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Review

Pharmacologic and Nonpharmacologic Therapies for the Gut Microbiota in Type 2 Diabetes

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Key Messages

- Impaired glucose homeostasis is associated with a shift in gut microbiota composition and decreased capacity for butyrate production.
- Gut microbiota metabolites, such as short-chain fatty acids, amino acids and bile acids, are involved in the regulation of host metabolism.
- Strategies to reverse gut microbiota dysbiosis, including fecal transplantation, prebiotics, probiotics and drugs, are being investigated.

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ABSTRACT

The gut microbiota is an important regulator of host metabolism. Metagenome analyses have demonstrated that the gut microbiota differs between patients with type 2 diabetes and healthy subjects, and several studies have shown that impaired glucose metabolism is associated with decreased levels of butyrate-producing bacteria. Gut microbiota-produced metabolites, such as short-chain fatty acids, amino acid derivatives and secondary bile acids, participate in metabolic and immunologic processes and, hence, pose putative links between the gut microbiota and glucose homeostasis. Strategies to prevent and treat type 2 diabetes through manipulation of the gut microbiota are being developed. These include replacement of the gut microbiota by fecal transplantation, consumption of fibres to promote the function and growth of beneficial bacteria and treatment with probiotic bacterial strains. Furthermore, it has been shown that many drugs, including drugs used for treatment of diabetes, have major impacts on gut microbiota and, thereby, potentially on glucose metabolism. In particular, the commonly used drug metformin has been shown to influence the functional capacity of the gut microbiota, and recent evidence indicates that this may contribute to the antidiabetes effect of metformin.

Mots clés:
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acides gras à chaîne courte

RÉSUMÉ

Le microbiote intestinal est un régulateur important du métabolisme de l'hôte. Les analyses du métagénome ont démontré que le microbiote intestinal diffère entre les patients atteints du diabète de type 2 et les sujets sains, et plusieurs études ont démontré que la perturbation du métabolisme glucidique est associée à la diminution des concentrations de bactéries productrices de butyrate. Les métabolites produits par le microbiote intestinal, comme les acides gras à chaîne courte, les dérivés des acides aminés et les acides biliaires secondaires, participent aux processus métaboliques et immunologiques et, par conséquent, créent des liens présumés entre le microbiote intestinal et l'homéostasie des glucides. Des stratégies pour prévenir et traiter le diabète de type 2 par manipulation du microbiote intestinal sont en cours d'élaboration. On pense ici notamment au remplacement du microbiote par la transplantation fécale, à la consommation de fibres pour favoriser le fonctionnement et la croissance de bactéries bénéfiques, et au traitement par souches bactériennes probiotiques. De plus, il a été démontré que plusieurs médicaments, y compris les médicaments utilisés dans le traitement du diabète, ont des

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répercussions majeures sur le microbiote intestinal et, de ce fait, des répercussions possibles sur le métabolisme des glucides. Notamment, il a été démontré que le médicament le plus fréquemment utilisé, la metformine, influence la capacité fonctionnelle du microbiote intestinal qui, selon de récentes données probantes, peut contribuer à l'effet antidiabétique de la metformine.

Introduction

The human gut harbors a complex ecosystem containing trillions of bacteria, collectively called the gut microbiota. In total, more than 1,000 microbial genomes have been identified, and they encode at least 150 times more genes than the human genome (1). This genetic richness enables the capacity for an extensive range of metabolic processes that cannot be performed by the host. Over the past decades, our knowledge about the gut microbiota and its impact on host physiology during health and disease has increased tremendously. This progress has, to a large extent, been driven by the development of high-throughput sequencing techniques, allowing the study of taxonomy and the functional capacity of complex ecosystems such as the gut microbiota.

The gut bacteria are exposed to nutritional, chemical and immunologic gradients along the gastrointestinal tract. In the small intestine, the levels of oxygen, bile acids and antimicrobial factors are high, giving rise to a sparse community of species able to cope with these conditions. In contrast, low oxygen levels in the colon facilitate a dense and diverse community of anaerobic bacteria with the ability to ferment complex carbohydrates (2). Most gut bacteria belong to the phyla Bacteroidetes and Firmicutes, but species from Actinobacteria, Proteobacteria and Verrucomicrobia are usually represented also (3). Proteobacteria and the Firmicutes genus *Lactobacillus* are enriched in the small intestine, while genera, such as *Ruminococcus*, *Bacteroides*, *Prevotella* and *Akkermansia species*, are enriched in the colon (4).

The gut microbiota transforms dietary components as well as host-derived molecules into a broad range of metabolites that may affect host physiology in many different ways (5,6). The main energy source for the gut microbiota is dietary carbohydrates. Fermentation of indigestible carbohydrates by colonic bacteria gives rise to short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate (5). SCFAs constitute an energy source for both the host and the gut bacteria. They also serve as signaling molecules involved in the regulation of host metabolism and energy balance. In addition to carbohydrates, the gut microbiota ferments amino acids derived from dietary proteins. Intermediate metabolites produced from amino acids may also function as signaling molecules affecting host physiology and health (7). Examples of host-derived factors metabolized by the gut microbiota include bile acids and components of the mucus layer.

The gut microbiota is important for the regulation of immune homeostasis and participates in the development of both innate and adaptive immunity. It is vital for the regulation and differentiation of several types of immune cells, including antigen-presenting cells and T cells (8). The immune system affects metabolic homeostasis, so the interaction between the gut microbiota and the immune system may be involved in the development of metabolic disease (9). The gut microbiota also contributes to the maintenance of gut barrier function by supporting structural development of the gut mucus layer, desmosomes and tight junctions (10). Gut-barrier function is essential to controlling transfer of bacteria-derived factors into the body. In the context of metabolic disease, decreased gut integrity may result in increased levels of lipopolysaccharide (LPS) in the blood (endotoxemia), which induce low-grade inflammation in metabolic tissues and thereby exacerbate the development of insulin resistance.

The composition of the gut microbiota differs greatly at the species level among individuals. Despite this baseline interindividual variation, the metabolic function of the gut microbiota is relatively similar in healthy individuals (11). However, several chronic diseases are associated with perturbed gut microbiota function. These alterations may be a consequence of the disease but could also be linked to disease etiology and pathophysiology. Imbalance in the gut microbiota with negative health consequences for the host is referred to as *dysbiosis*. Dysbiosis is generally thought to be caused by unfavorable environmental factors, such as an unhealthy diet, antibiotic treatment or chronic infection. Conversely, changes in environmental factors may be able to reverse dysbiosis and, ultimately, the associated disease, making the gut microbiota a potential therapeutic target.

The Gut Microbiota in Patients With Type 2 Diabetes

Type 2 diabetes is characterized by increased hepatic glucose production, insulin insensitivity and, ultimately, insufficient insulin secretion. The disease pathogenesis is heterogeneous, involving dysregulation of glucose metabolism and systemic as well as local inflammation. Dysbiosis has recently emerged as a factor potentially involved in the development of type 2 diabetes.

A number of studies have analyzed the gut microbiota composition in patients with type 2 diabetes. In a small 2010 study, significant differences at the phylum level were observed between patients and control subjects, indicating an association between diabetes and compositional changes in intestinal microbiota (12). Subsequently, 2 metagenome studies comprising larger patient cohorts were performed in China and Sweden and confirmed that patients with type 2 diabetes and control subjects differed in gut microbiota composition (13,14). When comparing the metagenomes of the Chinese and the Swedish populations, it was shown that the cohorts clustered separately, probably reflecting genetic and dietary habits. Still, both studies reported decreased levels of butyrate-producing bacteria in patients with diabetes. The data from these 2 studies were later reanalyzed in a meta-analysis focused on common diabetes medications as confounding factors (15). It was found that many of the observed differences could be attributed to metformin treatment. However, the reduced abundance of butyrate producers was independent of metformin use, and species belonging to *Roseburia*, *Subdoligranulum* and a cluster of *Clostridiales* were also decreased in a subgroup of treatment-naive patients with type 2 diabetes. It has been suggested that some of the antidiabetes effects of metformin may be mediated via the gut microbiota (see section below).

To investigate early changes in gut microbiota composition and function during the development of type 2 diabetes while avoiding the influence of antidiabetes drugs, studies have been performed in individuals with impaired glucose homeostasis but not yet diagnosed with diabetes. A study including 277 Danish subjects without diabetes showed that people with insulin resistance had gut microbiotas with increased capacity for branched-chain amino acid (BCAA) production as well as increased levels of serum BCAAs (16).

Prevotella copri and *Bacteroides vulgatus* were identified as the most important species driving the association between biosynthesis of BCAAs and insulin resistance. High levels of BCAAs are known to activate the target of rapamycin complex 1, resulting in insulin resistance through the phosphorylation of insulin receptor substrate 1 (17). Furthermore, in a recent case-control study, patients with prediabetes were matched with control subjects who had normal glucose regulation (18). In total, 36 operational taxonomic units were differentially abundant. *Clostridium* was decreased, whereas *Dorea*, *Sutterella* and *Streptococcus* species were increased in patients with prediabetes. In particular, members of the order Clostridiales and *Akkermansia muciniphila* were strongly decreased in subjects with prediabetes. Importantly, this study demonstrates that loss of butyrate producers seems to precede diabetes because it is observed in people with prediabetes.

In summary, although cohort-dependent differences and confounding factors such as medications make it hard to identify a type 2 diabetes microbiome signature, impaired glucose homeostasis is associated with decreased capacity for butyrate production. Furthermore, it is also possible that stochastic variations in the individual gut microbiota is the reason a common signature has not yet been observed (19).

Mechanisms Linking the Gut Microbiota and Type 2 Diabetes

Several metabolites produced by the gut microbiota, including SCFAs, amino acids and bile acids, are involved in the regulation of host metabolism and gut integrity (Figure 1). These interactions pose putative links between the gut microbiota and glucose homeostasis.

Short-chain fatty acids

Microbially produced SCFAs constitute 5% to 10% of the total energy intake in healthy subjects, and butyrate is the preferred energy source for colonocytes (5). SCFAs also regulate the host's glucose metabolism by acting as signaling molecules. By binding to G protein-coupled receptors GPR41 and GPR43, expressed on the enteroendocrine L cells, they stimulate secretion of the incretin hormones glucagon-like peptide-1 (GLP-1) and peptide YY in humans and rodents. GLP-1 stimulates insulin secretion, while peptide YY reduces appetite (5). SCFAs also stimulate gut motility and secretory activity by acting on enteric neuron cells (20) and reduce inflammation by acting on immune cells in the lamina propria (21) in a rodent model. The majority of butyrate is utilized directly in the gut, whereas some propionate and substantial amounts of acetate reach systemic circulation and, thereby, affect adipose tissue, brain and liver. SCFAs have been shown to induce overall beneficial metabolic effects in these peripheral tissues (5).

The presence of SCFAs is also linked to enhanced gut integrity and improvement of intestinal barrier function through increased expression of the tight junction proteins claudin-1 and zonula occludens-1 and through rearrangement of occludins (22). In addition, butyrate has also been shown to reduce inflammation by decreasing LPS translocation and reversing the aberrant expression of zonula occludens-1 in a rat model of hepatic ischemia (23). Another mechanism linking SCFAs with microbiota homeostasis has been described, wherein butyrate activates peroxisome proliferator-activated receptor-gamma in mouse colonocytes (24). This peroxisome proliferator-activated receptor-gamma activation was associated with increased beta oxidation and decreased levels of oxygen available to the gut bacteria. It also decreased the levels of nitrate in the gut lumen. The reduced bioavailability of electron acceptors prevented dysbiotic expansion of facultative anaerobes such as *Escherichia* and *Salmonella* in the colon, hence promoting a healthy gut microbiota.

Imidazole propionate

A novel link between microbial amino acid metabolism and the pathogenies of type 2 diabetes was recently demonstrated by Koh et al (25). The gut microbiota of patients with type 2 diabetes was shown to have increased capacity to produce the histidine-derived metabolite imidazole propionate (ImP) compared to the gut microbiota of healthy controls. Patients with type 2 diabetes also had increased plasma levels of ImP. Administration of ImP decreased glucose tolerance in mice and, like BCAAs, it was shown that ImP impairs insulin signaling in hepatocytes through a signaling pathway involving the activation of rapamycin complex 1.

Bile acids

Microbial processing of bile acids may regulate host metabolism. Bile acids are produced in hepatocytes and are secreted into the gut to facilitate absorption of dietary lipids. In addition, they act as signaling molecules controlling glucose homeostasis. By binding to the nuclear farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5, bile acids regulate the incretin hormone GLP-1 secretion in intestinal L cells (26). They also decrease gluconeogenesis and promote glycogen production in the liver, increase energy expenditure in brown adipose tissue and muscles, stimulate insulin secretion from beta cells in the pancreas and attenuate inflammation in immune cells (26). Experiments in mice show that FXR signaling in the ileum and the liver is also involved in negative-feedback control of bile-acid synthesis (27). The gut microbiota transforms host-derived primary bile acids by deconjugation, dehydrogenation and dehydroxylation in the distal small intestine and in the colon, thus increasing the diversity of the bile-acid pool. These modifications can affect receptor affinity. Hence, the microbiota is capable of regulating bile-acid signaling. Given that bile acids also affect bacterial composition, the gut microbiota and bile acids have a reciprocally regulatory relationship (28).

Gut-barrier integrity

Gut-barrier integrity and interactions between the gut microbiota and the innate immune system are important for metabolic health. Obese mice with impaired glucose metabolism and humans with type 2 diabetes have increased levels of LPS in the blood (29). Gut microbiota-derived LPS in the blood aggravates inflammation of the metabolic tissues of mice (30) and may, thereby, contribute to disease progression. The type of dietary fat also affects the level of endotoxemia. Mice fed a lard diet rich in saturated fatty acids have increased endotoxemia compared to mice fed a fish-oil diet rich in polyunsaturated fatty acids (31). In addition to lipids, dietary fibres are important regulators of gut integrity. Dietary supplementation with oligofructose reduces both plasma LPS levels and intestinal permeability (32). In the setting of dietary fibre deficiency, mouse experiments have shown that the gut microbiota may use host-secreted mucus glycoproteins as a nutrient source, resulting in degradation of the colonic mucus barrier (33). The gut microbiota influences gut epithelial and mucus layer homeostasis (34). Germ-free mice have impaired epithelial cell turnover and a thin adherent colonic mucus layer, properties that can be reversed upon gut colonization or when exposed to bacterial products.

A. muciniphila has been shown to promote epithelial integrity in human enterocytes (35) and *Lactobacillus plantarum* in Il-10 knockout mice (36). *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence mucus production in gnotobiotic mice (37) and *Lactobacillus casei* mucin glycosylation in human intestinal cells (38).

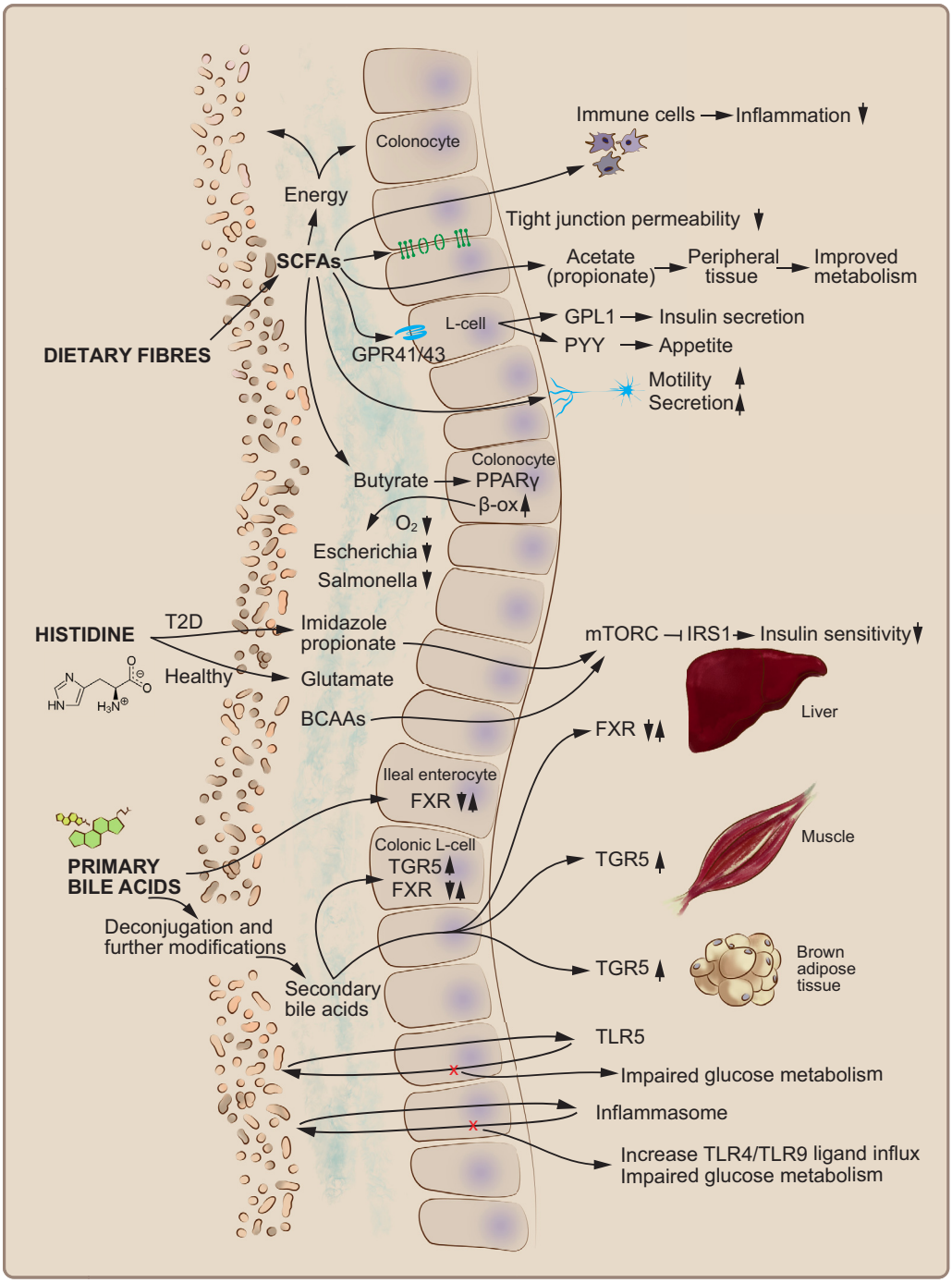

Figure 1. Mechanisms linking the gut microbiota and type 2 diabetes. Short-chain fatty acids regulate host glucose homeostasis by supplying colonocytes with energy, regulating immune cell function, decreasing gut permeability, improving peripheral tissue metabolism, stimulating incretin hormone production, increasing secretion and gut motility by stimulating enteric neuron signaling and increasing colonocyte beta oxidation by activating peroxisome proliferator-activated receptor-gamma (PPARγ). Imidazole propionate and branched-chain amino acids (BCAAs) impair insulin signaling by activation of rapamycin complex 1 (mTORC1). Bile acids regulate metabolism by binding to farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5) in several different tissues. Interaction between the gut microbiota and innate immunity components maintains systemic glucose homeostasis. *GPL1,* G protein-coupled bile acid receptor 1; *IRS1*, insulin receptor substrate 1; *PYY,* peptide YY; *TLR5,* toll-like receptor 5.

Innate immunity

The gut microbiota may affect host metabolism by interacting with factors involved in innate immunity. For example, mice deficient in toll-like receptor 5 (TLR5) have been shown to exhibit hyperlipidemia, hypertension, insulin resistance and obesity. The transfer of gut microbiota from TLR5-deficient donors into germ-free recipient mice induced metabolic perturbations in the recipients, indicating that dysbiosis may contribute to the impaired metabolic state of TLR5-deficient mice (39). Another example of an innate immunity component that affects metabolic health through interaction with the gut microbiota is the inflammasome. The inflammasome is a multiprotein complex involved in the activation of inflammatory response. Mice lacking constituents of the inflammasome that were fed a methionine/choline-deficient diet had a modified gut microbiota compared with wild-type mice (40). They also exhibited increased influx of TLR4 and TLR9 agonists into portal circulation, resulting in impaired glucose metabolism and

increased steatohepatitis. The disease phenotype could be partially transferred by cohousing, indicating that the microbiota from inflammasome-deficient mice can induce metabolic perturbations.

Therapies for Diabetes in the Microbiome

Type 2 diabetes may be counteracted by restoring gut microbiota homeostasis. Several strategies to reverse gut microbiota dysbiosis, including fecal transplantation, prebiotics, probiotics and drugs, have been investigated. In addition, more recent approaches point to the integration of individual-specific gut microbiota features into personalized interventions, as in the case of algorithms able to predict individual postprandial glycemic indexes based on personal data and gut microbiota composition (41).

Fecal microbiota transplant

The most radical way to manipulate gut microbiota composition is fecal microbiota transplant (FMT). FMT is the process of transmitting fecal bacteria from a healthy donor into a recipient. FMT treatment is used clinically for some disease conditions and is highly efficient for treatment of recurring *Clostridium difficile* infection. Treatment of other diseases such as ulcerative colitis has so far been less successful. It has been shown that metabolic profiles of mice (42) and humans (43,44) can be readily transmitted by FMT into germ-free recipient mice. Studies in which patients with metabolic syndrome receive fecal microbiota from lean donors have also been performed, resulting in improved insulin sensitivity and increased abundance of butyrate-producing bacteria in the gut (45). The responses to FMT have varied widely and were dependent on the baseline microbiota composition of the recipients (45). It must also be emphasized that the positive metabolic effect was transient, and repeated treatments would be required to sustain the effect.

Dietary fibres

Prebiotics are dietary fibres and other food components that promote the growth or function of beneficial bacteria. Consumption of prebiotics may affect glucose metabolism, and high intake of soluble dietary fibre has been shown to improve glycemic control, decrease hyperinsulinemia and lower plasma lipid concentrations in patients with type 2 diabetes (46). However, it should be emphasized that the response to fibres differs in human cohorts and that the outcomes of human intervention trials are often inconsistent. Studies performed on mouse models usually have stronger effects but also use higher amounts of fibres compared to human trials (47). Therefore, data concerning the effect of dietary fibres obtained in experimental models do not always apply to humans.

However, interindividual differences in gut microbiota composition have been shown to result in varying responses to dietary interventions. For example, in a study in which healthy subjects consumed barley kernel-based bread with high fibre content for 3 days, the subjects could be stratified into responders and nonresponders with respect to improvement in glucose metabolism (48). Metagenomic analysis revealed that responders had increased levels of *Prevotella copri* and increased potential to ferment complex polysaccharides compared with nonresponders. Future dietary therapies for metabolic disorders may involve an analysis of the gut microbiota to customize dietary fibre content according to bacterial composition. Synergistic therapies including both bacteria (probiotics) and suitable prebiotics may also be applied (synbiotics).

Probiotics

Probiotics are microorganisms that confer a health benefit on the host when consumed. Meta-analysis of probiotic treatment studies has shown that probiotics decrease glucose, glycated hemoglobin, insulin and homeostatic model assessment of insulin levels in participants with diabetes but not in participants with other risk factors. Additionally, formulations with more than 1 microbial strain are more efficient, especially if the probiotic is given in capsules (49).

Traditionally, lactic acid-producing species within the *Bifidobacterium* and *Lactobacillus* genera have been used as probiotics. However, studies comparing the gut microbiota composition between patients with type 2 diabetes and healthy subjects have identified other bacterial species that are consistently associated with a healthy phenotype, and they are currently being evaluated as novel probiotics.

A. muciniphila is a mucin-degrading bacterium. Humans studies have shown that an abundance of *A. muciniphila* is inversely correlated with obesity and diabetes (50). Administration of *A. muciniphila* to mice improved metabolism, restored gut barrier function and decreased inflammation in mice (51). Furthermore, administration of a pasteurized preparation of *A. muciniphila*, as well as a specific membrane protein isolated from *A. muciniphila*, reduced fat mass and improved insulin resistance and dyslipidemia in obese mice with diabetes (52). This suggests that treatment of metabolic disorders with *A. muciniphila* may not be restricted to the use of live bacteria. Another bacterium with potential value in the treatment of type 2 diabetes is *Faecalibacterium prausnitzii*, which has anti-inflammatory properties, and many studies have reported that its abundance is decreased in the gut microbiota of patients with metabolic syndrome and diabetes (13,14). However, it remains to be investigated whether *F. prausnitzii* contributes to improved glucose metabolism and whether it can be applied as a therapeutic agent.

In addition to naturally occurring bacteria, genetically engineered commensal species have been developed to deliver therapeutic factors to the gut. An example relevant to the treatment of diabetes is the use of *Lactococcus lactis* as a vector for producing GLP-1 (53). Oral administration of this strain resulted in decreased blood glucose and increased levels of insulin in rats. Although there are safety concerns that must be addressed prior to clinical application, the possibility of combining a suitable bacterial vector with a customized gene product may be useful in future diabetes therapy.

Influence of Drugs on the Gut Microbiota

Many human drugs may also affect gut bacteria. A broad screening of more than 1,000 drugs recently showed that 24% of the substances, including members of all major therapeutic classes, inhibit the growth of 1 or more gut bacterial strains in vitro (54). Similarly, many drugs including proton-pump inhibitors (55), nonsteroidal anti-inflammatory drugs (56), atypical antipsychotics (57) and several types of antidiabetes drugs induce changes in gut microbiota composition in vivo.

Antidiabetes drugs that influence the gut microbiota include biguanides, alpha-glucosidase inhibitors, incretin-based drugs, GLP-1 receptor agonists, dipeptidyl peptidase-4 inhibitors and thiazolidinediones (58). In most cases, a causal link between changes in gut microbiota composition and the antidiabetes effect of the drug has not been demonstrated. However, administration of the alpha-glucosidase inhibitor acarbose in patients with type 2 diabetes increases *Lactobacillus*, *Bifidobacterium* (59,60) and other SCFA-producing bacteria (59). Acarbose also increases the abundance of *Dialister* species, which correlates negatively with glycated

hemoglobin levels in patients with prediabetes, indicating a potential role in the regulation of glucose metabolism (59). The outcome of acarbose treatment of patients with type 2 diabetes is dependent on gut microbiota compositions prior to medication. Patients with a great abundance of *Bacteroides* species have been shown to exhibit greater improvement in metabolic parameters after acarbose treatment than patients with a gut microbiota dominated by *Prevotella* species (61). This indicates that stratification of patients based on gut microbiota composition could be helpful in choosing medication for patients with type 2 diabetes. Another antidiabetes drug with impact on gut microbiota composition is the GLP-1 receptor agonist liraglutide, which has been shown to reduce microbial diversity and decrease levels of *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* species in mice fed high-fat diets (62).

Influence of metformin on the gut microbiota

Metformin is the most commonly used medication for treatment of type 2 diabetes, and its impact on the gut microbiota has been more extensively studied than that of any other antidiabetes drug. Metformin decreases hyperglycemia at least partially by suppressing liver glucose production, but although this drug has been used for several decades, its mechanism of action has not yet been fully described. Recent research suggests that changes in gut microbiota may contribute to the antidiabetes effect of metformin.

To investigate the impact of antidiabetes drugs on the gut microbiota, Forslund et al (15) performed a meta-analysis of metagenomic data comprising 3 different populations. The authors showed that metformin influenced gut microbiota composition by decreasing the abundance of *Intestinibacter* and increasing that of *Escherichia* species. On a functional level, the study showed that metformin increases the potential of butyrate and propionate production. The impact of metformin on the gut microbiota was further investigated in a double-blind study by Wu et al (63). Metformin or placebo was given to treatment-naive patients with type 2 diabetes. Whole-genome shotgun sequencing revealed that metformin induced significant changes in the abundance of more than 80 bacterial strains. Most of these strains belonged to *Firmicutes* and gamma-*Proteobacteria* species. At the genus level, *Intestinibacter* was decreased, and *Escherichia* species were increased after metformin treatment. Pathway-enrichment analysis revealed that metformin treatment was linked to the enrichment of genes involved in bacterial environmental responses, drug resistance, central carbohydrate metabolism, amino acid metabolism and LPS biosynthesis. These results were confirmed in patients from the placebo group, who were switched to metformin treatment. Metabolomics analysis showed that metformin significantly increased butyrate and propionate in men while no differences

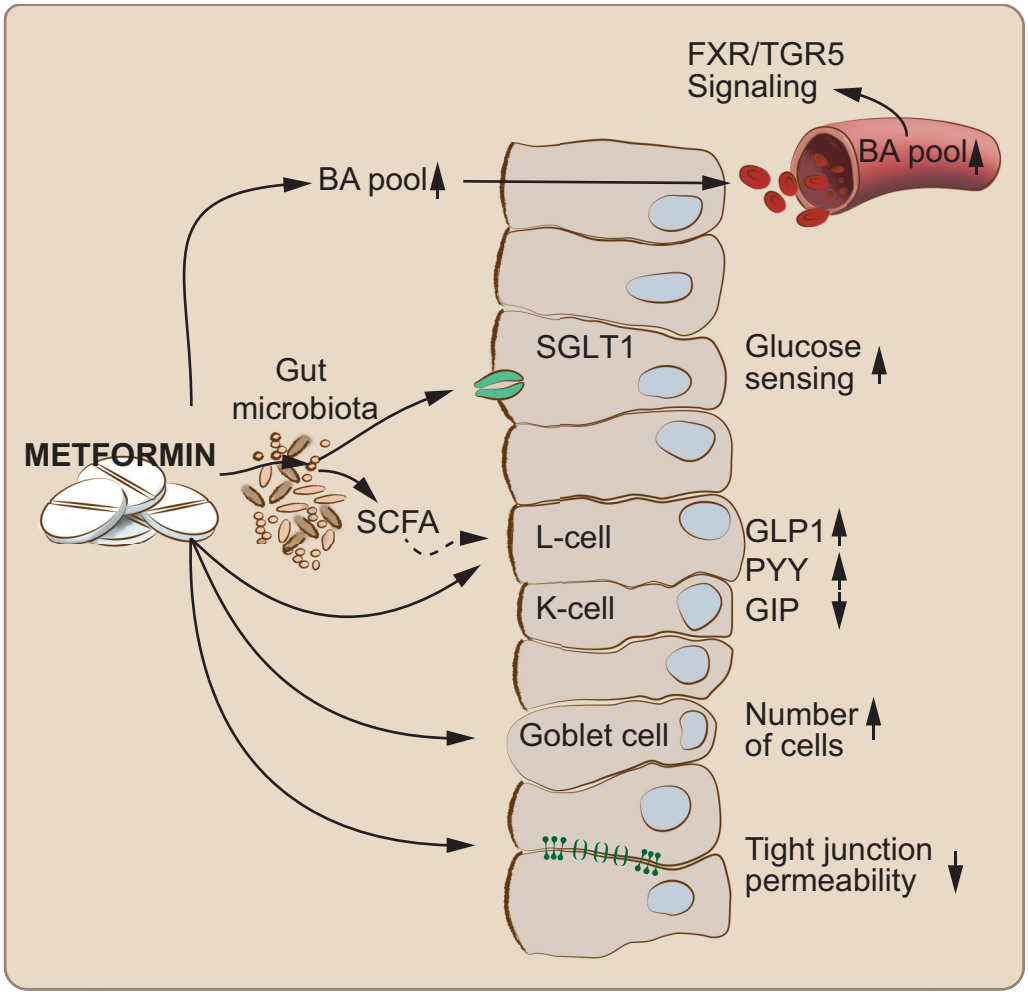

Figure 2. Mechanisms linking metformin, gut physiology and host glucose metabolism. Metformin may improve host glucose homeostasis by several mechanisms. Farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5) signaling is regulated by increasing the bile-acid pool. Glucose sensing is improved, and incretin hormone secretion is regulated by increased production of short-chain fatty acids (SCFAs). The number of goblet cells is increased and tight junction permeability is decreased. *GIP,* Gastric inhibitory polypeptide; *GLP1,* G protein-coupled bile acid receptor 1; *PYY,* peptide YY; *SGLT1,* sodium-glucose cotransporter type 1.

were observed when results from men and women were combined. Importantly, transfer of fecal samples to germ-free mice showed that glucose tolerance was improved in mice that received metformin-altered microbiota. In summary, metformin treatment has been shown to influence the gut microbiota and may reverse the dysbiotic decrease in butyrate production observed in patients with type 2 diabetes.

Mechanisms linking metformin, gut microbiota and metabolic health

Several intestinal functions linked to glucose homeostasis and potentially associated with development of type 2 diabetes are affected by metformin. The impact of metformin on gut physiology can often be attributed changes in the gut microbiota. Bauer et al (64) recently identified (Figure 2) a glucose-sensing pathway in the proximal small intestine involving sodium-glucose cotransporter 1 (SGLT1). High-fat feeding in mice changed the microbiota and compromised glucose sensing, while metformin treatment counteracted these microbial changes and restored glucose sensing. The metformin effect was shown to be mediated through the gut microbiota.

As previously discussed, SCFAs are produced by gut bacteria and are associated with a healthy metabolic phenotype. Metformin treatment increases the abundance of SCFA-producing bacteria such as *Allobaculum*, *Bacteroides*, *Blautia*, *Butyricoccus*, *Lactobacillus*, *Akkermansia* and *Phascolarctobacterium* species in rat models (65,66). Metformin also increases the levels of SCFAs producing bacteria, such as *Akkermansia*, *Lactobacillus*, *Bifidobacterium*, *Prevotella*, *Megasphaera*, *Shewanella*, *Blautia* and *Butyrivibrio* species in humans with type 2 diabetes (15,63,67), although contradictory reports indicate that the effect may be diet dependent (68).

Metformin is also a potent regulator of incretin hormones related to glucose homeostasis. GLP-1 and peroxisome proliferator-activated receptor-gamma are upregulated by metformin in both healthy subjects and in patients with type 2 diabetes (69,70). It has also been shown that plasma levels of glucose-dependent insulinotropic polypeptide are decreased by metformin in rats fed high-fat/high-sucrose diets (71). Hence, incretin hormone regulation may contribute to the positive effect on glucose metabolism induced by metformin. The mechanism by which metformin affects incretin production is largely unknown, but the effect may be mediated by metformin-induced changes in gut microbiota function.

Metformin also increases the bile-acid pool in the intestine, mainly through reduced ileal absorption (72), and has been shown to increase serum levels of bile acids in patients with type 2 diabetes (63). Studies in rats with diabetes also indicate that metformin may help to improve glucose metabolism by regulating the levels of serum bile acids (73). The role of gut microbiota in this process has not yet been investigated.

Finally, metformin may contribute to better glucose metabolism by improving gut barrier function. Metformin restores tight junctions and reverses increased gut permeability and serum LPS levels in mice fed high-fat diets (74). It has also been shown to increase the number of mucin-producing goblet cells (75).

Taken together, metformin restores dysbiosis during type 2 diabetes and increases the abundance of bacteria associated with healthy glucose metabolism, such as *A. muciniphila* and *Lactobacillus*. Furthermore, it improves the function of several intestinal processes associated with glucose metabolism, including glucose sensing, production of incretin hormones, bile-acid metabolism and gut barrier function. Metformin also induces gut microbiota-mediated production of SCFAs. Despite the fact that many mechanistic aspects still remain to be elucidated, the gut microbiota appears to be an important component in the action of metformin.

Conclusion and Future Perspectives

In recent years it has become increasingly acknowledged that type 2 diabetes is associated with gut microbial dysbiosis. Impaired glucose homeostasis is associated with changes in gut microbiota composition and function. These changes may be reversed in response to fibre, probiotics or drugs. However, it is not known to what extent the gut microbiota actually contributes to the disease phenotype in type 2 diabetes and whether future therapies targeted toward the gut microbiota may substantially affect glucose homeostasis. It also remains to be investigated how potential therapeutic agents should be designed to reverse dysbiosis in a sustainable and possibly personalized manner. Treatment with bacteria has been shown to improve glucose homeostasis in humans transiently. To achieve long-term effects, future therapies may involve symbiotic combinations of bacteria and fibres or other substances that promote the growth and function of beneficial bacteria.

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Author Contributions

Robert Caesar wrote the article.

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