

Effects of Metformin on the Gut Microbiota in Obesity and Type 2 Diabetes Mellitus

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Abstract: Metformin is a first-line treatment for type 2 diabetes mellitus (T2DM); however, its underlying mechanism is not fully understood. Gut microbiota affect the development and progression of T2DM. In recent years, an increasing number of studies has focused on the relationship between metformin and gut microbiota, suggesting that metformin might exert part of its hypoglycemic effect through these microbes. However, most of these results were not consistent due to the complex composition of the microbiota, the differences between species, the large variation between individuals, and the differences in experimental design, bringing great obstacle for our better understanding of the effects of metformin on the gut microbiota. Here, we reviewed the published papers concerning about the impacts of metformin on the gut microbiota of mice, rats, and humans with obesity or T2DM, and summarized the changes of gut microbiota composition caused by metformin and the possible underlying hypoglycemic mechanism which is related to gut microbiota. It was found that the proportions of some microbiota, such as phyla Bacteroidetes and Verrucomicrobia and genera *Akkermansia*, *Bacteroides* and *Escherichia*, were significantly affected by metformin in several studies. Metformin may exert part of hypoglycemic effects by altering the gut microbiota in ways that maintain the integrity of the intestinal barrier, promote the production of short-chain fatty acids (SCFAs), regulate bile acid metabolism, and improve glucose homeostasis.

Keywords: metformin, gut microbiota, obesity, type 2 diabetes mellitus

Introduction

T2DM is a group of clinical syndromes mainly characterized by disorders of glucose metabolism caused by the combined effects of genetic and environmental factors. The significant pathophysiological features of T2DM are the impaired regulatory function of insulin on glucose metabolism (insulin resistance) with reduced (or relatively reduced) insulin secretion caused by pancreatic islet β -cell dysfunction.¹ In recent years, with the improvement of people's living standards and dietary changes, the prevalence of obesity and T2DM has increased year by year, and the population with obesity and T2DM has become younger. The latest data from the International Diabetes Federation show that there were approximately 463 million diabetic patients worldwide in 2019. Approximately 87.5% of the T2DM patients are overweight, according to the 2017 National Diabetes Statistics Report of the CDC. The pathogenesis of T2DM is not fully understood, and it has many similarities with obesity, such as lipid metabolic disorders, stress, and insulin resistance. Obesity, especially the accumulation of abdominal visceral fat, aggravates insulin resistance in patients, which is a major risk factor for the development of T2DM.^{2,3}

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Metformin has been used to treat diabetes since the early 1950s and has become a first-line treatment for T2DM. Metformin alone or in combination with other hypoglycemic drugs can effectively control the blood glucose of diabetic patients, especially obese or overweight diabetic patients. The major mechanisms of hypoglycemic action of metformin include inhibiting hepatic gluconeogenesis and reducing hepatic glucose output, increasing glucose uptake and utilization in peripheral tissues (muscle and fat), and improving the energy metabolism in muscle, fat, and liver through the activation of AMP-activated protein kinase.⁴ The oral bioavailability of metformin is approximately 30–60%, the concentration in the intestine is 100–300 times higher than that in the serum, and approximately 50% of the intake dose is found in the stool. After oral administration, the half-life of metformin is approximately 3–4 hours, which is inconsistent with the duration of its hypoglycemic effect.^{5,6} In addition, intravenous administration of metformin could not improve blood glucose. These results all suggest that metformin has key effects on the digestive tract.

In recent years, with the development of detection technology, people have realized that the gut microbiota plays an important role in the development and progression of obesity and diabetes. More and more studies focused on relationship between metformin and gut microbiota. However, it is still unclear how metformin affects the gut microbiota as the inconsistent results of the studies. Therefore, we reviewed the literatures concerning the effect of metformin on gut microbiota of mice, rats, and humans with obesity or T2DM, and discussed the hypoglycemic mechanism of metformin which is related to gut microbiota.

T2DM and the Gut Microbiota

The gut microbiota refers to the microbiota colonizing the surface of the intestinal mucosa. As an environmental factor that is closely associated with the human body, the gut microbiota is involved in human growth and development, physiological processes, and even disease states. There are approximately 10^{14} bacteria in the human intestinal tract, which is approximately 10 times the number of human cells throughout the body, and these bacteria carry 100 times the number of genes as the human genome.⁷ These large numbers of bacteria are mainly from nine phyla, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Spirochaetes, and VadinBE97, among which Firmicutes, Bacteroidetes,

Proteobacteria, and Actinobacteria account for the majority of human gut microbiota.⁸ Intestinal microbes help the body extract nutrients and energy from food. They produce a variety of biologically active compounds, such as vitamins and short-chain fatty acids, and they participate in the regulation of a variety of metabolic processes.⁹

Many studies have analyzed the composition of the gut microbiota of T2DM patients. Larsen et al found differences in the gut microbiota at the phylum level between T2DM patients and normal individuals, eg, the non-T2DM group had a significantly higher proportion of Firmicutes than the T2DM group.¹⁰ Two metagenome-wide association studies involving relatively large populations were conducted in China and Sweden, confirming the differences in the composition of the gut microbiota between T2DM patients and control groups.^{11,12} Although no consensus had been reached on which bacteria change significantly in T2DM patients, all these studies found a decrease in the number of butyrate-producing bacteria and an increase in conditional pathogens.

It has been found that intestinal microbes participate in the development and progression of T2DM by affecting the integrity of the intestinal barrier, production of SCFAs, and the metabolism of bile acids. Lipopolysaccharide (LPS) is an endotoxin present in the cell wall of gram-negative bacteria that is released after bacterial lysis and can cause a series of nonspecific inflammatory responses. LPS is one of the stressors inducing intestinal mucosal injury and hinders the function of the intestinal barrier. High-fat diet could alter the composition of the gut microbiota in mice and increase the concentration of LPS in mouse plasma, leading to intestinal permeability changes and impaired glucose tolerance.¹³ Changes in the gut microbiota under metabolic disease conditions can down-regulate connexins and claudins in intestinal epithelial cells, increase intestinal mucosal permeability, promote LPS release, induce massive production of inflammatory cytokines, and decrease the insulin sensitivity of the body. LPS derived from intestinal microbes is an important factor involved in the development and progression of inflammatory and metabolic diseases.¹⁴ Gut microbiota can decompose nondigestible food (eg, carbohydrates) into SCFAs. Of these SCFAs, acetic acid, propionic acid, and butyric acid are the most abundant. SCFAs have important metabolic functions and are essential for intestinal health. Butyrate and propionate can activate intestinal gluconeogenesis and have beneficial effects on glucose and energy homeostasis.¹⁵ SCFAs could also regulate

glucose metabolism through stimulating the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY in intestinal epithelial L cells.¹⁶ Yang et al found that the levels of acetic acid, propionic acid, and butyric acid in the stool of diabetic mice were significantly lower than those in the normal and high-fat groups.¹⁷ Bile acid is the main component of bile, which not only promotes the digestion and absorption of fat but also participates in glycolipid and energy metabolism. Bile acids regulate lipid and glucose metabolism through the activation of the 7-transmembrane bile acid receptor and nuclear farnesoid X receptor (FXR).¹⁸ The gut microbiota interacts with bile acids. On the one hand, these bacteria participate in the metabolism of bile acids and transform the primary bile acid of the host by debinding, dehydrogenating, and dehydroxylating in the distal small intestine and colon, thereby increasing the diversity of the bile acid pool composition. These modifications can affect the affinity of the bile acids to their receptors. The binding of secondary bile acids to G protein-coupled receptor TGR5 in intestinal epithelial L cells can induce the secretion of GLP-1, regulate the homeostasis of glucose metabolism, and lead to the disorder of glycolipid metabolism.¹⁹ On the other hand, bile acids also affect the composition of the gut microbiota.^{20,21} For example, secondary bile acids played a role in colonization resistance against *Clostridium difficile*.^{22,23} The concentrations and compositions of bile acids changed in both T2DM patients and animal models. The postprandial total bile acid (TBA), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) were higher in T2DM patients than in normal subjects.²⁴ The ratio of 12 α -OH to non-12 α -OH BAs in serum increased in rats with high-fat diet and streptozotocin-induced type 2 diabetes.²⁵

Metformin and the Gut Microbiota

In recent years, with the advancement of gene sequencing technology, an increasing number of studies has focused on the impact of metformin on the gut microbiota. We searched PubMed with the keywords “Metformin AND Gut Microbiota”, and pick out all of the studies that clearly described the effect of metformin on composition of gut microbiota in obesity or type 2 diabetes. All these studies were published until 2020. Finally, we found a total of 34 relevant studies and summarized them in Tables 1–3.

A total of fifteen papers were published to investigate the effect of metformin on the gut microbiota in mice with obesity or T2DM (Table 1). C57BL/6 mice were used in most studies,

while some studies also studied db/db mice, KKAY mice, and ICR mice. In the studies of C57BL/6 mice, the majority of mice were aged 3–8 weeks and were given a high-fat diet (HFD) for different durations (4 days–33 weeks) to induce obesity, followed by metformin treatment (75–300 mg/kg/d) for 4 days to 14 weeks.^{26–36} Two studies were conducted to study the effect of metformin treatment (20 or 113.75 mg/kg) for 8 or 11 weeks on diabetic db/db mice.^{37,38} Two other studies gave mice a HFD for 3 or 4 weeks to induce obesity in KKAY mice and ICR mice, followed by metformin treatment.^{39,40} Due to the complexity of the gut microbiota and the experimental design of the different studies, such as the different HFD durations and the different dosages and durations of metformin treatment, such studies are bound to have low replicability, but in the published studies, the effects of metformin on certain gut microbiota have been consistent. For example, the proportions of phyla Bacteroidetes^{27,34,36,39} and Verrucomicrobia^{26,27,29,34,39} and genera *Akkermansia*^{26,28,29,31,32,34,37–39} and *Bacteroides*^{29,34,37,39} were significantly increased in the metformin treatment group. However, studies in ICR mice showed that the proportion of Bacteroidetes in the gut microbiota of the metformin treatment group was significantly decreased,⁴⁰ suggesting that the effect of metformin on the gut microbiota may be related to the mouse strain.

A total of 9 studies conducted in Wistar rats (five papers), Sprague-Dawley rats (two papers), Otsuka Long-Evans Tokushima fatty rats (one paper), and Zucker diabetic fatty rats (one paper) were included (Table 2). Two studies gave the rats HFD for 2 or 10 weeks to induce an obese rat model,^{41,42} five studies treated rats with HFD for 2–12 weeks followed by injection of streptozotocin (STZ) to induce diabetes,^{43–47} and two studies used spontaneously diabetic rat models.^{48,49} The dose of metformin ranged from 30 to 215.12 mg/kg/d, and the treatment period ranged from 4 weeks to 12 weeks. Similar to the results in mice, Verrucomicrobia was significantly increased in HFD-induced Wistar obese rats after treatment with metformin.⁴¹ However, in diabetic Wistar rats induced by HFD combined with STZ, metformin treatment showed the opposite effects on Verrucomicrobia.⁴³ Like in mice, the increase in genus *Akkermansia* caused by metformin was also found in rats treated with HFD and diabetic rats.^{41,45}

We found ten studies on the effect of metformin on the gut microbiota in obese or T2DM patients, two of which were conducted in obese patients^{50,51} and eight in T2DM patients^{52–59} (Table 3). Among them, four studies

Table 1 The Effects of Metformin on Composition of Gut Microbiota in T2DM or Obese Mice

	Animal Model	Control Group (Number)	Metformin Treatment Group (Number)	Effect of Metformin on Gut Microbiota	Country	Method for Detection	Author, Year and References
1	C57BL/6 mice (4 weeks old)	HFD for 8 weeks + HFD for 6 weeks (n=6)	HFD for 8 weeks + HFD with metformin treatment (300mg/kg/d) for 6 weeks (n=6)	Phylum: Verrucomicrobia↑ Genus: <i>Akkermansia</i> ↑	Korea	16S rRNA	Shin et al 2014 ²⁶
2	C57BL/6 mice (6 weeks old)	HFD for 18weeks + HFD for 10weeks (n=N/A)	HFD for 18weeks + HFD with metformin treatment (300mg/kg/d) for 10weeks (n=N/A)	Phylum: Bacteroidetes↑, Verrucomicrobia↑ Family: <i>Bacteroidaceae</i> ↑, <i>Verrucomicrobiaceae</i> ↑, <i>Clostridiales</i> family XIII↑ Species: <i>Akkermansia muciniphila</i> ↑, <i>Clostridium cocleatum</i> ↑	Korea	16S rRNA, qPCR	Lee et al 2014 ²⁷
3	C57BL/6 mice (3 weeks old)	HFD for 12 weeks + HFD for 6 weeks (n=3)	HFD for 12 weeks + HFD with metformin treatment (300mg/kg/d) for 6 weeks (n=3)	Genus: <i>Lactococcus</i> , <i>Coprococcus</i> , <i>Ruminococcus</i> , <i>Staphylococcus</i> , <i>Akkermansia</i> , <i>Streptococcus</i> , <i>Oscillospira</i> , <i>Lactobacillus</i> , <i>Allobaculum</i> , <i>Clostridium</i> , <i>Dorea</i> changed	USA	16S rRNA	Bornstein et al 2017 ²⁸
4	Obese diabetic db/db mice (8 weeks old)	Distilled water for 8 weeks (n=10)	Metformin treatment (20mg/kg/d) for 8 weeks (n=10)	Genus: <i>Bacteroidales</i> ↑, <i>Lactobacillus</i> ↑, <i>Allobaculum</i> ↑, <i>Bacteroides</i> ↑, <i>Akkermansia</i> ↑, <i>Staphylococcus</i> ↓, <i>Corynebacterium</i> ↓, <i>Jeotgaliococcus</i> ↓, <i>Aerococcus</i> ↓, <i>Enterococcus</i> ↓, <i>Facklamia</i> ↓	China	16S rRNA	Chen et al 2018 ³⁷
5	C57BL/6N mice (6 weeks old)	HFD for 23 weeks + HFD for 16 weeks (n=6)	HFD for 23weeks + HFD with metformin treatment (250mg/kg/d) for 16 weeks (n=6)	Phylum: Verrucomicrobia↑, Firmicutes/Bacteroidetes ratio↓ Genus: <i>Akkermansia</i> ↑, <i>Bacteroides</i> ↑, <i>Butyricimonas</i> ↑, <i>Parabacteroides</i> ↑	Korea	16S rRNA, qPCR	Lee et al 2018 ²⁹
6	KKAY mice (8–9 weeks old)	HFD for 3 weeks + HFD with saline solution for 8 weeks (n=6)	HFD for 3 weeks + HFD with metformin treatment (314mg/kg/d) for 8 weeks (n=6)	Phylum: Bacteroidetes↑, Verrucomicrobia↑, Actinobacteria↓, Firmicutes↓ Genus: <i>Bacteroides</i> ↑, <i>Blautia</i> ↑, <i>Escherichia-Shigella</i> ↑, <i>Akkermansia</i> ↑, <i>Aerococcus</i> ↓, <i>Lactobacillus</i> ↓, <i>Corynebacterium</i> ↓, <i>Staphylococcus</i> ↓	China	16S rRNA	Gao et al 2018 ³⁹
7	C57BL/6 mice	HFD for 4 weeks + HFD for 10 weeks (n=8)	HFD for 4 weeks + HFD with metformin treatment (100mg/kg/d) for 10 weeks (n=8)	Species: <i>Bacteroidetes fragilis</i> ↑, <i>Escherichia coli</i> ↓	Korea	16S rRNA, qPCR	Wang et al 2018 ³⁰
8	C57BL/6 mice (3 weeks old)	HFD for 4 weeks + STZ+ HFD with saline for 5 weeks (n=6)	HFD for 4 weeks + STZ + HFD with metformin treatment (200mg/kg/d or 75mg/kg/d) for 5 weeks (n=6)	Phylum: Firmicutes↓ Genus: <i>Akkermansia</i> ↑ Species: <i>Bacteroides spp.</i> ↓	China	16S rRNA	Zheng et al 2018 ³¹
9	C57BL/6 mice (6 weeks old)	HFD with saline for 3 weeks (n=5)	HFD with metformin treatment (300mg/kg/d) 3 weeks (n=5)	Genus: <i>Enterococcus</i> , <i>Lactococcus</i> , <i>Streptococcus</i> , <i>Akkermansia</i> changed	China	16S rRNA	Ji et al 2019 ³²

(Continued)

Table 1 (Continued).

	Animal Model	Control Group (Number)	Metformin Treatment Group (Number)	Effect of Metformin on Gut Microbiota	Country	Method for Detection	Author, Year and References
10	db/db mice (8 weeks old)	Saline solution for 11 weeks (n=5)	Metformin treatment (113.75mg/kg/d) for 11 weeks (n=5)	Genus: <i>Butyrivimonas</i> ↑, <i>Lactobacillus</i> ↑, <i>Coprococcus</i> ↑, <i>Ruminococcus</i> ↑, <i>Akkermansia</i> ↑ Genus: <i>Prevotella</i> ↓, <i>Proteus</i> ↓	China	16S rRNA, qPCR	Zhang et al 2019 ³⁸
11	ICR mice (8 weeks old)	HFD+STZ +HFD for 4 weeks (n=10)	HFD+STZ+HFD with metformin treatment (200mg/kg/d) for 4 weeks (n=10)	Phylum: Firmicutes↑, Bacteroidetes↓, Proteobacteria↓ Species: <i>Lactobacillus</i> spp.↑, <i>Lactobacillales</i> ↑	China	16S rRNA	Chen et al 2019 ⁴⁰
12	C57BL/6J mice (6–8 weeks old)	FFC for 4 days (n=6)	FFC with metformin treatment (300mg/kg/d) for 4 days (n=6)	Species: <i>Lactobacillus animalis</i> ↓, Unclassified members of <i>Burkholderia</i> and <i>Romboutsia</i> genera↓, An unclassified member of <i>Alloprevotella</i> family↓	Austria	16S rRNA	Brandt et al 2019 ³³
13	C57BL/6 mice (3 weeks old)	HFD for 12 weeks + HFD for 12 weeks (n=14)	HFD for 12 weeks + HFD with metformin treatment (300mg/kg/d) for 12 weeks (n=14)	Phylum: Bacteroidetes↑, Verrucomicrobia↑, Firmicutes/ Bacteroidetes ratio↓ Genus: <i>Bacteroides</i> ↑, <i>Akkermansia</i> ↑, <i>Parabacteroides</i> ↑, <i>Christensenella</i> ↑, <i>Clostridiales</i> other↑, <i>AF-12</i> ↓, <i>Muribaculum</i> ↓, <i>Clostridiaceae</i> other↓, <i>Lachnoclostridium</i> ↓, <i>Coprococcus</i> ↓, <i>Dorea</i> ↓, <i>Papillibacter</i> ↓, <i>Oscillospira</i> ↓, <i>Ruminococcus</i> ↓, <i>Desulfovibrio</i> ↓, <i>Desulfovibrionaceae</i> ↓	Ireland	16S rRNA	Ryan et al 2020 ³⁴
14	C57BL/6J mice (5 weeks old)	HFD for 14 weeks (n=12)	HFD with metformin treatment (200mg/kg/d) for 14 weeks (n=12)	Family: <i>Lachnospiraceae</i> ↓	USA	16S rRNA	Chung et al 2020 ³⁵
15	C57BL/6J mice (78 weeks old)	HFD for 11 weeks (n=5–7)	HFD with metformin treatment (100mg/kg/d) for 11 weeks (n=5–7)	Phylum: Bacteroidetes↑ Genus: Genera belonging to <i>S24_7</i> , <i>Ruminococcaceae</i> , and <i>Lactococcus</i> ↑, Genera belonging to <i>Veillonellaceae</i> , <i>Coriobacteriaceae</i> , <i>Lactobacillus</i> , <i>Dorea</i> , <i>SMB53</i> , <i>Roseburia</i> , and <i>Dehalobacterium</i> ↓	USA	16S rRNA	Ahmadi et al 2020 ³⁶

Notes: ↑, Increase; ↓, decrease.

Abbreviations: N/A, not available; STZ, streptozotocin; HFD, high-fat diet; FFC, fat-fructose- and cholesterol-rich diet.

compared the changes in the gut microbiota of newly diagnosed T2DM patients (without drug treatment before the visit) who received metformin for different follow-up times (from 3 days to 2 years). Four studies were conducted in T2DM patients who had received drug treatment and compared the gut microbiota of patients with vs without metformin treatment. The results in humans are quite

different from the results of animal studies. Clinical trials on diabetic patients are affected by many complex factors, such as experimental design, diet, ethnic origins, course of disease, comorbidities, and combined medications. In addition, there were significant differences in intestinal microbial diversity between humans and animals.⁶⁰ Results of some bacteria were consistent in humans and

Table 2 The Effects of Metformin on Composition of Gut Microbiota in T2DM or Obese Rats

	Animal Model	Control Group (Number)	Metformin Treatment Group (Number)	Effect of Metformin on Gut Microbiota	Country	Method for Detection	Author, Year and References
1	Wistar rats (8–10 weeks old)	HFD for 10 weeks + HFD for 8 weeks (n=10)	HFD for 10 weeks + HFD with metformin treatment (200mg/kg/d) for 8 weeks (n=10)	Phylum: Proteobacteria↑, Verrucomicrobia↑ Genus: <i>Allobaculum</i> ↑, <i>Bacteroides</i> ↑, <i>Blautia</i> ↑, <i>Butyrivococcus</i> ↑, <i>Lactobacillus</i> ↑, <i>Phascolarctobacterium</i> ↑, <i>Parasutterella</i> ↑, <i>Klebsiella</i> ↑, <i>Prevotella</i> ↑, <i>Akkermansia</i> ↑, <i>Clostridium</i> XIVa↓, <i>Flavonifractor</i> ↓, <i>Lachnospiraceae_incertae_sedis</i> ↓, <i>Roseburia</i> ↓, <i>Clostridium</i> XI↓	China	16S rRNA	Zhang et al 2015 ⁴¹
2	OETF rats (4 weeks old)	Distilled water for 12 weeks (n=7)	Metformin treatment (100mg/kg/d) for 12 weeks (n=7)	Species: <i>Akkermansia</i> spp.↑, <i>Prevotella</i> spp.↓	Korea	DGGE, qPCR	Wang et al 2017 ⁴⁸
3	Wistar rats (200g)	HFD for 4 weeks+ STZ + HFD for 2 weeks +HFD for 4 weeks (n=8)	HFD for 4 weeks + STZ + HFD for 2 weeks + HFD with metformin treatment (200mg/kg/d) for 4 weeks (n=9)	Phylum: Bacteroidetes↑ Verrucomicrobia↓, Proteobacteria↓ Genus: <i>02d06</i> , <i>rc4_4</i> ↑ <i>Sutterella</i> , <i>Prevotella</i> changed	China	16S rRNA, qPCR	Liu et al 2018 ⁴³
4	Wistar rats (200g)	HFD for 4 weeks + STZ + HFD for 2 weeks +HDF for 4 weeks (n=6)	HFD for 4 weeks + STZ + HFD for 2 weeks +HFD wit metformin treatment (200mg/kg/d) for 4 weeks (n=7)	Phylum: Bacteroidetes changed	China	16S rRNA	Liu et al 2018 ⁴⁴
5	Zucker diabetic fatty fa/fa (ZDF) rats (7 weeks old)	HFD for 4 weeks + HFD with saline for 4 weeks (n=8)	HFD for 4 weeks + HFD with metformin treatment (215.15mg/kg/d) for 4 weeks (n=8)	Phylum: Firmicutes↑ Genus: <i>Lactobacillus</i> ↑ Species: <i>Lactobacillus intestinalis</i> ↑, <i>Lactobacillus johnsonii</i> ↑	China	16S rRNA	Zhang et al 2019 ⁴⁹
6	Wistar rats	HFD for 4 months + HFD with saline for 6weeks (n=8)	HFD for 4 months +HFD with metformin treatment (150 mg/kg/d) for 6 weeks (n=8)	Species: <i>Bifidobacteria</i> ↑, <i>Akkermansia</i> ↑, <i>Shewanella</i> ↑, <i>Allobaculum</i> ↑, <i>Peptostreptococcaceae</i> ↑, <i>Intestinibacter</i> ↑, <i>Prevotella</i> ↓, <i>Deferribacteres</i> ↓	China	16S rRNA	Li et al 2019 ⁴²
7	Sprague-Dawley rats (100–120g)	HFD for 12 weeks + STZ + HFD for 2 days + HFD with distilled water for 6weeks (n=6)	HFD for 12 weeks + STZ + HFD for 2days + HFD with metformin treatment (100 mg/kg/d) for 6 weeks (n=6)	Order: <i>Clostridiales</i> ↑, <i>Lactobacillales</i> ↑, <i>Enterobacteriales</i> ↓ Genus: <i>Lactobacillus</i> ↑, <i>Roseburia</i> ↑, <i>Akkermansia</i> ↑, <i>Desulfovibrio</i> ↓, <i>Lachnospiraceae</i> NK4A136↓	China	16S rRNA	Cui et al 2019 ⁴⁵

(Continued)

Table 2 (Continued).

	Animal Model	Control Group (Number)	Metformin Treatment Group (Number)	Effect of Metformin on Gut Microbiota	Country	Method for Detection	Author, Year and References
8	Sprague-Dawley rats (170–190g)	HFD for 6 weeks + STZ + HFD for 1 week + HFD with saline for 8 weeks (n=6)	HFD for 6 weeks + STZ + HFD for 1 week + HFD with metformin treatment (120 mg/kg/d) for 8 weeks (n=6)	Genus: <i>Eubacterium_xylanophilum</i> ↑, <i>Oscillibacter</i> ↑, <i>Anaerotruncus</i> ↑, <i>Bacteroidales_S24-7_group</i> ↓	China	16s rRNA	Liu et al 2020 ⁴⁶
9	Wistar rats (180–200g)	HFD for 2 weeks + STZ + HFD for 2 weeks + HFD for 12 weeks (n=5)	HFD for 2 weeks + STZ + HFD for 2 weeks + HFD with metformin treatment (30mg/kg/d) for 12 weeks (n=5)	Species: <i>Lactobacillus</i> spp.↑, <i>Bifidobacterium</i> spp.↑, <i>Escherichia</i> spp.↓, <i>Clostridium perfringens</i> ↓	Thailand	qPCR	Khat-udomkii et al 2020 ⁴⁷

Notes: ↑, Increase; ↓, decrease.

Abbreviations: N/A, not available; STZ, streptozotocin; DGGE, denaturing gradient gel electrophoresis.

rodents. Hiel et al reported that the proportion of *Akkermansia* significantly increased in obese patients on metformin, which was consistent with the results in animal studies.⁵⁰ The proportion of *Akkermansia muciniphila* was also increased in metformin-treated T2DM patients.⁵⁴ Forslund et al found that *Escherichia* spp. significantly increased in T2DM patients treated with metformin over those not treated with metformin.⁵² Similarly, Wu et al showed that *Escherichia* significantly increased in newly diagnosed T2DM patients after treatment with metformin.⁵³ Two studies on obese patients also found that metformin significantly increased the proportion of the *Escherichia/Shigella* group.^{50,51}

Dysbiosis in gut microbiota is strongly associated with the pathogenesis of obesity and T2DM. The composition of gut microbiota exhibited significant differences among obese, T2DM and healthy individuals.^{61,62} High fat diet and STZ induction change the gut microbiota of rats or mice.^{63,64} Besides, metformin also had different effects on the gut microbiota of obese/diabetic and healthy humans/animals. Metformin decreased the bacterial diversity in mice fed a high-fat diet, but had no effect on bacterial diversity in mice fed a normal diet.²⁷ The various influencing factors make the change of gut microbiota more complex. In order to better understand the gut microbiota in the clinical use of metformin, we just reviewed the literatures concerning the impacts of metformin on composition of gut microbiota in T2DM or obese rodents or patients.

There were also some studies which investigate the effect of metformin on gut microbiota in healthy subjects

or normal rats/mice. The short-term effect of metformin on composition of gut microbiota has been evaluated in healthy Caucasian volunteers, and seven days of treatment with metformin was associated with a significant decrease in the families *Peptostreptococcaceae* and *Clostridiaceae_1* and four genera within these families.⁶⁵ Another clinical trial conducted in 27 healthy young Danish men showed that metformin reduced the abundance of *Intestinibacter* spp. and *Clostridium* spp., while increased the abundance of *Escherichia/Shigella* spp. and *Bilophila wadsworthia*.⁶⁶ Metformin also altered the gut microbiota of healthy mice, and it was found that microbes from the *Verrucomicrobiaceae* and *Prevotellaceae* classes were enriched, while those from *Lachnospiraceae* and *Rhodobacteraceae* were depleted.⁶⁷

The Mechanisms by Which Metformin Exerts Hypoglycemic Effects Through the Gut Microbiota Maintaining Intestinal Barrier Integrity

The interaction between the intestinal barrier integrity and the gut microbiota is very important in the development and progression of metabolic diseases. Shin et al found that metformin inhibited the increase in serum LPS induced by HFD and believed that it was due to the change in the gut microbiota composition.²⁶ Similarly, metformin reduced the blood LPS level and protected intestinal barrier function in a HFD-induced mouse model of obesity and insulin resistance.⁶⁸ In addition, exogenous administration of LPS can block the enhancement of blood

Table 3 The Effects of Metformin on Composition of Gut Microbiota in T2DM or Obese Patients

	Control Group (Number)	Metformin Treatment Group (Number)	Effect of Metformin on Gut Microbiota	Country	Method for Detection	Author, Year and References
1	Metformin-untreated T2DM patients (n= 106)	Metformin-treated T2DM patients (n=93)	Species: <i>Escherichia</i> spp.↑, <i>Intestinibacter</i> spp.↓	Germany	Metagenomic	Forslund et al 2015 ⁵²
2	Metformin-untreated T2DM patients (n=14)	Metformin-treated T2DM patients (n=14)	Genus: <i>Prevotella</i> ↑, <i>Megasphaera</i> ↑, <i>Butyrivibrio</i> ↑, <i>Oscillospira</i> ↓, <i>Barnesiellaceae</i> ↓, <i>Clostridiaceae</i> 02d06↓ Species: <i>Akkermansia muciniphila</i> ↑	Colombia	16S rRNA	Cuesta-Zuluaga et al 2017 ⁵⁴
3	Treatment-naive newly diagnosed T2DM (n=22)	Treatment-naive newly diagnosed T2DM + 4 months of metformin treatment (1700 mg/d) (n=22)	Genus: <i>unclassified Enterobacteriaceae</i> ↑, <i>Pectobacterium</i> ↑, <i>Pantoea</i> ↑, <i>Serratia</i> ↑, <i>Raphidiopsis</i> ↑, <i>Dickeya</i> ↑, <i>Helicobacter</i> ↑, <i>Shewanella</i> ↑, <i>Erwinia</i> ↑, <i>Cronobacter</i> ↑, <i>Rheinheimera</i> ↑, <i>Dermacoccus</i> ↑, <i>Yersinia</i> ↑, <i>Bacillus</i> ↑, <i>Pseudomonas</i> ↑, <i>Salmonella</i> ↑, <i>Klebsiella</i> ↑, <i>Enterobacter</i> ↑, <i>Citrobacter</i> ↑, <i>Escherichia</i> ↑, <i>Dethiosulfovibrio</i> ↓, <i>Deferribacter</i> ↓, <i>Bartonella</i> ↓, <i>Acetivibrio</i> ↓, <i>Hippea</i> ↓, <i>Pseudogulbenkiania</i> ↓, <i>Pseudoflavonifractor</i> ↓, <i>Subdoligranulum</i> ↓, <i>Intestinibacter</i> ↓	Sweden	Metagenomic	Wu et al 2017 ⁵³
4	Metformin-untreated T2DM patients (n=23)	Metformin-treated T2DM patients (n=23)	Family: <i>Enterobacteriaceae</i> ↑	Sweden	qPCR, T-RFLP	Hung et al 2018 ⁵⁶
5	Treatment-naive newly diagnosed T2DM (n=22)	Treatment-naive newly diagnosed T2DM + 3 days of metformin treatment (1000 mg b.i.d) (n=22)	Genus: <i>Bacteroides</i> ↓ Species: <i>Bacteroides fragilis</i> ↓	China	Metagenomic	Sun et al 2018 ⁵⁵
6	African American men with T2DM who do not take metformin (n=11)	African American men with T2DM who take metformin (n=21)	Genus: <i>Bifidobacterium</i> ↑, <i>Catenibacterium</i> ↑, <i>Parabacteroides</i> ↑	USA	16S rRNA	Barengolts et al 2018 ⁵⁷
7	Obese women before metformin treatment (n=20)	Obese women after 2 months of low-calorie diet and metformin treatment (1000 mg/d) (n=20)	Genus: <i>Escherichia/Shigella</i> ↑	Iran	16S rRNA	Ejtahed et al 2019 ⁵¹
8	T2DM patients before metformin treatment (n=26)	T2DM patients after more than 3 months of metformin treatment (n=51)	Phylum: <i>Actinobacteria</i> ↑, <i>Fusobacteria</i> ↑ Class: <i>Betaproteobacteria</i> ↓, <i>Gammaproteobacteria</i> ↓ <i>Erysipelotrichi</i> ↓ Genus: <i>Turicibacter</i> ↑, <i>Fusobacterium</i> ↑	China	16S rRNA	Zhang et al 2019 ⁵⁸
9	Metformin-naive obese patients (n=53)	Metformin-treated obese patients (n=42)	Genus: <i>Akkermansia</i> ↑, <i>Clostridium</i> cluster XIVa↑, <i>Clostridium</i> cluster XIVb↑, <i>Escherichia/Shigella</i> ↑, <i>Klebsiella</i> ↑, <i>unclassified Enterobacteriaceae</i> ↑, <i>Clostridium</i> cluster XI↓, <i>Clostridium</i> cluster XVIII↓, <i>Roseburia</i> ↓, <i>unclassified Lachnospiraceae</i> ↓	Belgium	16S rRNA	Hiel et al 2020 ⁵⁰
10	Treatment-naive T2DM patients (n=14)	T2DM patients after 1–2 years of metformin treatment (n=14)	Order: <i>Bacteroidales</i> ↑, <i>Acidobacteriales</i> ↑ Family: <i>Koribacteraceae</i> ↑ Genus: <i>Pelomonas</i> spp.↑	Mexico	16S rRNA	Chávez-Carbajal et al 2020 ⁵⁹

Notes: ↑, Increase; ↓, decrease.

Abbreviations: N/A, not available; T-RFLP, terminal restriction fragment length polymorphism.

glucose control and insulin signaling caused by metformin. These results indicate that metformin might improve glucose metabolism by maintaining intestinal barrier function.

Promotion of SCFA Production

SCFAs produced by the gut microbiota can exert beneficial effects on peripheral tissues, such as adipose tissue, skeletal muscle, and liver, by controlling substrate metabolism and function, thereby improving insulin sensitivity. The ability of the gut microbiota to produce butyrate and propionate is enhanced in patients treated with metformin. Metformin treatment can increase the abundance of SCFA-producing bacteria in mice and rats, such as *Allobaculum*, *Bacteroides*, *Blautia*, *Butyricicoccus*, *Lactobacillus*, *Akkermansia*, *Phascolarctobacterium*, *Butyricimonas*, *Coprococcus*, and *Ruminococcus*.^{26,27,29,38,41} A similar finding was observed in T2DM patients: after taking metformin, the relative abundance of SCFA-producing bacteria increased (eg, *Akkermansia*, *Lactobacillus*, *Bifidobacterium*, *Prevotella*, *Megasphaera*, *Shewanella*, *Blautia*, and *Butyrivibrio*).^{52–54}

Regulation of Bile Acid Metabolism

It was found that metformin can improve glucose metabolism by regulating the total bile acid level in the serum of diabetic rats. Wu et al showed that metformin treatment for 4 months significantly increased plasma bile acid, while bile acid in stool samples remained unchanged. In the second month of metformin treatment, the level of bile salt hydrolase (bsh) produced by the gut microbiota increased.⁵³ Sun et al analyzed the gut microbiota in patients who were newly diagnosed with T2DM and were treated for the first time with metformin for 3 days. They found that metformin increased the level of glycochenodeoxycholic acid (GUDCA) by regulating the gut microbiota (such as inhibition of *Bacteroides fragilis* growth), thereby inhibiting the FXR signaling pathway to reduce blood glucose and maintain blood glucose homeostasis.⁵⁵ It was found that metformin reduced the reabsorption of bile acid in the distal ileum, leading to the increasing bile salt concentration within the colon, which might account for the effects of metformin on the colonic microbiota.^{69,70}

Regulating Specific Bacteria to Maintain Glucose Homeostasis

Through summarizing and comparing the results in patients and rodent models, we found that metformin

significantly increases the abundance of the phylum Verrucomicrobia, genus *Akkermansia*, and species *Akkermansia muciniphila*.^{26,27,31,41,42,45,48,53,54} Zhang et al found that *Akkermansia muciniphila* was reduced in patients with prediabetes (impaired glucose tolerance and/or impaired fasting glucose) and newly diagnosed T2DM patients, suggesting that a low abundance of this bacterium might be a biomarker of glucose intolerance.⁷¹ Lee et al found that *Akkermansia muciniphila* was negatively correlated with serum glucose level in HFD-fed mice treated with metformin.²⁷ *Akkermansia muciniphila* is one of the most abundant single species in the human gut microbiota, a well-known utilizer of mucin, and has been negatively correlated with obesity, diabetes, and cardiovascular diseases.⁷² It plays an important role in maintaining the integrity of the mucosa and could reduce the migration of the pro-inflammatory LPS and improve blood glucose.⁷³

Other Possible Mechanisms

It was reported by Horakova et al that metformin decreased glucose transport from the intestinal lumen into the blood.⁷⁴ Morita et al evaluated the accumulation of [¹⁸F]fluorodeoxyglucose in different portions of the intestine from 24 pairs of patients with T2DM who were receiving treatment with metformin or were not.⁷⁵ The results suggested that metformin could promote the transport of glucose from the circulation into stool. Bauer et al proposed a new mechanism of the hypoglycemic effect of metformin associated with the gut microbiota in rodents.⁷⁶ It was showed that high-fat diet changed the upper small intestinal microbiota and compromised glucose sensing in rats. Metformin treatment in the upper small intestine restored sodium–glucose cotransporter 1 (SGLT1) expression and glucose sensing while shifting the upper small intestinal microbiota partly by increasing the abundance of *Lactobacillus*. In addition, metformin-treated microbiota transplants restored glucose SGLT1 sensing.

Conclusions

Metformin has been shown to alter the composition of the gut microbiota in mouse and rat models induced by HFD and in T2DM patients, manifested as changes in the relative proportions of certain bacteria of different taxonomic levels. Metformin can improve glucose homeostasis and exert hypoglycemic effects by affecting the gut microbiota, through which it maintains intestinal barrier function, increases the production of SCFAs, regulates bile acid metabolism, and affects glucose homeostasis. The relationship among T2DM,

metformin, and the gut microbiota is gradually being understood, but the results of different studies are slightly different. It is still unclear how much of the hypoglycemic effect of metformin comes from its alteration of the gut microbiota and what role the microbiota plays in the development and progression of T2DM. Therefore, we expect future in-depth studies to take on the gut microbiota as a new hypoglycemic target.

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Disclosure

The authors declare no conflict of interest.

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