linked to IR, promoting the onset and progression of DM[92]. Besides animal experimentation, clinical studies have also shown that TLR4 is a pivotal receptor of the natural immune system, with a core role of triggering the inflammatory response, including TNF-α, interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, and IL-1β[93]. Genetic variants in the TLR4 gene or IRAK1 and TIRAP genes might have an indispensable role in the onset and progression of IR and T2DM through disruption of the inflammatory reaction[92]. TLR5, as another member of the TLR family, which is mainly expressed in intestinal mucosa, has an indispensable role in the onset and progression of metabolic syndromes[94]. TLR5-deficient mice exhibit hyperphagia and develop metabolic diseases, for instance, hyperlipidemia, hypertension, IR, and obesity[95]. TLR5 knockout mice exhibited an increase in body mass and epididymal fat pad size in varying degrees compared to their wild-type counterparts, which was linked to increased contents of serum triglycerides and cholesterol, as well as increases in the proinflammatory proteins interferon-γ and IL-1β in adipose tissue[96]. Moreover, by transplanting the gut microflora from TLR5-deficient mice to their wild-type GF counterparts, the increased contents of proinflammatory cytokines and features of metabolic diseases, for instance, IR and obesity, have been documented[96]. The elevated inflammatory mediators in DM cause oxidative and ER stress in pancreatic islet β cells, thereby influencing insulin sensitivity and glucose homeostasis, aggravating DM[97]. TLR2 can identify components of bacterial cell walls and lipid-containing molecules, thereby transducing inflammatory signaling by activating NF-κB and producing proinflammatory cytokines in cells[98]. More importantly, unlike TLR5-deficient mice, TLR2-deficient mice exhibit increased insulin sensitivity and faster clearance of glucose, accompanied by attenuated expression of inflammatory cytokines[99,100]. Therefore, TLRs have multiple effects on the expression of inflammatory cytokines in T2DM. The distribution of TLRs in tissues and organs may decide their role in the onset and progression of T2DM.

INTERACTIONS BETWEEN BILE ACIDS AND GUT MICROBIOTA

As the end-product of cholesterol metabolism, bile acids (BAs) are derived from cholesterol catabolism in the liver and are essential for the solubilization, absorption, and metabolism of lipid- and fat-soluble vitamins[101], as well as xenobiotics, including drugs and environmental contaminants[102]. However, BAs are now identified as key endogenous steroids that play critical roles in regulating and maintaining lipid, glucose, and energy metabolism, protecting against inflammation in the liver, intestine, and heart, and preventing DM and obesity (Figure 3)[103]. Clinical data prove that dysregulation of BA homeostasis and dysbiosis can induce metabolic disorder, for instance, disorders of lipid, glucose, and energy metabolism, as well as inflammatory cytokine generation, which are closely linked with T2DM[102]. BA synthesis occurs via two pathways. The rate-limiting enzyme cytochrome P450 cholesterol 7-ahydroxylase (CYP7A1) produces the majority of the BA pool, and is responsible for the classical pathway. There is also an alternative pathway (3%-18% of total BA synthesis in healthy humans), which is initiated by cytochrome P450 27-ahydroxylase (CYP27A1)[104]. All of the products of BAs are the primary BAs including cholic acid (CA) and chenodesoxycholic acid (CDCA), which are reabsorbed through the enterohepatic circulation. Around 95% of primary BAs are actively reabsorbed through the apical sodium-dependent BA transporter (ASBT/SLC10A2) and secreted at the basolateral membrane by the heterodimeric organic solute transporters α and β[104]. Only 5% of primary BAs reach the large intestine and are converted into secondary BAs by the gut microbiota, for instance, deoxycholic acid (DCA), lithocholic acid (LCA), and ursodesoxycholic acid (UDCA) in humans, and DCA, LCA, muricholic acid (MCA), hyodeoxycholic acid, and murideoxycholic acid in mice[105]. Further research has proved that these gut bacteria have bile salt hydrolase, including Lactobacillus, Bifidobacterium, Firmicutes, Enterococcus, Clostridium, and Bacteroides, which transform primary BAs into secondary BAs at millimolar concentrations in the intestine, and determine BA composition in the circulating pool and total BA pool size[104,106]. Thus, interactions between BAs and gut bacteria remarkably affect the
Bile acids were synthesized by cytochrome P450 (CYP) 7A1/CYP27A1. Gut microbiota regulates the BA pool size and composition, thereby participating in energy metabolism, glucose homeostasis, lipid metabolism, and inflammation of the host by activating farnesoid X receptor/TGR5 in various tissues. BA: Bile acid; CYP: Cytochrome P450; FXR: Farnesoid X receptor; GPBAR1: G-protein-coupled bile acid receptor 1; CA: Cholic acid; CDCA: Chenodexycholic acid; GLP-1: Glucagon-like peptide 1; DCA: Deoxycholic acid; LCA: Lithocholic acid; MCA: Muricholic acid.

Figure 3  The critical role of dysbiosis/bile acids/farnesoid X receptor/TGR5 axis in diabetes mellitus. Bile acids were synthesized by cytochrome P450 (CYP) 7A1/CYP27A1. Gut microbiota regulates the BA pool size and composition, thereby participating in energy metabolism, glucose homeostasis, lipid metabolism, and inflammation of the host by activating farnesoid X receptor/TGR5 in various tissues. BA: Bile acid; CYP: Cytochrome P450; FXR: Farnesoid X receptor; GPBAR1: G-protein-coupled bile acid receptor 1; CA: Cholic acid; CDCA: Chenodexycholic acid; GLP-1: Glucagon-like peptide 1; DCA: Deoxycholic acid; LCA: Lithocholic acid; MCA: Muricholic acid.

health of the host and contribute to the pathogenesis of metabolic diseases, for instance, liver disease, obesity, and DM. However, it is still unclear how BA pool alterations affect DM.

Farnesoid X receptor (FXR) and G-protein-coupled BA receptor 1 (GPBAR1, also known as TGR5) play critical roles in gut microbiota-mediated BA signaling. FXR and TGR5 are expressed in various tissues, including the liver, intestine, kidneys, adrenal glands, brown/white adipose tissue, and immune cells[105]. FXR also competes for other nuclear receptors, for instance, PPAR and NF-κB, thereby regulating lipid and glucose metabolism and the inflammatory response in (patho)physiological conditions in humans[104,107]. By raising FXR-deficient mice in GF conditions, Gonzalez et al [108] confirmed that the gut microflora regulates FXR signaling by acting on the conversion of primary BAs into secondary BAs, and by regulating their synthesis. BAs are natural ligands of FXR and TGR5, including agonists (CDCA, DCA, CA and LCA) and antagonists (MCA and possibly UDCA). Selectivity reduces the gut microflora production of β-MCA induced by dysbiosis in obese mice, a rodent-specific FXR antagonist, which reduces BA feedback regulation and increases BA synthesis in the liver by alleviating FXR repression in the ileum. This emphasizes that changes in the BA pool size and composition caused by dysbiosis are closely linked to obesity and DM[108,109]. Some studies have illustrated that: (1) In the intestine, FXR reduces postprandial glucose absorption, which is delayed in FXR-deficient mice; and (2) BAs regulate the production and secretion of GLP-1 via the activation of TGR5 and FXR in enteroendocrine L cells. BAs also control lipoprotein metabolism via hepatic FXR
activation. FXR reduces lipogenesis by repressing hepatic sterol responsive element binding protein (SREBP)-1c expression in SHP-dependent and FGF15/19-dependent manners[110]. FXR represses microsomal triglyceride transfer protein and apolipoprotein B gene expression, thereby reducing very-low-density lipoprotein secretion [111]. FXR and TGR5 are expressed in several immune cell types, including monocytes, macrophages, and Kupffer cells, and human dendritic cells also have a critical role in the onset and progression of DM and related complications. For example, TGR5 activation reduces HFD-induced glucose intolerance, IR, and inflammation by inhibiting NLRP3 inflammasome activation via the TGR5-cyclic AMP-protein kinase A axis in mice[112]. These results indicate that the gut microflora regulates the BA pool size and composition, thereby participating in energy metabolism, glucose homeostasis, lipid metabolism, and inflammation of the host. The dysbiosis/BAs/FXR/TGR5 axis might play an important role in this process.

DIETARY NATURAL PRODUCTS AND GUT MICROBIOTA

Various synthetic drugs with antidiabetic effects are in current clinical use. However, the application of these drugs is usually limited by their various undesirable adverse effects, including weight gain, hypoglycemia, fluid retention, heart failure, urinary tract infection, and dyspepsia[2]. In contrast, numerous studies have indicated that herbal medicines and their active ingredients possess antidiabetic properties with few adverse effects, and are worthy of investigation for clinical application. An increasing number of studies have illustrated that the extracts of fruits, vegetables, herbs, and other plant foods alleviate T2DM by modulating the gut microbiota (Table 1)[113].

Dietary fibers

Dietary fibers are compounds of natural origin present in plants. Chemically, these compounds are defined as nondigestible carbohydrates (with ≥ 3 monomeric units), for instance, polysaccharides and oligosaccharides[114]. Some prospective cohort studies have shown that individuals with high intake of dietary fiber are inversely linked to the risk of DM compared with low intake[115]. Although most dietary fibers belong to prebiotics, which are not digested and absorbed by the human gut and remain intact while passing through the gastrointestinal tract, they selectively arouse the growth and activity of potential beneficial bacteria in the gut. The human gut microbiota encodes several types of carbohydrate-active enzymes, including glycoside hydrolases, polysaccharide lyases, glycosyltransferases, and carbohydrate esterases, which are capable of degrading dietary fiber and then generating small-molecular-weight metabolites (degradation products), which may display antidiabetic effects in T2DM[116]. Studies on anti-T2DM activity of fibers have shown that dietary fibers increase the abundance of some species, for instance, Eubacterium rectale, Roseburia, Prevotella, Ruminococcus bromii, Bacteroides, and Bifidobacterium, and decrease the number of some Gram-negative bacteria, for instance, Desulfovibrio and Enterobacteriaceae (LPS-producing bacteria)[117]. More importantly, these changes induced by dietary fiber intervention enhance the production of SCFAs, which can bind to the GPR and enhance the level of the enteroendocrine hormones PYY and GLP-1 in gut epithelial L-cells, thereby improving IR, appetite regulation, and energy intake/expenditure, as well as lipid oxidation[118]. The alleviating effect of pumpkin polysaccharide in HFD-fed mice is linked with increased SCFA production and selective enhancement of some bacteria, for instance, Bacteroidetes, Prevotella, and Deltaproteobacteria[119]. Administration of insulin can reduce the fasting blood glucose level, increase GLP-1 level, and alleviate glucose intolerance as well as blood lipid contents in rats with T2DM induced by HFD and streptozotocin[120]. SCFA-producing bacteria have a key role in the process, including Lachnospiraceae, Phascolarctobacterium, and Bacteroides[120]. A double-blind, randomized, controlled clinical trial of 60 patients with T2DM found that supplementation with 10 g/d inulin powder promoted gut health by increasing the proportion of Akkermansia muciniphila[121].

Polyphenols

Dietary polyphenols are natural compounds that occur in many plant foods, such as fruits and vegetables. These compounds constitute a large heterogeneous collection of compounds, but with structural units common to all phenolic compounds (hydroxylated aromatic rings or phenol rings)[122]. Numerous studies have proved that the beneficial effects of dietary polyphenols may reduce the risk of T2DM and/or its complications[123]. However, it is proved that most polyphenols are not digested and

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absorbed by the small intestine, but remain in the colon, and are metabolized by gut microflora including demethylation, dihydroxylation, and decarboxylation[124]. Focusing on the changes of the intestinal microbiota, polyphenols improve intestinal health by promoting the growth of beneficial bacteria and inhibiting the pathogenic bacteria[117]. More importantly, the main antidiabetic actions of dietary polyphenols include: Protection of pancreatic β-cells against stimuli-induced oxidative stress; inhibition of the activities of various enzymes (for instance, α-amylases, α-glucosidases, and pancreatic lipase); promotion of β-cell proliferation and survival; and repression of advanced glycation end products formation[117]. An ethanolic extract of Lessonia nigrescens (rich in phenolics and flavonoids) displays its hypoglycemic effect by increasing the abundance of Bacteroidetes and decreasing Firmicutes in the intestines[118]. A recent study illustrated that the antidiabetic effects of polyphenols were also linked to changes in the markers of gut barrier function, for instance, ZO-1 and occludin[125]. Grape pomace extract (mixture of polyphenols consisting of anthocyanins, flavanols, and flavanol glycosides) improves fat mass gain, adipose tissue inflammation, impaired glucose tolerance, and IR by reducing the concentrations of Clostridium sensu stricto, Lactococcus, Desulfovibrioaceae, and Streptococcae, and increasing the abundance of Allobaculum, Prevotella, Roseburia, and Erysipelotrichaceae, and improving gut barrier function[126]. Besides these extracting mixtures, the effects of several polyphenol monomeric compounds in T2DM/obesity are also closely linked to the alternation of gut microflora. Resveratrol attenuates HFD-induced nonalcoholic steatohepatitis and ameliorates the intestinal barrier dysfunction and inflammation in rats[127]. Quercetin reverted gut microflora imbalance and related endotoxemia-mediated TLR4 pathway induction, with subsequent repression of inflammasome response and reticulum stress pathway activation, leading to the blockage of lipid metabolism gene expression deregulation in obese mice[63].

**Alkaloids**

Alkaloids have antimalarial, antihyperglycemic, antiasthma, anticancer, and antibacterial activities[128]. Many of them have been utilized in traditional or modern medicines for drug discovery. Recent studies have illustrated that the pharmacological activity of alkaloids is mainly mediated by the gut microflora[129]. Because most alkaloids usually exhibit low oral bioavailability, their absorption into the bloodstream is difficult. The gut microbiota has a variety of enzymes, consisting of β-glucuronidase,

### Table 1 Main preclinical and human data reporting the effects of dietary natural products on gut microbiota and associated mechanisms in diabetes mellitus and related complications

| Name                  | Model                                      | Key findings                                                                 | Ref.               |
|-----------------------|--------------------------------------------|------------------------------------------------------------------------------|--------------------|
| Pumpkin polysaccharide| HFD (mice)                                 | Increases SCFAs production; selectively enhances the abundance of *Bilophila* and *Prevotella* | Liu et al[119]     |
| Inulin                | T2DM in rats induced by HFD and streptozotocin and clinic trial | Increases SCFA-producing bacteria including *Lachnospiraceae*, *Planococcus*, *Bacteroidetes*, and *Akermania* | Li et al[112] and Food and Drug Administration, HHS[113] |
| Lessonia nigrescens ethanolic extract | T2DM mice (streptozotocin injection) | Increases the ratio of *Bacteroidetes*/Firmicutes in the intestine | El Kaoutari et al[115] |
| Grape pomace extract  | HFD (mice)                                 | Reduces the abundance of *Desulfovibrio* and *Lactococcus*, and increases the abundance of *Allobaculum* and *Roseburia*; improves the gut barrier function | Bowey et al[123] |
| Resveratrol           | NASH rat model                             | Reverts gut microbiota imbalance and related endotoxemia-mediated TLR4 pathway induction | Li et al[124] |
| Quercetin             | HFD (mice)                                 | Modulates the ratio of *Firmicutes*/Bacteroidetes; increases SCFA content in feces; regulates BCAAs biosynthesis and catabolism in liver and adipose tissue; reduces the increased expressions of inflammatory mediators and alleviates gut permeability by decreasing LPS level in plasma; modulates the bile acid cycle and subsequently the ileal FXR signaling pathway | Solon-Biet et al[61] |
| Berberine             | db/db mice High fat diet (mice and rats) FXR knockout (FXR+/−) mice | Increases the ratio of *Firmicutes* to *Bacteroidetes* and the number of *Roseburia*; decreases the levels of proinflammatory cytokines, including TNF-α and IL-6 | Tesar and Kottke[129], He et al[130], Song et al[131], Wang et al[132], and Zhang et al[133] |
| Capsaicin             | ob/ob mice                                 | Modulates the ratio of *Firmicutes*/Bacteroidetes and related endotoxemia-mediated TLR4 pathway induction | Christodoulou et al[128] |

HFD: High-fat diet; SCFAs: Short-chain fatty acids; T2DM: Type 2 diabetes mellitus; NASH: Nonalcoholic steatohepatitis; BCAAs: Branched-chain amino acids; FXR: Farnesoid X receptor; LPS: Lipopolysaccharide; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin 6.
Figure 4 Possible mechanisms of gut microbiota in type 2 diabetes mellitus. A variety of independent mechanisms that influence the development of diabetes mellitus via the gut microbiota are summarized. Short-chain fatty acids, branched-chain amino acids, endotoxin leakage, bile acid signaling, and gut permeability might be considered to participate in the process of type 2 diabetes mellitus. SCFAs: Short-chain fatty acids; BCAAs: Branched-chain amino acids; LPS: Lipopolysaccharide.

β-glucosidase, β-galactase, nitroreductase, azoreductase, 7α-hydroxylase, and protease, and various carbohydrates, which can metabolize alkaloids into many different metabolites that are closely linked to DM[130]. Capsaicin improves glucose homeostasis and insulin tolerance in obese diabetic ob/ob mice by increasing the ratio of Firmicutes to Bacteroidetes and the number of Roseburia, which could decrease the contents of proinflammatory cytokines, for instance, TNF-α and IL-6[131]. Among the alkaloids, the most widely studied is berberine. As an isoquinoline alkaloid, berberine occurs in various medicinal plants, including Coptis chinensis Franch and Phellodendron chinense Schneid. Numerous experimental models have proved that the antiobesity and anti-hyperlipidemic effects of berberine are closely related to changes in the gut microbiome[128]. Researchers have shown that the blood level of BBR in hyperlipidemic patients was higher than that in healthy individuals owing to the differential microbiota composition[132]. Further studies have shown that the effects of berberine on DM are multidimensional: (1) Modulation of the ratio of Firmicutes to Bacteroidetes, thereby increasing SCFA content in feces[133]; (2) Regulation of BCAA biosynthesis and catabolism in liver and adipose tissue[134]; (3) Reduction of the increased expression of inflammatory mediators by decreasing LPS level in plasma and alleviation of gut permeability[135]; and (4) Modulation of the BA cycle and subsequently the ileal FXR signaling pathway[136].

In summary, numerous natural products, for instance, fruits, vegetables, and medicinal plants, possess prebiotic effects and have been illustrated to ameliorate T2DM by modulating gut microflora composition and abundance, reducing gut permeability, and subsequently increasing production of SCFAs and BCAAs, decreasing the level of LPS, and inhibiting inflammation. Current studies mainly focus on modulating the action of natural products and their bioactive components on the gut microbiota for preventing and managing T2DM. However, because the composition of natural products is so complex that the gut microflora may also influence host metabolism of natural products, further studies should focus on the metabolism of natural products and their bioactive components by the gut microbiota. This is important for the pharmacokinetic parameters, enhancing drug efficacy, and finding a novel lead compound via gut microflora-related mechanisms.

CONCLUSION

Trillions of microorganisms colonize the human gut, which are collectively termed the microbiome and provide us with genetic and metabolic attributes pertinent to the maintenance of our body homeostasis. Animal and epidemiological studies have
demonstrated significant differences in the intestinal microbiota composition and abundance between diabetic and nondiabetic individuals. Moreover, by analyzing the metabolic product of the gut microbiota and their effects on host metabolism, SCFAs, BCAAs, endotoxin leakage, BA signaling, and gut permeability might be remarkably linked to initiation and aggravation of T2DM (Figure 4). The effects of some natural products on T2DM are also related to the regulation of the gut microflora and subsequent changes in metabolites. However, the available data in this field remain limited, for instance, most are small-sample clinical studies or rodent model studies. We conclude that the gut microbiota influences the onset and progression of diabetes through a variety of independent mechanisms. Moreover, by using isolating and culturing techniques, and the combination of multiomics, some new molecular markers of metabolites and mechanisms will be identified, which are related to the interaction of metabolites of the gut microflora and the host. This may provide a new insight into the role of the gut microflora and help us to make more accurate predictions for the future treatment of T2DM.

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